Food Chemistry 251 (2018) 9–17

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of exogenous ATP on the postharvest properties and pectin degradation of mung bean sprouts (*Vigna radiata*)

Lin Chen[a,b], Yige Zhou[a,b], Zhenyun He[a,b], Qin Liu[a,b], Shaojuan Lai[c], Hongshun Yang[a,b,*]

[a] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China
[c] Guangzhou Pulu Medical Technology Co., Ltd, Guangzhou, Guangdong 510800, PR China

ARTICLE INFO

Keywords:
Quality maintenance
Antioxidant effect
Discolouration amelioration
Transcriptional expression
Nanostructure
Polysaccharide

ABSTRACT

The effects of exogenous ATP on the postharvest quality, browning and softening of mung bean (*Vigna radiata*) sprouts were evaluated. ATP treatment significantly alleviated the quality loss and browning events during the storage of 3 days. It also reduced the oxidant damage by inducing high activities of peroxidase (9.3–13.9%) and superoxide dismutase (8.8–10.3%) which scavenged the reactive oxygen species (ROS) effectively. Transcriptional results indicated that ATP treatment decreased *VrPL1*, *VrPME* and *VrPG1* gene expression levels more than 2 folds at some time points. Furthermore, the atomic force microscope (AFM) images revealed that the pectin degradation was notably slowed by ATP treatment and the width and height of pectin backbone were better maintained (47.1% and 45.6% higher than control without ATP treatment). The cooperative effects of ROS scavenging and decreased expressions of pectin-related genes might contribute to the deferred pectin deterioration and firmness loss by ATP treatment.

1. Introduction

Postharvest fruits and vegetables are subject to various physiological and biochemical changes and eventually lead to quality deterioration including browning, softening and nutrition loss. The discolouration mainly comes from the oxidation of polyphenols by polyphenol oxidase (PPO) which is widespread in plants. Accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) during ripening will further stimulates the browning disorders (Lin et al., 2014). Besides impairing the appearance of fruits and vegetables, browning may also negatively affect other postharvest properties including flavour, texture and taste (Jiang, Duan, Joyce, Zhang, & Li, 2004; Zhang & Yang, 2017). Softening, as another major property of quality disorders in fruits and vegetables, primarily results from the cell wall modifications (Razzaq, Singh, Khan, Khan, & Ullah, 2016). Previous studies showed that pectin constituted up to 60% of wall mass in many fruits which exhibited the important role of the polyuronides during ripening (Cybulska, Zdunek, & Kozioł, 2015). Furthermore, Xin, Chen, Lai, and Yang (2017) investigated the nanostructures of different pectins in cherry by atomic force microscope (AFM), indicating the correlation between sodium carbonate-soluble pectin (SSP) and firmness. In order to save the loss of postharvest properties of fruits and vegetables, diverse strategies have been explored recently.

Abundant studies indicate that energy homeostasis plays an important role in the processes of ripening and senescence of plants (Yao, Zhu, Yi, Qu, & Jiang, 2015). The browning and softening of postharvest fruits and vegetables may occur due to the energy deficit and decreased energy generation efficiency (Chen, Lin et al., 2015; Wang et al., 2013). Exogenous adenosine triphosphate (ATP) treatment has been confirmed to improve the intracellular ATP and energy charge (EC) levels effectively and further maintain the level of unsaturated fatty acid and integrity of membrane by inhibiting the ROS accumulation (Wang et al., 2013). Besides as an energy supplier, ATP also acts as a signal molecule to mediate different cellular processes. The extracellular ATP may activate a specific recognition mechanism to elevate the contents of cytosolic free calcium ion (Ca^{2+}). This causes generation of intracellular second messengers including nitric oxide (NO), Ca^{2+} and ROS which activate a variety of biological responses including induction of gene expressions (Tanaka, Gilroy, Jones, & Stacey, 2010). Furthermore, it has been verified that Ca^{2+} and NO effectively maintain the firmness of postharvest crops by preventing the morphology changes of pectin or reducing the activities of pectin degradation enzymes (Liu, Chen et al., 2017; Zaharah & Singh, 2011). Therefore, exogenous ATP may meliorate postharvest qualities and softening of fruits and vegetables by energy supply and signal regulation.

Mung bean sprouts (*Vigna radiata*) are commonly consumed as

* Corresponding author at: Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.foodchem.2018.01.061
Received 27 April 2017; Received in revised form 28 December 2017; Accepted 7 January 2018
Available online 08 January 2018
0308-8146/

cooked food or fresh vegetable salad. However, bean sprouts are highly perishable due to their inherent properties and easily represent browning and softening during the storage. The commercial values of mung bean sprouts are lost after storage of 3 days at room temperature (Goyal, Siddiqui, Upadhyay, & Soni, 2014). Exogenous ATP is generally recognised as safe and can be used for long-term oral supplementation as functional health products (Wilson et al., 2013). Also, its precursor adenosine monophosphate (AMP) is permitted as food additive by European Union. However, to our best knowledge, little information is available on the role of exogenous ATP in quality regulation of postharvest mung bean sprouts.

The objectives of present work were to test the effects of ATP supply on the storage, quality properties, pectin-related gene expression and cell wall nanostructure to verify this novel postharvest technology in preservation area of sprouts.

2. Materials and methods

2.1. Sprouts materials and postharvest treatment

Mung bean sprouts were purchased from a local supermarket in Singapore and immediately transported to the laboratory. Sprouts which were uniform in size without physical injuries and infections were selected. The sprouts were then washed by tap water and air-dried at room temperature. Based on the results of a preliminary experiment, the sprouts were immersed in 1 mM ATP solution and deionised (DI) water (control treatment) for 5 min. After treatments, the sprouts were stored in trays packed with polyethylene bags in dark at room temperature and sampled for 3 days (Goyal et al., 2014). The hypocotyls of different treated sprouts were collected and cut into small pieces, frozen, ground and stored in liquid nitrogen immediately for further biochemical analysis. Parameters of storage properties (weight loss, respiration, relative conductivity and firmness) were measured during the daily sampling at the same time each day.

2.2. Measurement of postharvest storage properties

The weight loss was calculated by comparing the measured weights at day 1, 2 and 3 with the original weight (day 0). Tissue water contents were also measured by oven-dry method for dry basis conversion (Liu, Liu, Yao, & Ma, 2016). Two grams of sprout samples from two groups at each sampling day were dried in an oven at 110 °C to constant weights and the measurements were replicated for three times. The results were recorded as percent of dry weight (DW) and applied for the following dry basis calculation.

Respiration rate of mung bean sprouts was measured according to the method of Wu, Zhang, and Adhikari (2012) with some modifications. Ten sprouts were weighed and then sealed in 80 mL glass containers for 1 h at room temperature. The respiration gas samples of living sprouts in the sealed containers were sampled and injected directly into a headspace gas analyser (GS3 Micro, Systech Instruments, Thame, UK) by a needle connected with the analyser and the carbon dioxide (CO_2) contents were recorded. The respiration rate was expressed as mL $CO_2 \cdot h^{-1} \cdot kg^{-1}$.

The relative conductivity was measured according to the method of Liu, Hao, Liu, and Li (2011) with slightly modification. Living integrated mung bean sprouts (about 2 g) without milling in each group were dipped into DI water for 12 h with shaking. The electrolyte leakage (C_1) was determined as conductivity of living sprouts by a conductivity meter (ES-14, Horiba, Kyoto, Japan). The above solutions including integrated sprouts were then boiled for 20 min and the electrolyte leakage (C_2) was measured as total electrolyte leakage (conductivity of dead sprouts). The relative conductivity was calculated using the formula: $(C_1/C_2) \times 100\%$.

A Texture Analyser (TA-XT2i, Stable Micro System, Surrey, UK) was used to determine the firmness of the middle hypocotyl (Liu, Wu et al., 2017). The sprouts were subject to the compression test and the maximum compression distance was set as 1 mm. The pre-test speed, test speed and post speed were set at 1, 1, and 1 mm/s, respectively. The force-versus-distance curves were obtained and the maximum peak force during compression was recorded. At least 10 replicates from each group were tested.

2.3. Analysis of browning process

Method of Chinnici, Natali, and Riponi (2014) was applied to determine the degree of browning (DOB) of mung bean sprouts with some modifications. Briefly, 3 g samples were homogenised with 6 mL DI water and centrifuged at $12,000 \times g$ for 20 min. Two millilitres of supernatant were added to 3 mL ethanol and then centrifuged at $12,000 \times g$ for 20 min. The extracted brown pigment was then measured at 440 nm which was the wavelength for maximum absorption.

The total phenolic contents were measured by the method described by Chen, Lin et al. (2015). One gram sample was homogenised in 5 mL methanol containing 1% (v/v) hydrochloric acid at 4 °C. The homogenates were then extracted in dark environment for 20 min followed by centrifugation at $12,000 \times g$ for 10 min. The absorbance of supernatant was recorded at 280 nm. The total phenolic contents were determined by using standard gallic acid and expressed as $mg \cdot g^{-1}$ DW.

The enzymes in sprouts were extracted by the method of Chen et al. (2014). One gram sample was homogenised in 5 mL ice-cold 50 mM phosphate buffer, (pH 7.5). The homogenates were centrifuged at $12,000 \times g$ for 20 min and the supernatants were collected as extract solutions. PPO activity was determined by monitoring the increased absorbance of quinone derivative produced by the oxidation of pyrocatechol by PPO at 420 nm following the method of Chen, Lin et al. (2015). Briefly, 100 μL enzyme extract was mixed with 3 mL reaction solution contained 10 mM pyrocatechol and 50 mM phosphate buffer (pH 7.5). The increased absorbance at 420 nm was recorded for 5 min and the activity was expressed as unit (U)$\cdot min^{-1} \cdot g^{-1}$ DW.

2.4. Postharvest quality analysis

Total soluble solids (TSS) and titratable acid (TA) were determined according to the method of Laorko, Li, Tongchitpakdee, Chantachum, and Youravong (2010) with some modifications. Homogenised juice of 2 g sprouts was filtered and then a few drops of filtrate were tested by a digital refractometer (RX-5000α, ATAGO, Tokyo, Japan). The °Brix values of each sample were recorded. For TA measurement, 1 g sprouts were homogenised in 3 mL DI water and the extracts were filtered and diluted for 10 times. Sodium hydroxide solution (2 mM) was titrated against the diluted extracts (10 mL). Several drops of 1% (m/v) phenolphthalein dissolved in ethanol were added as indicator. The following equation was used to calculate the TA content with reference to malic acid:

$$\text{TA content} = \frac{a \times V \times c \times 0.067 \times 1000}{m}$$

where a is dilution ratio, V is titre volume of NaOH (mL), c is concentration of NaOH ($mM \cdot mL^{-1}$), 0.067 is the conversion factor ($g \cdot mM^{-1}$) and m is the dry weight of sprout sample (g).

Measurements of soluble sugar and protopectin contents were performed by the method of Li, Hu, and Xu (2015). Phenol–sulfuric acid method was applied to determine the soluble sugar and the sugar levels were calculated by a glucose standard curve (30–300 $\mu g \cdot L^{-1}$, $R^2 > 0.99$; regression equation: $y = 0.0071x + 0.0064$, where y is absorbance value and x is the glucose concentration). Briefly, 1 g sprouts were homogenised in 5 mL DI water and the mixture was boiled for 30 min for soluble sugar extraction. The cooled extracts were then filtered and diluted with DI water to 10 mL. Two millilitres appropriately diluted soluble sugar extract, 1 mL 9% (m/v) phenol solution and 5 mL concentrated sulfuric acid were mixed, kept for 30 min at room

temperature and tested at 485 nm.

Furthermore, carbazole colorimetry method was used to measure the contents of protopectin in mung bean sprouts and the standard curve was calibrated by galacturonic acid (20–100 μg·mL^{-1}, $R^2 > 0.99$; regression equation: $y = 0.005x - 0.013$, where y is absorbance value and x is the galacturonic acid concentration). One gram sprouts were milled and boiled in 20 mL 80% ethanol solution for 30 min to remove sugar components. The residual was collected after centrifugation at 12,000 × *g* for 10 min and the procedure was repeated for three times. Soluble pectin component in collected residual was removed by 20 mL DI water at 50 °C for 30 min. After centrifugation, 10 mL concentrated sulfuric acid was added and the mixture was boiled for 1 h to extract the protopectin. The cooled supernatant was collected by centrifugation. Two millilitres of diluted protopectin extracts were mixed with 5 mL concentrated sulfuric acid and 0.2 mL 0.15% (m/v) carbazole solution. The mixtures were kept at darkness for 30 min and the absorption was tested at 530 nm.

Soluble protein contents were measured according to Chen et al. (2014) and bovine serum albumin (BSA) was used as standard (20–100 μg·mL^{-1}, $R^2 > 0.99$; regression equation: $y = 0.0048x + 0.0131$, where y is absorbance value and x is the BSA concentration). Protein extracts were prepared as the method of enzyme (PPO) extraction presented in Section 2.3. Ascorbic acid (AsA) contents were determined by monitoring the red chelates integrated from reduced ferrous ion and bathophenanthroline (Hu et al., 2015). Standard curve of AsA was manufactured to calibrate the results (10–60 μg·mL^{-1}, $R^2 > 0.99$; regression equation: $y = 0.0365x + 0.0721$, where y is absorbance value and x is the AsA concentration).

2.5. Antioxidant system assays

Previous method was applied to test the contents of H_2O_2 (Huang, Jian, Jiang, Duan, & Qu, 2016). Enzyme solutions were extracted by the method described in Section 2.3. Peroxidase (POD) was assayed in a reaction mixture containing 25 mM guaiacol, 30 mM H_2O_2 and aliquot enzyme solution in 50 mM phosphate buffer (pH 7.5). One unit of POD activity was expressed as 1 OD change per min per g DW (Yao et al., 2014). A superoxide dismutase (SOD) assay kit (Cayman Chemical, MI, USA) was used to test the SOD activities. The amount of enzyme required to demonstrate 50% dismutation of the superoxide anion (O_2^-) was defined as one unit of SOD activity.

2.6. Expression analysis of pectin-related genes by quantitative RT-PCR

Pectin-related genes in mung bean were selected by the method of Fabi et al. (2014). Suitable pectin-related genes in papaya and *Arabidopsis thaliana* were selected and compared with the whole genome of mung bean sprouts in GenBank. Best matched pectin-related genes in mung bean were chosen for further studies. The gene information was obtained from the website of National Center of Biotechnology Information (NCBI) and the probable biological properties of the deduced polypeptides were calculated by ExPasy proteomics Server (http://web.expasy.org/protparam/).

Expression patterns of pectin-related genes of mung bean were determined by quantitative RT-PCR. Total RNA was isolated from the frozen hypocotyl samples by using a Total RNA Mini-Preps Kit (Bio Basic, Ontario, Canada). RNase-free DNase (Sangon Biotech, Shanghai, China) was added to degrade DNA in RNA extracts. The concentration of RNA was measured by a spectrophotometer (BioDrop, Biochrom, Cambridge, UK) and the integrity was verified by 1% (w/v) agarose gel electrophoresis. cDNA was synthesised by M-MuLV reverse transcriptase, Oligo dT primer (Sangon Biotech, Shanghai, China) and around 1 μg RNA according to the instruction. The mung bean *actin* gene (GenBank accession: XM_014661327; sense primer: 5′-CGTGTTTCCTTCTGTTGTTGG-3′; antisense primer: 5′-CCTCTTTCCCTTAGCCTTGTC-3′) was used as internal control (Li, Shi, & Leng, 2015). Primer pairs of target genes were designed by Primer 5 software and the specificities were confirmed by Primer Blast in NCBI (Table S1). The reaction condition and system were conducted by a previous report (Chen, Yang et al., 2015). The relative expression levels were calculated as $2^{-\Delta\Delta CT}$ [$\Delta C_T = C_{T,\ Target}\text{-}C_{T,\ actin}$; $\Delta\Delta CT = \Delta C_{T,\ treatment}\text{-}\Delta C_{T,\ control\ (0d)}$]. Heatmap was manufactured by HemI 1.0, and the data was normalised by log2 transformation.

2.7. Nanostructural characterisation of SSP

Preparation of SSP fractions was conducted by a previous method (Liu et al., 2017). Briefly, 1 g sprouts were milled and boiled in 20 mL ethanol (80% v/v) for 20 min. The cooled samples were filtered through a nylon filter cloth (300 meshes) by vacuum pump and the extraction was repeated for 3 times. Residual collected from the surface of nylon filter cloth was incubated in 20 mL 90% (v/v) dimethyl sulphoxide (DMSO) solution at 4 °C overnight. The residue was then dipped into 20 mL mixture of chloroform and ethanol (2:1, v/v) for 10 min and washed with acetone for 3 times. The residual cell wall materials were then incubated in 20 mL DI water at 25 °C for 4 h and the residual was collected by centrifugation. The process was repeated twice more before addition of 20 mL 50 mM cyclohexane diamine tetracetic acid (CDTA) at 25 °C for 4 h. After the process was repeated for 3 times, the residue was further extracted by 20 mL 50 mM sodium carbonate containing 2 mM CDTA. The extraction process was also repeated for 3 times and the supernatants were collected and combined together as SSP. The SSP solution was dialysed (molecular cut-off 3000 Da) against DI water thrice for 24 h.

The concentrations of purified SSP solutions were tested by carbazole colorimetry method and quantified by galacturonic acid standard curve. Two millilitres of SSP solutions were mixed with 5 mL concentrated sulfuric acid and 0.2 mL 0.15% (m/v) carbazole solution and the mixtures were tested at 530 nm after keeping at darkness for 30 min. The SSP solutions were further diluted to around 5 μg·mL^{-1} as working solution. Ten microlitres of SSP working solutions were pipetted on mica sheets and dried by an aurilave. A TT-AFM (AFM workshop, Signal Hill, CA, USA) equipped with a Sensaprobe TM190-A-15 tip (Applied Nanostructures, Mountain View, CA, USA) with resonance of 190 kHz and force constant of 45 N/m was used to investigate the nanostructural characteristics of pectin in mung bean sprouts (Chong, Lai, & Yang, 2015). The image was made by AFM under tapping mode, scan rate of 0.3 Hz and scan lines of 512. The AFM images were analysed by the software Gwyddion (Yu, Li, Ng, Yang, & Wang, 2018). Qualitative data was obtained through sectional analysis of pectin backbone chains (thick chains) from AFM images, such as width and height. Side chains (thin chains) were confirmed by the method of Round, Rigby, MacDougall, and Morris (2010). The overlap of a thick chain and a thin chain which exhibited height increase less than 1.5 times was screened as a side chain point of backbone. At least 20 measurements from 10 images were carried out for data analysis.

2.8. Statistical analysis

Data were statistically analysed by analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) method to assess the effects of ATP treatment on mung bean sprouts. Additionally, differences with $P \leq 0.05$ were considered significant.

3. Results

3.1. Storage properties of mung bean sprouts

During the postharvest storage, weight loss, respiration rate and relative conductivity in mung bean sprouts exhibited rising tendencies (Fig. 1a-c). Weight loss constantly rose from 0% to 27.3% and respiration rate approximately enhanced by three times at the end of

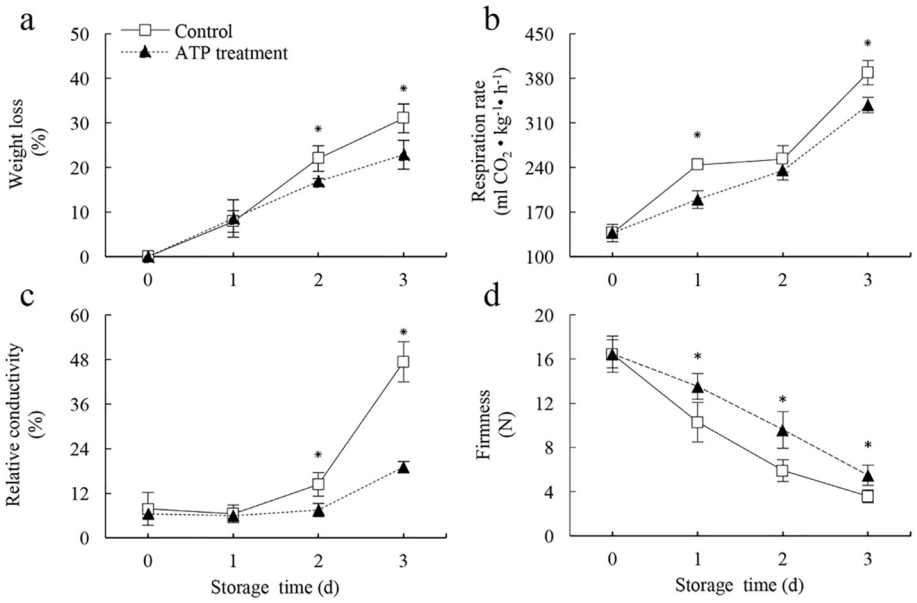

Fig. 1. Effect of ATP treatment on the (a) weight loss, (b) respiration rate, (c) relative conductivity and (d) firmness of mung bean sprouts.

storage. In addition, the relative conductivity increased considerably from 7.8% to 47.4% during the three-day storage. Compared with the control group, ATP treatment significantly delayed the raise of storage properties. Weight loss, respiration rate and relative conductivity were decreased by 26.3–27.4%, 13.1–22.5% and 48.0–59.8% respectively ($P \leq .05$). On the other hand, firmness of mung bean sprouts continuously decreased from 16.7 to 3.9 N (Fig. 1d). After the ATP treatment, the firmness was improved by 31.5–62.3%.

3.2. Hydrogen peroxide contents and antioxidant enzymes

The H_2O_2 content in mung bean sprouts increased continuously after harvest and the ATP treatment considerably alleviated the H_2O_2 accumulation by 29.2% at day 3 (Fig. 2a). Activities of antioxidant enzymes POD and SOD were both induced in the process of senescence (Fig. 2b and c). Exogenous ATP improved the POD activity by 9.3% and 13.9% at day 1 and day 2, respectively. Also, the SOD activities were increased by 8.8% and 10.3% at day 1 and day 3 after ATP treatment.

3.3. Browning process in mung bean sprouts

Effect of exogenous ATP on the browning events in mung bean sprouts was monitored (Fig. 3a and b). More severe browning and aging features were observed in control group than ATP treatment group. Further results revealed that ATP treatment significantly ($P \leq .05$) decreased the DOB from 1.0 to 0.62 OD at the end of the storage (Fig. 3c). Total phenolics showed a reduced trend, while the exogenous ATP could maintain the contents of total phenolics at relative high levels which was more than two times than the control group at the third day (Fig. 3d). PPO activities decreased along with storage time (Fig. 3e). Furthermore, the PPO activities were significantly ($P \leq .05$) decreased ranging from 11.1% to 34.3%.

3.4. Effect of ATP treatment on the postharvest qualities

Fig. 4 shows the effects of ATP treatment on the TSS, contents of soluble sugar, protopectin, TA, soluble protein and AsA of mung bean sprouts. Results demonstrated that all the quality-related characteristics in mung bean sprouts were reduced during the storage. For TSS, control group showed significantly ($P \leq .05$) higher contents at the first and third days compared with ATP treatment group (Fig. 4a). Contents of soluble sugar exhibited a similar changing tendency with TSS (Fig. 4b).

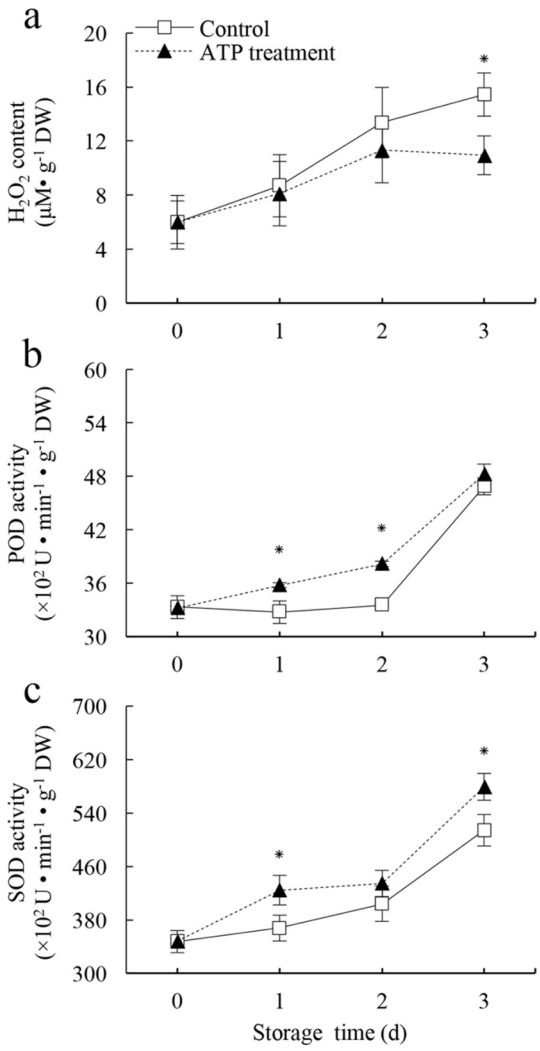

Fig. 2. Effect of ATP treatment on the content of (a) hydrogen peroxide (H_2O_2), activities of (b) peroxidase (POD) and (c) superoxide dismutase (SOD).

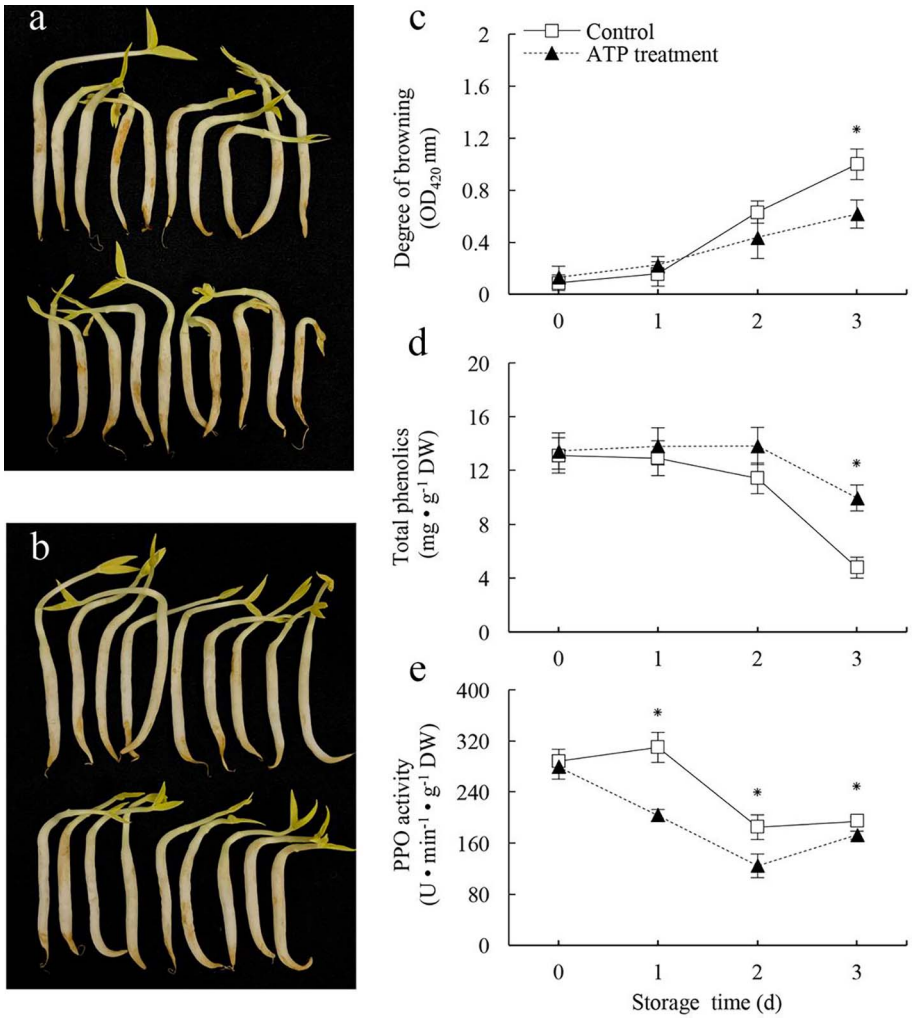

Fig. 3. Effect of ATP treatment on the browning in (a) control and (b) treated groups (3 days), (c) degree of browning (DOB), (d) total phenolics and (e) polyphenol oxidase (PPO) activity of mung bean sprouts.

In addition, sprouts of control group contained a higher concentration of soluble sugar after storage for 3 days. For contents of protopectin, TA and soluble protein, exogenous ATP could notably ($P \leq .05$) maintain higher levels by 16.4%, 25.8% and 74.1% respectively at the third day of storage (Fig. 4c-e). In addition, AsA was slightly rose after ATP treatment (Fig. 4f).

3.5. Expression pattern of pectin-related gene

A total of six pectin-related genes including genes of two pectate lyases (PLs), one pectinesterase (PME) and three Polygalacturonases (PGs) were identified by the BLASTP on NCBI. Characteristics of these genes and the deduced enzymes are summarised in Table S2. According to the detailed information, the length of the six pectin-related proteins varied from 399 to 584 residues and the various isoelectric point (pI) values (ranging from 5.13 to 9.28) and molecular weight (MW) values (ranging from 42.2 to 64.0 kDa) were also recorded. Furthermore, most of these proteins (except PL1) were stable with an instability index < 40.

Heatmap demonstrated the effects of exogenous ATP on the expression patterns of pectin-related genes in mung bean sprouts (Fig. 5A). The relative expression levels of these genes (except *VrPG1*) changed more than 2 times during the postharvest storage (Fig. 5B). Moreover, 3 genes (*VrPL2*, *VrPG1* and *VrPG2*) were up regulated and 2 genes (*VrPL1* and *VrPG3*) were down regulated. For *VrPME*, its expression level decreased at 24 h and then recovered until to 72 h. In addition, the expression levels of most pectin-related genes in ATP treatment group were significantly ($P \leq .05$) lower than those in control group. For *VrPL1*, *VrPME* and *VrPG1*, exogenous ATP decreased the expression level more than 2 times compared with the control group at some time points.

3.6. Nanostructure of SSP molecules

The nanostructures of SSP in different groups were shown in Fig. 6A. The branched chain (Br) structure could be found in all the three groups. Also, thick chains (Tk) and thin chains (Tn) were presented in each group. Loose structures (Ls) were observed in both control and ATP treatment group at day 3 (Fig. 6Ad and f). In addition, short straight fraction (Ss) of SSP was also found in the two groups.

The width and height of the SSP thick chains were also investigated, as shown in Fig. 6B. The control group before treatment showed the highest width and height which were 259.94 and 8.82 nm, respectively. The two parameters decreased to 120.21 and 4.25 nm after storage of 3 days. However, ATP treatment could maintain the width and height at relative high levels which were 176.86 and 6.19 nm, respectively after 3 days.

4. Discussion

Quality deterioration caused by senescence considerably affects the postharvest storage of various crop products such as fruits and vegetables. Exogenous ATP has become a new strategy and drawn extensive attentions in postharvest field recently for its functions of energy

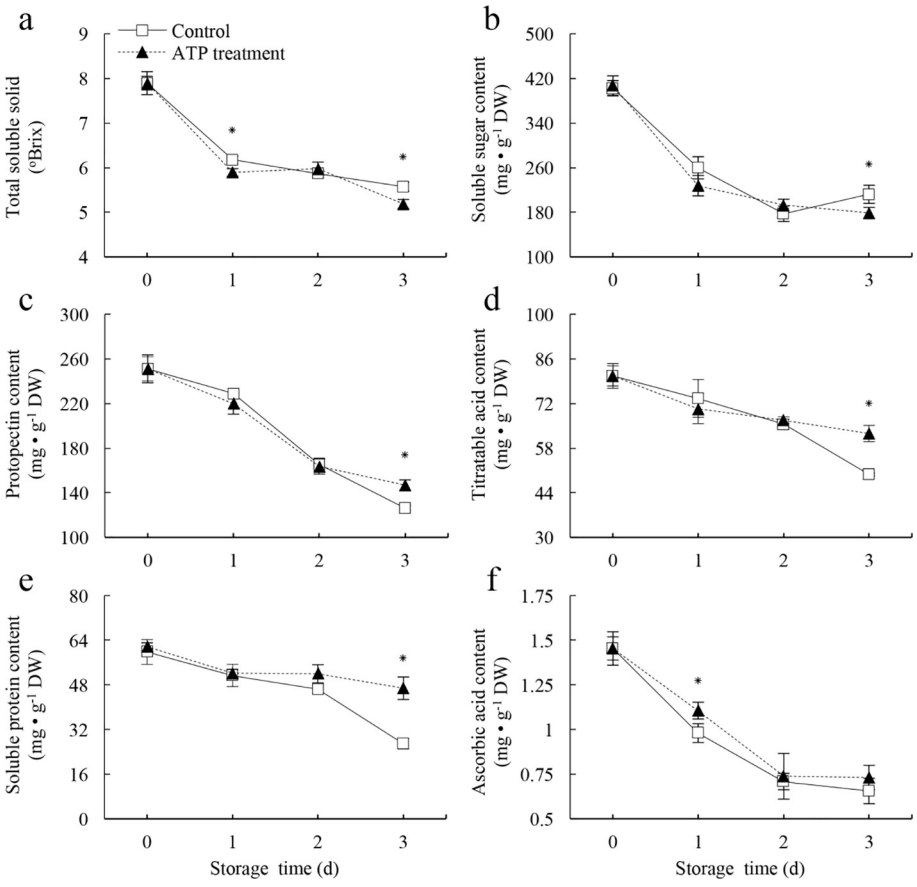

Fig. 4. Effect of ATP treatment on the (a) total soluble solid (TSS), contents of (b) soluble sugar, (c) protopectin, (d) titratable acid, (e) soluble protein and (f) ascorbic acid.

homeostasis and signal transmission (Tanaka et al., 2010; Wang et al., 2013). In this study, we checked the effect of ATP treatment on the postharvest storage quality and browning of mung bean sprouts and preliminarily examined the underlying mechanism of softening event from transcriptional and nanostructural levels. Results showed that ATP treatment effectively maintained the weight and firmness and decreased the rates of respiration and relative conductivity (Fig. 1). Respiration is a key component in ATP synthesis pathway which is associated with energy status in cells. Exogenous ATP could alleviate the respiration by improving the energy status (Chen, Lin et al., 2015). Furthermore, relative conductivity reflects the integrity of cell membrane which can be damaged by severe stresses such as senescence. Our relative conductivity results were similar with the previous studies that ATP treatment could prevent the destruction of membrane and further delay the senescence event (Yao et al., 2014).

Oxidative damage has been proved as the major cause of membrane permeability changes (Mondal, Malhotra, Jain, & Singh, 2009). Thus, the antioxidant system in mung bean sprouts were tested (Fig. 2). H_2O_2 level was evaluated along with the process of aging. Anti-oxidation enzyme system was activated to scavenge the accumulated ROS. Moreover, exogenous ATP significantly ($P \leq .05$) decreased the H_2O_2 content by rising higher activities of POD and SOD compared with control group. The mechanism might be due to the signal transduction of ATP which could activate the Ca^{2+}, NO and ROS signal pathways in plants (Tanaka et al., 2010). The downstream defense response such as antioxidant system could be further induced by the second messenger trio (Ca^{2+}, NO and ROS). Nanoparticle-based approaches could further reveal the process (Yu & Yang, 2017).

Colour appearance is one of the most important factor to evaluate the quality and commercial value of fruits and vegetables. The browning process of mung bean sprouts during storage showed that the decreased content of total phenolics catalysed by PPO might contribute to the evaluated DOB (Fig. 3c-e). Furthermore, higher contents of total phenolics were observed in ATP treatment group (Fig. 3d). Phenolic compounds are one of the most important plant chemicals and widely used as antioxidants by human (Do et al., 2014). The elevated contents might result from the lower activities of PPO after ATP treatment. As a result, the DOB was remarkably decreased by exogenous ATP at the end of the storage. These results were consistent with the conclusion of Yi et al. (2008).

TSS, contents of TA, ascorbic acid, soluble sugar and protein were usually tested for evaluating the storage quality of postharvest fruits and vegetables (Chen, Lin et al., 2015). As shown in Fig. 4, the results demonstrated that these nutrition and flavour characteristics all exhibited decreasing tendencies which were similar with previous reports (Goyal et al., 2014). The nutrient elements were consumed for surviving purpose in postharvest crops. On the other hand, exogenous ATP could effectively slow the decrease of TA, soluble protein and AsA contents by different degrees and subsequently maintain better quality of mung bean sprouts (Fig. 4d-f). In addition, TSS in ATP-treated sprouts was lower than that in control group (Fig. 4a). Since soluble sugar constituted the majority content of TSS (Magwaza & Opara, 2015), the similar results of soluble sugar were recorded in Fig. 4b. The decreased TSS and soluble sugar in ATP treatment group could be partially explained by the fact that ATP treatment delayed the degradation of plant polysaccharides such as protopectin which was an important component in cell wall (Lin, Luo, & Chen, 2016). It has been proved that pectin played a key role to maintain the firmness of fruits and vegetables (Zhang, Chen, Zhang, Lai, & Yang, 2017). Thus, the softening events prevented by ATP treatment (Fig. 1d) might be due to the maintenance of stable structure and content of pectin.

The transcriptional information of pectin-related genes was checked to clarify the mechanism of pectin degradation during the storage. Results showed that most of these genes were up or down regulated

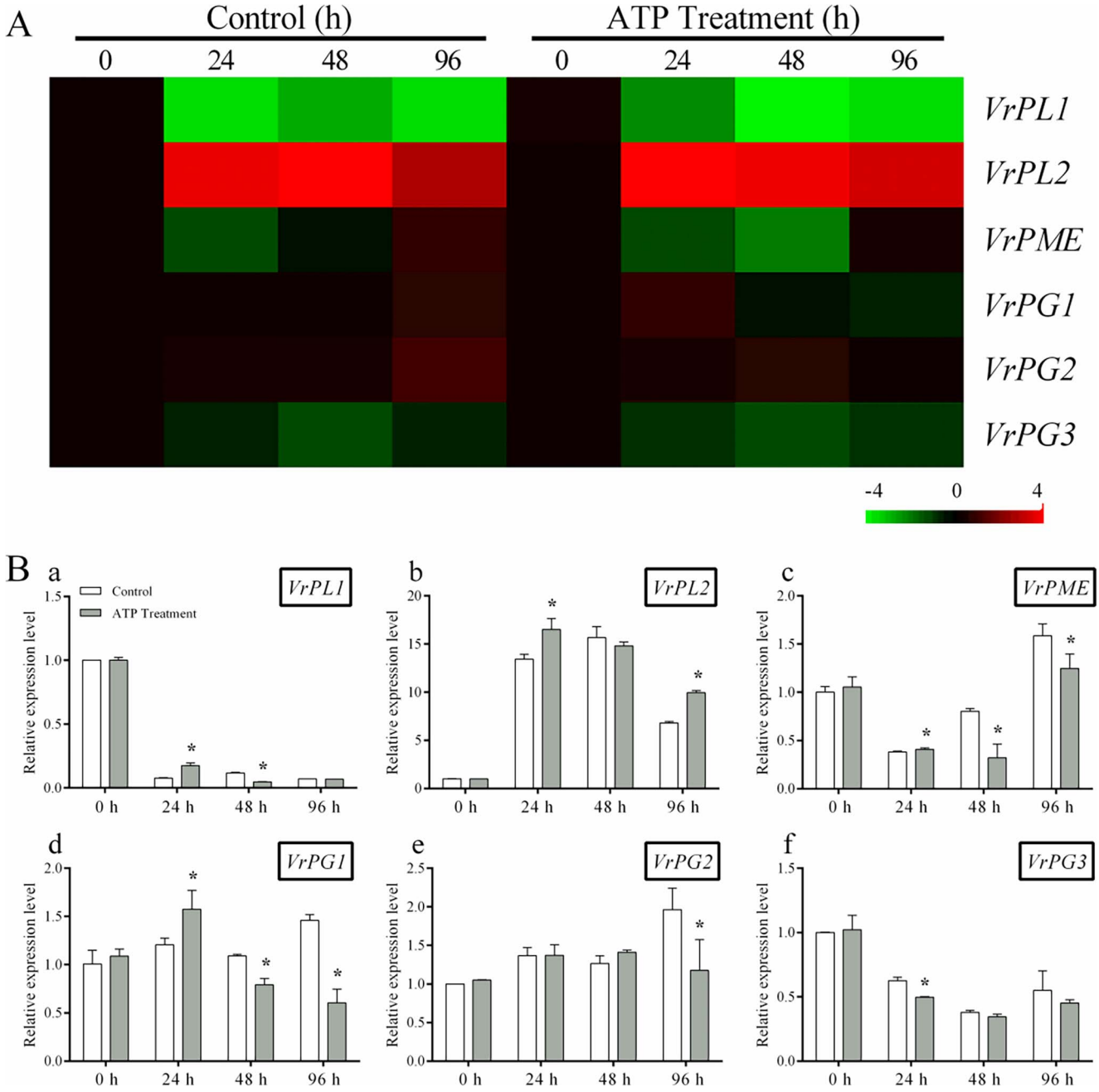

Fig. 5. Heatmap of pectin-related genes in mung bean. Levels of down expression (green) or up expression (red) are shown on a $\log_2$ scale for each gene (n = 3) (A); Relative expression of pectin-related genes in mung bean sprouts (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

over than 2 folds which meant these genes were involved in the process of pectin degradation (Fig. 5). Also, Fabi et al. (2014) had confirmed the central role of PGs during pulp softening by expression profiles of *cpPGs*. *VrPG1*, *VrPG2* and *VrPG3* were highly similar with *cpPG1*, *cpPG2* and *cpPG3* respectively, indicating their important roles during the softening of mung bean sprouts. Comparing with the expression levels of pectin-related genes in control group, ATP treatment could significantly ($P \leq .05$) decrease the expression of these genes. Guo et al. (2014) reported that the NO delayed the softening by regulating the cell wall softening-related enzymes such as PL, PME and PG. Thus, the ATP treatment might regulate the pectin-related genes and prevent the pectin degradation by activating the second messengers such as NO.

The nanostructures of SSP indicated that massive thick chains (Tk) interconnected with each other by branch structures (Br) to maintain the firmness of mung bean sprouts. However, the well-set structure was dissolved and the thick chains degradation occurred (Fig. 5A) after storage of 3 days. Data analysis further confirmed the significant reduction in width and height of SSP backbone (Fig. 5B). However, ATP treatment effectively delayed the pectin degradation with higher levels of width and height. Furthermore, abundant loose structures and short straight chains (Ss) could be found in control and ATP-treated groups at day 3 indicated the on-going process of pectin deterioration. Cell wall metabolism has still not fully explored because of the complex structures. Previous studies have demonstrated that accumulation of ROS could lead to the cleavage of polysaccharides bonds which result in the degradation of cell wall component (Dheilly et al., 2016). Also, pectin-related enzymes including PL, PME and PG highly contributed to the pectin deterioration (Fraeye et al., 2009). Based on our results, exogenous ATP might slow the pectin degradation by scavenging the ROS accumulation and reducing the expression level of pectin-related genes.

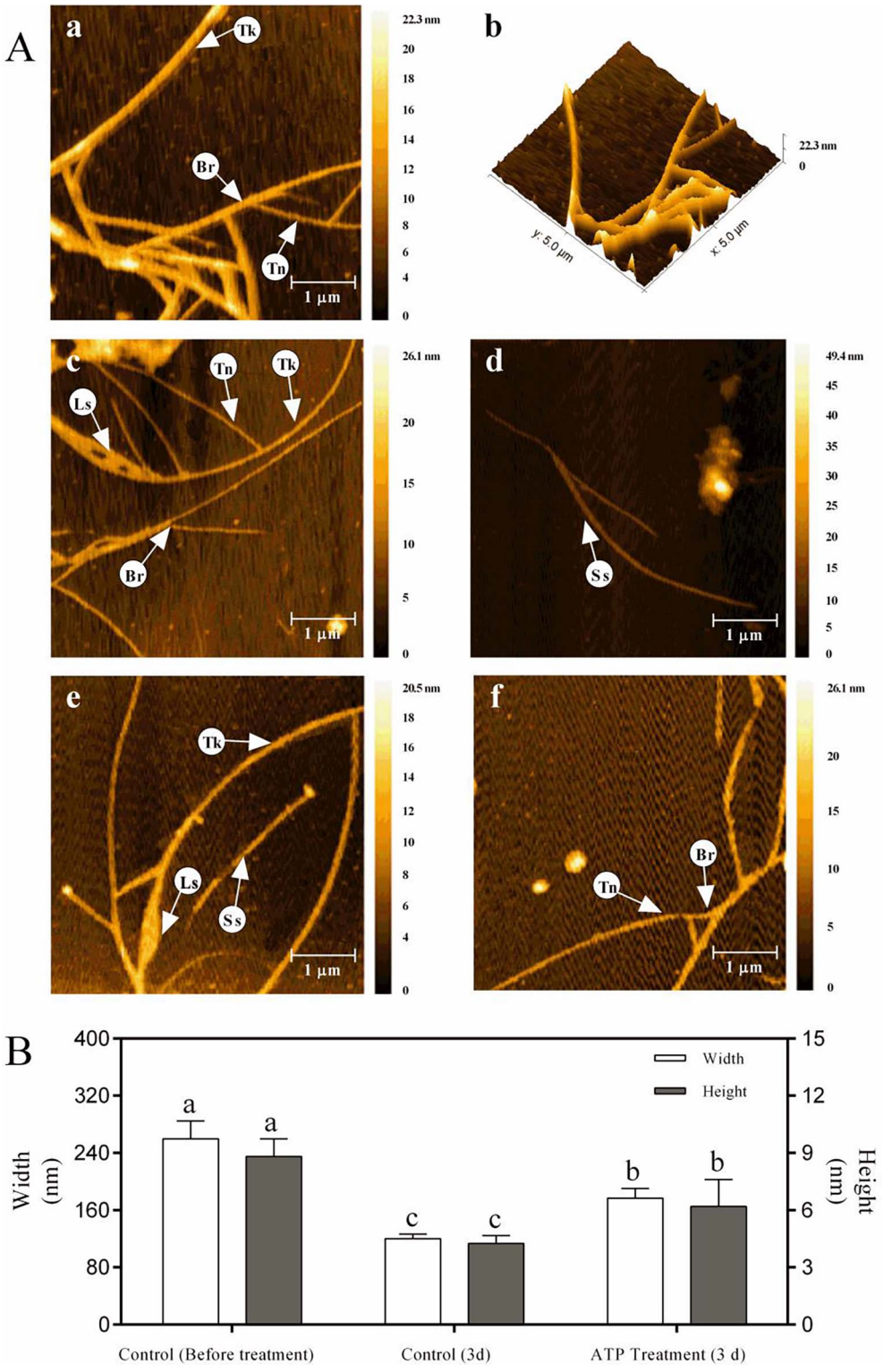

Fig. 6. Atomic force microscopy (AFM) images of sodium carbonate-soluble pectin (SSP) chains in mung bean sprouts (A) and changes in width and height of thick chains (B). Note: a: control group before treatment; b: three-dimensional image of the control group before treatment; c, d: control group at day 3; e, f: ATP treatment group at day 3. Br: branched chain; Tk: thick chain; Tn: thin chain; Ls: loose structure; Ss: short straight chain.

5. Conclusion

Browning and softening events are the main problems during the storage of mung bean sprouts. This research demonstrated that application of exogenous ATP could notably maintain the quality and delay the browning and softening of mung bean sprouts. The inhibition of browning might result from the decreased PPO activities after ATP treatment. Moreover, the amelioration of oxidant damage by inducing the antioxidant enzymes was also observed. Complicated softening event was investigated from transcriptional and nanostructural levels. Exogenous ATP decreased the expression level of pectin-related genes and the AFM results provided a supporting understanding of the degradative process. The results indicated that ATP treatment is a potential preservation processing of postharvest fruits and vegetables. However, the underlying mechanism of energy regulation such as carbohydrate metabolism during the storage should be studied in the future.

Acknowledgement

This study was funded by the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112), projects 31371851 and 31071617 supported by NSFC, Natural Science Foundation of Jiangsu Province (BK20141220), Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SYN201522) and an industry grant supported by Changzhou Qihui Management & Consulting Co., Ltd (R-143-000-616-597).

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this manuscript. We have no financial and personal relationships with other people or organisations that can inappropriately influence our work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.01.061.

References

Chen, M., Lin, H., Zhang, S., Lin, Y., Chen, Y., & Lin, Y. (2015). Effects of adenosine triphosphate (ATP) treatment on postharvest physiology, quality and storage behavior of longan fruit. *Food and Bioprocess Technology, 8*(5), 971–982.

Chen, L., Xu, M., Zheng, Y., Men, Y., Sheng, J., & Shen, L. (2014). Growth promotion and induction of antioxidant system of tomato seedlings (*Solanum lycopersicum* L.) by endophyte TPs-04 under low night temperature. *Scientia Horticulturae, 176*, 143–150.

Chen, L., Yang, Y., Liu, C., Zheng, Y., Xu, M., Wu, N., et al. (2015). Characterization of WRKY transcription factors in *Solanum lycopersicum* reveals collinearity and their expression patterns under cold treatment. *Biochemical and Biophysical Research Communications, 464*(3), 962–968.

Chinnici, F., Natali, N., & Riponi, C. (2014). Efficacy of chitosan in inhibiting the oxidation of (+)-catechin in white wine model solutions. *Journal of Agriculture and Food Chemistry, 62*(40), 9868–9875.

Chong, J. X., 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.

Cybulska, J., Zdunek, A., & Kozioł, A. (2015). The self-assembled network and physiological degradation of pectins in carrot cell walls. *Food Hydrocolloids, 43*, 41–50.

Dheilly, E., Le Gall, S., Guillou, M.-C., Renou, J.-P., Bonnin, E., Orsel, M., et al. (2016). Cell wall dynamics during apple development and storage involves hemicellulose modifications and related expressed genes. *BMC Plant Biology, 16*(1), 201.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., et al. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis, 22*(3), 296–302.

Fabi, J. P., Broetto, S. G., da Silva, S. L. G. L., Zhong, S., Lajolo, F. M., & do Nascimento, J. R. O. (2014). Analysis of papaya cell wall-related genes during fruit ripening indicates a central role of polygalacturonases during pulp softening. *PloS One, 9*(8), e105685.

Fraeye, I., Knockaert, G., Van Buggenhout, S., Duvetter, T., Hendrickx, M., & Van Loey, A. (2009). Enzyme infusion and thermal processing of strawberries: Pectin conversions related to firmness evolution. *Food Chemistry, 114*(4), 1371–1379.

Goyal, A., Siddiqui, S., Upadhyay, N., & Soni, J. (2014). Effects of ultraviolet irradiation, pulsed electric field, hot water and ethanol vapours treatment on functional properties of mung bean sprouts. *Journal of Food Science and Technology, 51*(4), 708–714.

Guo, Q., Wu, B., Chen, W., Zhang, Y., Wang, J., & Li, X. (2014). Effects of nitric oxide treatment on the cell wall softening related enzymes and several hormones of papaya fruit during storage. *Food Science and Techonology International, 20*(4), 309–317.

Hu, Y., Chen, C., Xu, L., Cui, Y., Yu, X., Gao, H., et al. (2015). Postharvest application of 4-methoxy cinnamic acid for extending the shelf life of mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology, 104*, 33–41.

Huang, H., Jian, Q., Jiang, Y., Duan, X., & Qu, H. (2016). Enhanced chilling tolerance of banana fruit treated with malic acid prior to low-temperature storage. *Postharvest Biology and Technology, 111*, 209–213.

Jiang, Y., Duan, X., Joyce, D., Zhang, Z., & Li, J. (2004). Advances in understanding of enzymatic browning in harvested litchi fruit. *Food Chemistry, 88*(3), 443–446.

Laorko, A., Li, Z., Tongchitpakdee, S., Chantachum, S., & Youravong, W. (2010). Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice. *Journal of Food Engineering, 100*(3), 514–521.

Li, W., Hu, Q., & Xu, J. (2015). Changes in physicochemical characteristics and free amino acids of hawthorn (*Crataegus pinnatifida*) fruits during maturation. *Food Chemistry, 175*, 50–56.

Li, S., Shi, R., & Leng, Y. (2015). De novo characterization of the mung bean transcriptome and transcriptomic analysis of adventitious rooting in seedlings using RNA-Seq. *PLoS ONE, 10*(7), e0132969.

Lin, Y., Lin, H., Zhang, S., Chen, Y., Chen, M., & Lin, Y. (2014). The role of active oxygen metabolism in hydrogen peroxide-induced pericarp browning of harvested longan fruit. *Postharvest Biology and Technology, 96*, 42–48.

Lin, X., Luo, C., & Chen, Y. (2016). Effects of vacuum impregnation with sucrose solution on mango tissue. *Journal of Food Science, 81*(6), E1412–E1418.

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

Liu, R., Hao, J., Liu, H., & Li, L. (2011). Application of electrolyzed functional water on producing mung bean sprouts. *Food Control, 22*(8), 1311–1315.

Liu, W., Liu, J., Yao, M., & Ma, Q. (2016). Salt tolerance of a wild ecotype of vetiver grass (*Vetiveria zizanioides* L.) in southern China. *Botanical Studies, 57*(1), 27.

Liu, Q., Wu, J., Lim, Z. Y., Aggarwal, A., Yang, H., & Wang, S. (2017). Evaluation of the metabolic response of *Escherichia coli* to electrolysed water by ^{1}H NMR spectroscopy. *LWT-Food Science and Technology, 79*, 428–436.

Magwaza, L. S., & Opara, U. L. (2015). Analytical methods for determination of sugars and sweetness of horticultural products—a review. *Scientia Horticulturae, 184*, 179–192.

Mondal, K., Malhotra, S. P., Jain, V., & Singh, R. (2009). Oxidative stress and antioxidant systems in Guava (*Psidium guajava* L.) fruits during ripening. *Physiology and Molecular Biology of Plants, 15*(4), 327–334.

Razzaq, K., Singh, Z., Khan, A. S., Khan, S. A. K. U., & Ullah, S. (2016). Role of 1-MCP in regulating 'Kensington Pride' mango fruit softening and ripening. *Plant Growth Regulation, 78*(3), 401–411.

Round, A. N., Rigby, N. M., MacDougall, A. J., & Morris, V. J. (2010). A new view of pectin structure revealed by acid hydrolysis and atomic force microscopy. *Carbohydrate Research, 345*(4), 487–497.

Tanaka, K., Gilroy, S., Jones, A. M., & Stacey, G. (2010). Extracellular ATP signaling in plants. *Trends in Cell Biology, 20*(10), 601–608.

Wang, H., Qian, Z., Ma, S., Zhou, Y., Patrick, J. W., Duan, X., et al. (2013). Energy status of ripening and postharvest senescent fruit of litchi (*Litchi chinensis Sonn.*). *BMC Plant Biology, 13*(1), 55.

Wilson, J. M., Joy, J. M., Lowery, R. P., Roberts, M. D., Lockwood, C. M., Manninen, A. H., et al. (2013). Effects of oral adenosine-5′-triphosphate supplementation on athletic performance, skeletal muscle hypertrophy and recovery in resistance-trained men. *Nutrition & Metabolism, 10*(1), 57.

Wu, Z., Zhang, M., & Adhikari, B. (2012). Application of high pressure argon treatment to maintain quality of fresh-cut pineapples during cold storage. *Journal of Food Engineering, 110*(3), 395–404.

Xin, Y., Chen, F., Lai, S., & Yang, H. (2017). Influence of chitosan-based coatings on the physicochemical properties and pectin nanostructure of Chinese cherry. *Postharvest Biology and Technology, 133*, 64–71.

Yao, F., Huang, Z., Li, D., Wang, H., Xu, X., Jiang, Y., et al. (2014). Phenolic components, antioxidant enzyme activities and anatomic structure of longan fruit pericarp following treatment with adenylate triphosphate. *Scientia Horticulturae, 180*, 6–13.

Yao, F., Zhu, H., Yi, C., Qu, H., & Jiang, Y. (2015). MicroRNAs and targets in senescent litchi fruit during ambient storage and post-cold storage shelf life. *BMC Plant Biology, 15*(1), 181.

Yi, C., Qu, H., Jiang, Y., Shi, J., Duan, X., Joyce, D., et al. (2008). ATP-induced changes in energy status and membrane integrity of harvested litchi fruit and its relation to pathogen resistance. *Journal of Phytopathology, 156*(6), 365–371.

Yu, X., Li, Y., Ng, M., Yang, H., & Wang, S. (2018). Comparative study of pyrethroids residue in fruit peels and fleshes using polystyrene-coated magnetic nanoparticles based clean-up techniques. *Food Control, 85*, 300–307.

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

Zaharah, S., & Singh, Z. (2011). Mode of action of nitric oxide in inhibiting ethylene biosynthesis and fruit softening during ripening and cool storage of 'Kensington Pride' mango. *Postharvest Biology and Technology, 62*(3), 258–266.

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.

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