

# Different molecular weights chitosan coatings delay the senescence of postharvest nectarine fruit in relation to changes of redox state and respiratory pathway metabolism

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## ABSTRACT

The aim of this study is to investigate the impact of different molecular weights of chitosan treatment (LM 30 kDa; HM 120 kDa) on fruit senescence related to redox state and respiratory pathway metabolism in post-harvest nectarine fruit stored at 25 °C for 8 days. The treatments of LM and HM chitosan both delayed senescence, which are due to inhibition of respiration rate, and enhanced the antioxidant system, as evidenced by the improvement of ASA-GSH cycle and total phenolics and flavonoids contents and decrease in H<sub>2</sub>O<sub>2</sub> and MDA accumulation. Meanwhile, fruit treated with HM chitosan manifested better quality and redox state than LM. It is noteworthy that the results showed that HM chitosan notably suppressed the activity of SDH enzyme and increased the total activity of G-6-PDH and 6-PGDH. Accordingly, changed respiratory pathways by HM chitosan coating contributed to senescence retardation and modification of redox status in postharvest nectarine fruit.

## 1. Introduction

Nectarine [*P. persica* (L.) Batsch, var. nectarine], a fruit with attractive appearance and pleasing flavor, contains beneficial secondary metabolites that increase the nutritional value of the fruit (e.g., vitamins and various phenolics). However, due to its typical climacteric properties, nectarine fruit exhibits a sharp senescence in postharvest period followed by quality deterioration including a decrease in firmness, loss of color and flavor (García-parra et al., 2011; Xi et al., 2017). The existing major method for prolonging the postharvest storage life of nectarines is low-temperature, whereas nectarine fruit is prone to chilling injury during cold storage, inducing some physiological disorders (Lurie & Crisosto, 2005; Zhao, Shu, Fan, Cao, & Jiang, 2018). Accordingly, it is still required to find ways to slow down senescence to reduce the post-harvest loss of nectarine fruit.

It is generally known that the senescence process of postharvest fruits is accompanied by the accumulation and production of reactive oxygen species (ROS) (Mittler, 2002). The elimination of reactive oxygen species falls into two major categories, namely, antioxidant substances and antioxidant enzymes (Sgherri & Navari-Izzo, 1995). Antioxidant substances primarily include reduced glutathione (GSH), vitamin E, polyphenols, and carotenoids. Antioxidant enzymes primarily include superoxide dismutase (SOD), ascorbate oxidase (APX), catalase (CAT), and glutathione oxidase (GPX) (Cao, Hu, Zheng, & Lu,

2010). GSH, an vital antioxidant substance in plant cells, can be regenerated by AsA-GSH with NADPH as a reducing agent (Sgherri & Navari-Izzo, 1995). The oxidation step of the phosphopentose pathway (HMP) is considered the main source of NADPH, which is involved in the regeneration of oxidized antioxidants (Mittler, 2016; Sgherri & Navari-Izzo, 1995). The enzymes in this cycle that produce NADP<sup>+</sup> are glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphate-glucanate-dehydrogenase (6-PGDH) (Slekar, Kosman, & Culotta, 1996). The embden-meyerhof-parnas (EMP)-tricarboxylic-acid-cycle (TCA) refers to major respiration and energy-generating pathway in plants, playing key roles in regulating senescence of postharvest fruit (Yang, Cao, Su, & Jiang, 2014). It is well known that the plant respiratory pathways correlate with changes in the external environment, by O<sub>2</sub>/CO<sub>2</sub> controlled atmospheres (CA), treatment of 50% O<sub>2</sub> + 50% CO<sub>2</sub> increases the rate of HMP and TCA in Broccoli (Li, Lv, Guo, & Wang, 2016).

Chitosan is a high-molecular-weight cationic polysaccharide, having been broadly used in fruit preservation coatings due to its natural degradable characteristics (Romanazzi, Feliziani, & Sivakumar, 2018; Sharma, Barman, & Siddiqui, 2016). Previous studies have shown that chitosan coating can enhance the antioxidant system to maintain postharvest fruit quality such as guava (Batista Silva et al., 2018), loquat (Song et al., 2016) and sweet cherry (Pasquariello et al., 2015). Recent studies have suggested that chitosan coating can reduce the

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response of fruit to environmental conditions by the reduction of gas exchange on the fruit surface, thereby delaying fruit ripening, and reduce water loss, respiratory rate and ethylene production (Cissé, Polidori, Montet, Loiseau, & Ducamp-Collin, 2015; Cosme Silva et al., 2017). It has been reported that low molecular weight chitosan (LMWC, 3.4–51.3 kDa) has a greater inhibitory effect on kiwifruit gray mold than high molecular weight chitosan (HMWC, 136.8–342.0 kDa) (Hua et al., 2019). Jongsri, Wangsomboondee, Rojsitthisak, and Seraypheap (2016) reported that chitosan coating can cover stoma on mangoes skin, leading to a decrease in the respiration rate as well as an increase in antioxidant capacity to maintain the quality. Moreover, a study revealed that the chitosan coating caused a significant change in the internal gas composition of the papaya during cold storage (Ali, Muhammad, Sijam, & Siddiqui, 2011). Yet, the effects of the modified atmosphere of chitosan coating on the metabolism of the respiratory pathway of fruit have not been reported.

At present, there are primarily commercially available practical grade chitosan materials with different molecular weights. Thus far, most studies on the application of chitosan in postharvest fruit preservation have focused on the chitosan concentration, and combined treatment (Jiang et al., 2018; Mannozi et al., 2018). However, up to now, few works on the effects of different molecular weights chitosan treatment on the postharvest nectarine fruit preservation and the metabolism of the respiratory pathway of fruit have been reported. In this study, two different molecular weight chitosan materials, high molecular weight chitosan (HM: 120 kDa) and low molecular weight chitosan (LM: 30 kDa) were applied in postharvest nectarine fruit. The aims of the present study were to assess the impacts of different molecular weights of chitosan treatment on quality attribute of harvested nectarine fruit, and impacts of the modified atmosphere of chitosan coating on the metabolism of the respiratory pathway and redox state which could be a potential mechanism by which chitosan can increase the antioxidant power of postharvest fruit.

## 2. Materials and methods

### 2.1. Fruit material and treatment application

Nectarine fruit (*Prunus persica* cv. 'Ruiguang 7') of commercial maturity (soluble solids content [SSC] was about 9%) was purchased from local market in Beijing, PR China. The fruit was transferred to the laboratory (Beijing, PR China) in 5 h via truck. 300 fruits with uniform size and no physical damage were selected, all nectarine fruit were randomly separated into three groups.

Two different molecular weight chitosan materials, high molecular weight chitosan (HM: 120 kDa) and low molecular weight chitosan (LM: 30 kDa) were purchased from market reagent companies (MACKLIN; CAS 9012-76-4). One percent (w/v) chitosan solution was prepared according to Jiang and Li (2001). Briefly, 50 g of chitosan was dissolved in 5 L distilled water containing 0.7% lactic acid (v/v). After stirring to fully dissolve, the pH of the chitosan solution was adjusted to 6.0 with 0.1 M NaOH and 0.1% (w/v) tween-80 was used to amend the solution. The fruit were put into one percent (w/v) HM and LM chitosan solution for one min, and the control fruit was immersed in distilled water containing 0.1% (w/v) tween-80 and 0.7% lactic acid (v/v) for one minute. After treatment, fruit were air-dried at room temperature for 30 min and stored at a temperature  $25 \pm 2^\circ\text{C}$ . The relative humidity (RH) was 80–90%. Every 2 d randomly selected fruits for measurement until 8 d. Three replicates for all assays.

### 2.2. Respiration rate

Respiratory rate was determined with reference to Jiang, Mayak & Halevy (1994). Six fruits were randomly selected from each group and placed in a sealed box with a rubber stopper for two hours. The sealed box has a capacity of 5.0 L. After the gas in the glass jar was

equilibrated with a 1.0 ml syringe, 1.0 ml of gas was taken from the glass jar and measured by gas chromatography (GC7890F, TianmeiShanghai, a FID detector, carrier gas:  $\text{N}_2$ , combustion gas: air. The measurement conditions are column temperature  $60^\circ\text{C}$ , injection temperature  $120^\circ\text{C}$ , detection temperature  $\text{CO}_2$   $360^\circ\text{C}$ , ethylene  $150^\circ\text{C}$ ). The respiratory rate was expressed in  $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ .

### 2.3. Fruit quality measurements

#### 2.3.1. Weight loss

The weight loss was expressed as the percentage loss (%) of sample compared to the initial weight when the fruit was coated and dried.

#### 2.3.2. Fruit firmness

The fruit hardness tester (GY-3, Zhejiang Top Instrument Co., Ltd.) was used to determine the fruit firmness. During the measurement, three points were equally selected on both sides of the suture of the fruit. Fruit firmness was expressed in  $\text{kg cm}^{-1}$ .

#### 2.3.3. Soluble solids content (SSC)

The PAL-1 digital sugar meter (Japan ATAGO) was used to determine the fruit soluble solids content. In the measurement, the SSC content was measured after juicing the pulp on both sides of the suture of each fruit. SSC content was expressed as %.

#### 2.3.4. Titratable acidity (TA)

For the determination of titratable acid, refer to AOAC (1984). The titratable acid content of fruits is expressed as a percentage of malic acid in fresh fruit weight.

#### 2.3.5. Pulp color

Color parameters  $L^*$  (lightness),  $a^*$  ('-green' to '+red') of nectarine pulp was determined using a reflectance spectrophotometer (Model NF333, Nippon Denshoku Industries, Tokyo, Japan).

#### 2.3.6. MDA assays

Extraction and assays of MDA was conducted by the thiobarbituric acid reaction method according to previously method (Xi et al., 2017). The MDA content was expressed as  $\mu\text{mol kg}^{-1}$ .

#### 2.3.7. $\text{H}_2\text{O}_2$ content

$\text{H}_2\text{O}_2$  content determination process was slightly modified according to the previous method (Jana & Choudhuri, 1982). Five grams of frozen pulp samples were homogenized in 5 ml of pre-chilled acetone solution. The mixture was then centrifuged at 12,000g for 20 min at  $4^\circ\text{C}$ . 1.0 ml of the supernatant was collected as a sample extract, and 0.1 ml of 10% (v/v) titanium tetrachloride-hydrochloric acid and 0.2 ml of concentrated ammonia was added to the extract. After mixing and reacting for 5 min, the reaction solution was then centrifuged again at 12,000g for 20 min at  $4^\circ\text{C}$ , the supernatant was discarded, a precipitate was left, and 3.0 ml of 2 M sulfuric acid was added to the precipitate to dissolve the precipitate. The reaction solution absorbance value was measured at 412 nm with a spectrophotometer. Using a standard curve for  $\text{H}_2\text{O}_2$  obtained by the same method, the  $\text{H}_2\text{O}_2$  content was expressed as  $\text{mmol kg}^{-1}$ .

### 2.4. Extraction and assay of ASA-GSH cycle enzymes activities

Processes for extracting and measuring APX and GR activities were according to a previous method (Ma & Cheng, 2004). APX enzyme activity results are expressed as  $\text{U mg}^{-1}$  protein, the amount of enzyme required to reduce the absorbance of the reaction system by 0.01 per minute at 290 nm was an enzyme activity unit ( $\text{U} = 0.01 \Delta\text{OD}_{290} \text{ min}^{-1}$ ). GR enzyme activity results are expressed as  $\text{U mg}^{-1}$  protein, the amount of enzyme required to reduce the absorbance of the reaction system by 0.01 per minute at 340 nm was an enzyme activity unit

( $U = 0.01\Delta OD_{340} \text{ min}^{-1}$ ). Protein content was measured using a previous method (Bradford, 1976).

## 2.5. ASA and GSH contents

Processes for extracting and measuring ASA content were according to a previous method (Chumyarn, Shank, Faiyue, Uthaibutra, & Saengnil, 2017). The content of ASA was calculated from the standard curve of ASA obtained by the same method, and the content was expressed in  $\text{mg kg}^{-1}$ .

Extraction and assays of reduced glutathione was conducted with slight modification according to Guri (1983). Five grams of frozen pulp sample was homogenized in 5 ml extraction solution containing 50 g  $\text{L}^{-1}$  trichloroacetic acid, 5 mM EDTA. The mixture was then centrifuged at 12,000g for 20 min at 4 °C. The supernatant was collected and added to a reaction solution containing 4 mM 3-Carboxy-4-nitrophenyl disulfide (DTNB), 0.1 M phosphate buffered (pH 7.7). After the reaction system was reacted at room temperature for 10 min, the mixture absorbance value was measured at 340 nm with a spectrophotometer. The content was of GSH calculated from the standard curve of GSH obtained by the same method, and the content was expressed in  $\text{mg kg}^{-1}$ .

## 2.6. Enzyme activities assay of respiratory pathway metabolism

### 2.6.1. Total activity extraction and analysis of glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH)

The total activity of G-6-PDH and 6-PGDH determination process is slightly modified according to Brown and Wary (1968). In brief, 1 g of frozen pulp samples were homogenized in 5 ml of pre-chilled extraction buffer containing 0.05 M phosphate buffer (pH 6.8), 0.2 M sucrose and 1  $\text{mg L}^{-1}$  bovine serum albumin. The mixture was then centrifuged at 12,000g for 20 min at 4 °C. The supernatant was discarded and 5 ml of 0.05 M Tris-HCl extraction buffer (pH 7.6) was added to the pellet. The mixture was then centrifuged again at 12,000  $\times$  g for 5 min at 4 °C. 0.2 ml of the supernatant was collected as a sample extract, and reaction buffer containing 0.2 ml 5 mM 6-P-G (Glucose 6-phosphate), 0.2 ml 5 mM  $\text{MgCl}_2$ , 0.2 ml 5 mM  $\text{NADPN}_2$  and 1 ml 0.05 M Tris-HCl buffer (pH 7.4) was added to the sample extract. Instead of the sample extract, 0.2 ml buffer was used as a control, the absorbance increase of the reaction system was quickly measured at 340 nm. The amount of enzyme required to increase the absorbance of the reaction system by 0.01 per minute was an enzyme activity unit ( $U = 0.01\Delta OD_{340} \text{ min}^{-1}$ ). G-6-PDH + 6-PGDH total activity results were expressed as  $U \text{ mg}^{-1}$  protein. Protein content was measured using a previous method (Bradford, 1976).

### 2.6.2. Succinic dehydrogenase (SDH) extraction and analysis

Extraction and assays of SDH activity was conducted with slight modification according to method described by Li et al. (2016). The extraction of SDH crude enzyme solution is the same as that of G-6-PDH and 6-PGDH. The crude enzyme extract was taken as a sample solution and stored at 4 °C for later use. 2.0 ml 0.05 M phosphate buffer (pH 7.4), 0.1 ml 1.2 M succinic acid disodium salt (pH 7.4) and 0.1 ml 0.9 mM 2, 6-dichloro-1-phenol (DCIP) mixed and reacted 10 min at 30 °C water bath as initial reaction system. Thereafter, 0.1 ml enzyme extract and 0.1 ml 3  $\text{mg L}^{-1}$  phenazine methosulfate were added to above initial reaction system starting enzymatic reactions. The absorbance of the reaction system at 600 nm was recorded from 15 s after the start-up and was recorded every 15 s, and data of at least six points were continuously recorded. According to the recorded data, the  $OD_{600}$  curve was plotted over time, and the initial linear part of the curve was calculated as  $\Delta OD_{600}$ . The amount of enzyme required to reduce the absorbance of the reaction system by 0.01 per minute was an enzyme activity unit ( $U = 0.01\Delta OD_{600} \text{ min}^{-1}$ ). SDH enzyme activity result was expressed as  $U \text{ mg}^{-1}$  protein. Protein content was measured using a

previous method (Bradford, 1976).

### 2.6.3. Phosphohexose isomerase (PGI) extraction and analysis

Process for extracting and measuring PGI activity were carried out with some modification according to a previous method (Li et al., 2016). Frozen fruit tissue samples (1 g) were homogenized in 5 ml of pre-chilled extraction buffer containing 0.05 mM Tris-HCl (pH 7.4). The mixture was then centrifuged at 12,000  $\times$  g for 30 min at 4 °C. 0.5 ml of the supernatant was collected as a sample extract, and 1.0 ml 10 mM glucose 6-phosphate disodium salt dihydrate was added to the sample extract. After 5 min of reaction in a 30 °C water bath, 2 ml 10% trichloroacetic acid was added to reaction solution. The reaction solution was then centrifuged again at 12,000  $\times$  g for 15 min at 4 °C. 1.0 ml of the supernatant was collected as a sample extract and 5 ml of 38% hydrochloric acid (HCL) and 2 ml of 0.1% resorcinol were added to the sample extract. Thereafter, reaction solution reacted 10 min in a 80 °C water bath. According to the measured absorbance at 520 nm after the reaction system cooling and standard curve of fructose obtained by the same method, the sample solution of 6-phosphofructose can be calculated. The PGI activity result was expressed as content of 6-P-F.

## 2.7. Determination of total phenolics and flavonoids contents

Total phenolics content was determined according to Folin-Ciocalteu method (Han, Mao, Wei, & Lu, 2017). According to standard curve of gallic acid obtained by the same method, the total phenolics content of frozen wound peel tissue samples was expressed as mg of gallic acid equivalents (GAE)  $\text{kg}^{-1}$ . Total flavonoids content was determined as described by Han et al. (2017). The total flavonoids content of frozen wound peel tissue samples was expressed as mg of rutin equivalents (RE)  $\text{kg}^{-1}$ .

## 2.8. Total antioxidant capacity

In the present study, 1 g of frozen tissue sample was homogenized in 5 ml of 80% (v/v) ethanol, the mixture was then centrifuged at 12,000  $\times$  g for 5 min at 4 °C and the supernatant was taken for antioxidant capacity determination, the ABTS, DPPH, FRAP,  $\text{Cu}^{2+}$  reducing power were used to measure antioxidant activities of the nectarine fruit.

The ABTS radical scavenging activity of sample was determined according to method reported by Re et al. (1999). Aqueous solution of Trolox was used for calibration. Results were expressed as mmol TE  $\text{kg}^{-1}$ .

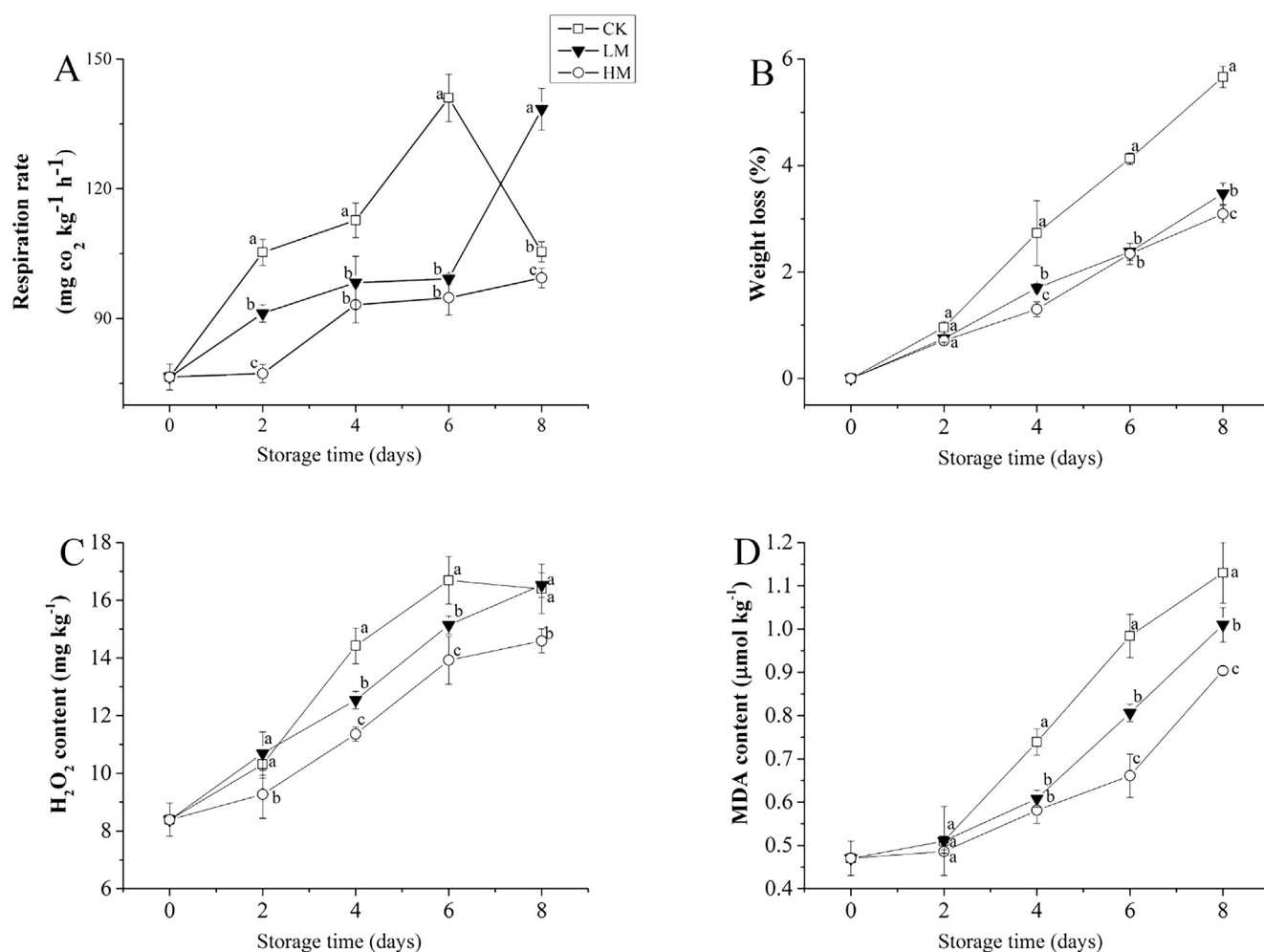
The DPPH inhibition assay was determined with some modification from a previous method (Brand-Williams, Cuvelier, & Berset, 1995). The reaction system consisted of supernatant, 0.2 mM DPPH, 80% (v/v) ethanol: 1:4:3 (v/v/v). The result was expressed as the clearance rate as DPPH inhibition (%).

Determination of FRAP was conducted from the method by Benzie and Strain (1996) with some modification. The reaction system prepared by 0.1 ml supernatant and 3 ml FRAP solution. The FRAP solution consisted of TPTZ (10 mM) – HCl (40 mM) solution, 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution and 0.3 M acetate buffer (pH 3.6) :1:1:10 (v/v/v). According to standard curve of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  obtained by the same method, the FPAP was expressed as mmol Fe(II)  $\text{kg}^{-1}$ .

The Cupric ion ( $\text{Cu}^{2+}$ ) reducing power was determined by a previous method the (Apak, Güçlü, Özyürek, & Karademir, 2004). Aqueous solution of Trolox was used for calibration. Results were expressed as mmol Trolox equivalents (TE)  $\text{kg}^{-1}$ .

## 2.9. Statistical analysis

The experiment was conducted in a completely randomized design, with triplicate per treatment. The analyses of data performed by one-way analysis of variance (ANONA) from SPSS 22.0 window. Significant



**Fig. 1.** Effect of LM and HM chitosan on respiration rate (A), Weight loss (B), H<sub>2</sub>O<sub>2</sub> content (C) and MDA content (D) of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates, and vertical bars indicate the standard deviations for each treatment (n = 3). Bars with different letters represent statistical differences ( $P < 0.05$ ) within the same day. Untreated fruits were used as control.

differences were calculated by least significant difference (Duncan) test and defined as  $P < 0.05$ .

### 3. Results

#### 3.1. Respiration rate

The respiratory rate of control nectarine fruit continued to increase and shown climacteric peak on day 6, followed by decline on day 8 (Fig. 1A). The application of the different molecular weights chitosan all significantly reduced the respiration rate during the first 6 d of the storage period, however the respiratory rate of LM chitosan coating fruit reached climacteric peak and had a higher level on day 8 than control fruit (Fig. 1A). Compared with the control and LM chitosan coating fruit, the respiratory rate climacteric peak of HM chitosan coating fruit was strongly suppressed throughout the storage period (Fig. 1A).

#### 3.2. Fruit quality parameters

##### 3.2.1. Firmness and weight loss

The firmness of nectarine fruit dropped with increasing storage time in all treatments (Table 1). Although the firmness of LM and HM chitosan fruit all significantly higher ( $p < 0.05$ ) than control, the application of HM chitosan coating treatment was the most effective in maintaining firmness of nectarine fruit during the storage period

(Table 1).

The weight loss of nectarine fruit gradually increased at a steady rate and reached 3.09–5.66% by the 8th day for all treatments (Fig. 1B). Generally, the weight loss of nectarine fruit in both chitosan treatments were significantly reduced ( $p < 0.05$ ) relative to the control, but to different extents, the value of weight loss in LM fruit was 38.69% lower than in the control at the end of storage, the HM was 45.4% (Fig. 1B).

##### 3.2.2. SSC and TA

As shown in Table 1, the SSC levels of the control fruit continued to increase and reached 12.96% on day 8. The treatments of different molecular weights chitosan coating of LM and HM all significantly suppressed ( $p < 0.05$ ) the increase of SSC, whereas the SSC of HM fruit appeared a decline on day 6. Similar changes in TA content of all nectarine fruit occurred during the storage period, both rising on day 2 and then slowly decreasing at the following days (Table 1). However, the HM fruit shown the highest of TA content on day 2, 6 and 8, and the TA content of LM fruit was higher ( $p < 0.05$ ) than control on day 4 and 8 (Table 1).

##### 3.2.3. Pulp color

With nectarine fruit senescence, the color of the pulp changes from white to red. With increasing storage time, the  $L^*$  value decreased and  $a^*$  value increased in all fruit (Table 1). Compared with the control, fruit treated with LM and HM chitosan coating had a notable delay in pulp color change during storage time. The  $a^*$  value of LM and HM chitosan



**Table 1**

Effect of LM and HM chitosan on a\*, L\*, SSC, TA and firmness of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates. Untreated fruits were used as control.

Storage (days)	Treatment	a*	L*	SSC (%)	TA (%)	Firmness (kg cm <sup>-1</sup> )
Day0	CK	6.8 ± 0.29 <sub>i</sub>	65.21 ± 0.38 <sub>a</sub>	9.68 ± 0.13 <sub>gh</sub>	0.616 ± 0.014 <sub>cd</sub>	13.24 ± 0.39 <sub>a</sub>
	LM	6.8 ± 0.29 <sub>i</sub>	65.21 ± 0.38 <sub>a</sub>	9.68 ± 0.13 <sub>gh</sub>	0.616 ± 0.014 <sub>cd</sub>	13.24 ± 0.39 <sub>a</sub>
	HM	6.8 ± 0.29 <sub>i</sub>	65.21 ± 0.38 <sub>a</sub>	9.68 ± 0.13 <sub>gh</sub>	0.616 ± 0.014 <sub>cd</sub>	13.24 ± 0.39 <sub>a</sub>
Day2	CK	11.18 ± 1.12 <sub>fg</sub>	61.07 ± 0.46 <sub>bc</sub>	10.40 ± 0.21 <sub>ef</sub>	0.661 ± 0.009 <sub>b</sub>	11.58 ± 0.41 <sub>cd</sub>
	LM	10.69 ± 0.96 <sub>gh</sub>	61.53 ± 0.6 <sub>bc</sub>	10.14 ± 0.16 <sub>efg</sub>	0.660 ± 0.020 <sub>b</sub>	12.19 ± 0.11 <sub>bc</sub>
	HM	8.95 ± 0.65 <sub>h</sub>	63.09 ± 0.59 <sub>ab</sub>	9.79 ± 0.14 <sub>fgh</sub>	0.708 ± 0.009 <sub>a</sub>	12.65 ± 0.37 <sub>ab</sub>
Day4	CK	13.35 ± 0.33 <sub>de</sub>	54.34 ± 1.64 <sub>e</sub>	10.06 ± 0.21 <sub>efg</sub>	0.589 ± 0.010 <sub>de</sub>	7.92 ± 0.04 <sub>f</sub>
	LM	11.9 ± 0.5 <sub>ef</sub>	59.19 ± 0.79 <sub>cd</sub>	11.30 ± 0.45 <sub>bc</sub>	0.627 ± 0.010 <sub>bc</sub>	10.22 ± 0.32 <sub>e</sub>
	HM	9.25 ± 0.69 <sub>jh</sub>	62.31 ± 1.42 <sub>b</sub>	11.70 ± 0.46 <sub>b</sub>	0.631 ± 0.020 <sub>bc</sub>	11.26 ± 0.28 <sub>d</sub>
Day6	CK	18.09 ± 1.17 <sub>b</sub>	51.78 ± 1.24 <sub>e</sub>	11.53 ± 0.25 <sub>bc</sub>	0.523 ± 0.030 <sub>f</sub>	5.2 ± 0.43 <sub>h</sub>
	LM	14.89 ± 1.69 <sub>cd</sub>	54.35 ± 1.51 <sub>e</sub>	11.03 ± 0.35 <sub>cd</sub>	0.516 ± 0.020 <sub>fg</sub>	6.79 ± 0.66 <sub>g</sub>
	HM	11.07 ± 0.52 <sub>g</sub>	58.08 ± 1.25 <sub>d</sub>	9.20 ± 0.20 <sub>h</sub>	0.572 ± 0.020 <sub>e</sub>	8.55 ± 0.12 <sub>f</sub>
Day8	CK	24.28 ± 0.55 <sub>a</sub>	48.19 ± 2.16 <sub>f</sub>	12.96 ± 0.76 <sub>a</sub>	0.391 ± 0.010 <sub>i</sub>	3.46 ± 0.24 <sub>i</sub>
	LM	16.81 ± 0.67 <sub>b</sub>	51.96 ± 1.91 <sub>e</sub>	11.60 ± 0.32 <sub>bc</sub>	0.430 ± 0.020 <sub>h</sub>	4.93 ± 0.61 <sub>h</sub>
	HM	16.26 ± 1.14 <sub>bc</sub>	54.21 ± 0.99 <sub>e</sub>	10.56 ± 0.21 <sub>de</sub>	0.485 ± 0.020 <sub>g</sub>	6.36 ± 0.54 <sub>g</sub>

a\* Values followed by the same letter in the column on the same day were not significantly different according to Duncan's Multiple Range Test ( $P < 0.05$ ). Data are accompanied by standard errors of the means ( $n = 3$ ).

coating were 30.76% and 33.03% lower than in the control at the end of storage, and the L\* value of LM and HM chitosan coating were 7.82% and 12.49% higher than in the control at the end of storage (Table 1).

### 3.2.4. H<sub>2</sub>O<sub>2</sub> and MDA contents

H<sub>2</sub>O<sub>2</sub> contents of nectarine fruit (including control and chitosan coating treated fruit) showed a continuous increase during the storage (Fig. 1C). HM chitosan coating treated fruit showed the lowest H<sub>2</sub>O<sub>2</sub> contents in all fruit. Although the H<sub>2</sub>O<sub>2</sub> contents of LM chitosan fruit had lower ( $p < 0.05$ ) level than control on days 4 and 6, there were no significant differences between H<sub>2</sub>O<sub>2</sub> contents of LM and control fruit on days 2 and 8 (Fig. 1C). Similar to the pattern of H<sub>2</sub>O<sub>2</sub> content changes in fruit, the content of MDA in fruit showed an continuous accumulation during the storage (Fig. 1D). The increase of MDA in fruit was significantly inhibited ( $p < 0.05$ ) by different molecular weights chitosan coating treatment and the HM chitosan coating treatment had stronger inhibition than LM (Fig. 1D).

### 3.3. The enzymes activities of ASA-GSH cycle

As shown in Fig. 2A, the activity of APX was significantly enhanced by different molecular weights chitosan coating treatment. On days 4 and 8 separately, the activity of APX in HM fruit was 3.6 and 1.57 times of that of the control, and that in the LM was 1.76 and 1.53 times of that of the control (Fig. 2A). In all groups, GR activity showed a intense rise and reached a maximum on day 4, but later decreased by the end of the storage time (Fig. 2B). Treatment with HM chitosan coating increased the peak value and maintained higher ( $p < 0.05$ ) GR activity compared with the control. However, no significant enhancement ( $p < 0.05$ ) of GR activity were observed in LM fruit compared with the control excepting day 6 and even had a lower level on day 4 (Fig. 2B).

### 3.4. GSH and ASA contents

The GSH content in control fruit exhibited slightly change during the first 4 days of storage and followed by decline on next days (Fig. 2C). However, the GSH content in LM and HM chitosan coating fruit showed an increase on day 2 and 4 respectively and led to higher level than control during the whole storage (Fig. 2C). Levels of ascorbic acid content in all fruit gradually decreased to the end of storage (Fig. 2D). Treatment with both molecular weights chitosan coating all led to higher ( $p < 0.05$ ) ascorbic acid content in nectarine fruit than control during the whole storage, except end of storage for LM (Fig. 2D).

### 3.5. The enzymes activities of respiratory pathway metabolism

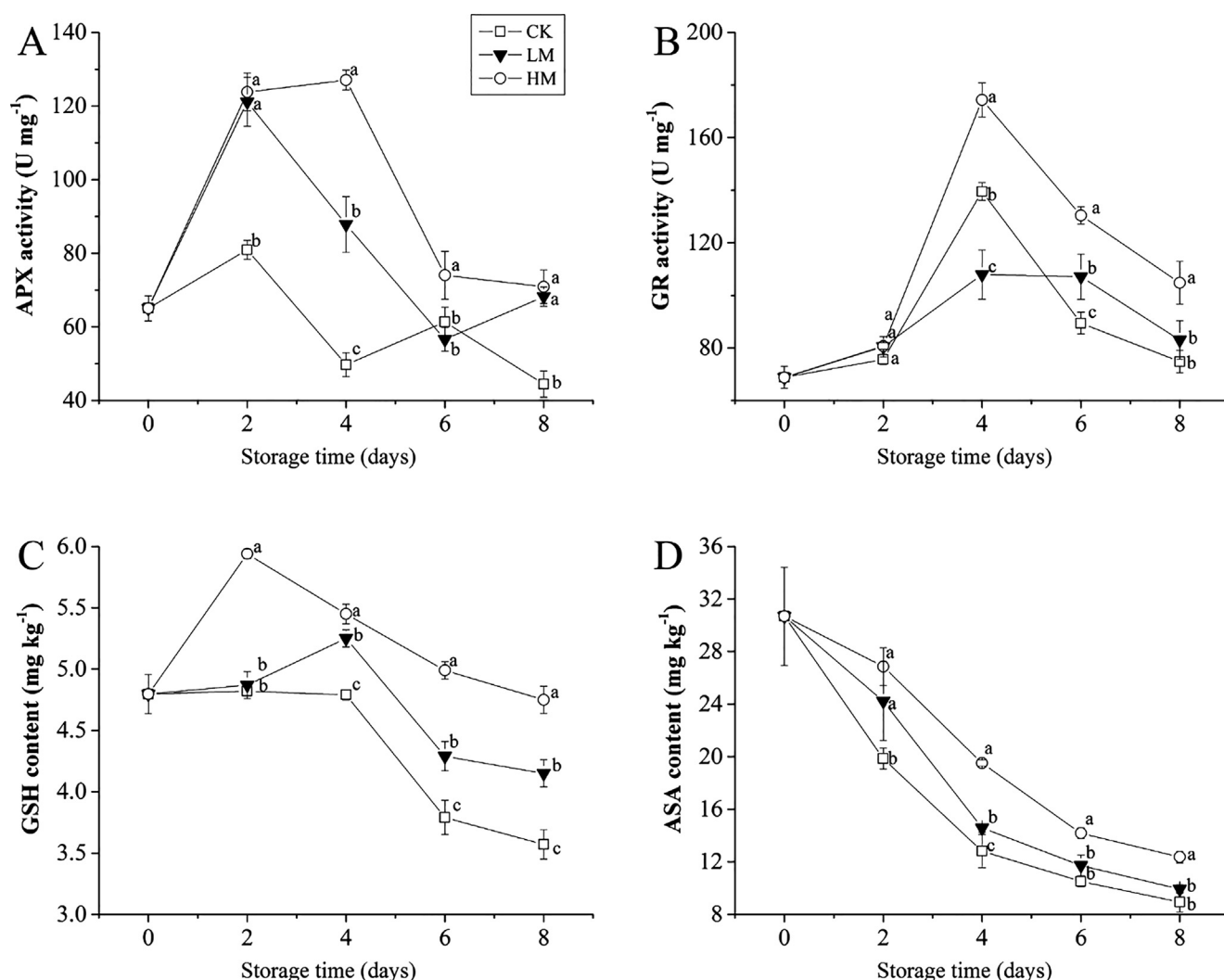
The 6-P-F content is a symbol of PGI activity. The contents of 6-P-F in control fruit were basically unchanged from 0 to 6 days, followed by a sharply decline from 6 to 8 days (Fig. 3A). However, the 6-P-F content in LM and HM chitosan coating fruit shown a notable increase on days 2 and 6 respectively, and HM had highest level of 6-P-F content on day 6 (Fig. 3A). Generally, the total activity of G-6-PDH and 6-PGDH of nectarine fruit continued to increase in all treatments during storage (Fig. 3B). The treatment of different molecular weights chitosan coating of LM and HM all significantly promoted ( $p < 0.05$ ) the total activity of G-6-PDH and 6-PGDH, whereas the HM was significantly higher than LM and control fruit on days 4 and 6 (Fig. 3B). The SDH activity shown a relatively steady level from 0 to 4 days, followed by a sharp decline from 4 to 6 days and then rising to the original level on day 8 (Fig. 3C). Activity of SDH in LM and HM chitosan coating treated fruit was notably decreased ( $p < 0.05$ ) from 0 to 4 days, and the HM had been lower level of SDH activity than control fruit throughout the storage period except day 6. Interestingly, there was a intense rise of SDH activity in LM fruit on day 8 and reached higher level than control fruit (Fig. 3C).

### 3.6. Total phenolics and flavonoids contents

As shown in Fig. 4A, the total phenolics content in control fruit decreased steadily during storage from 2 to 8 days. However, the total phenolics content in LM fruit was notable higher than control on days 2, 4 and 6, and the HM had a highest level of total phenolics content in all fruit on days 4, 6 and 8 (Fig. 4A). Compared with the control, the total flavonoids content in fruit treated with HM chitosan coating was significantly promoted ( $p < 0.05$ ) during storage. Interestingly, although the total flavonoids content in fruit treated with LM chitosan coating was higher than that of the control fruit on days 2 and 4, it showed a lower level on the end of storage time (Fig. 4B).

### 3.7. Total antioxidant capacity

In control fruit, the results indicated that the total antioxidant capacity determined using DPPH free radical scavenging assay sharply increased on day 2 and gradually decreased throughout the remainder of the storage time (Fig. 5A). There was a significant ( $p < 0.05$ ) difference between control and different molecular weights chitosan treatments. In LM and HM chitosan coating fruit, the DPPH was continuously significantly ( $p < 0.05$ ) higher than the control from 4 to 8 days (Fig. 5A). Similar to the pattern of DPPH changes in all



**Fig. 2.** Effect of LM and HM chitosan on APX activity (A), GR activity (B), GSH content (C) and ASA content (D) of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates, and vertical bars indicate the standard deviations for each treatment ( $n = 3$ ). Bars with different letters represent statistical differences ( $P < 0.05$ ) within the same day. Untreated fruits were used as control.

treatments, the ABTS free radical scavenging assay of the nectarine fruit showed a significant ( $p < 0.05$ ) difference between control and different molecular weights chitosan treatments during the storage period (Fig. 5B). The value of ABTS in LM was highest on days 4 and 6, and in HM was highest on day 8 (Fig. 5B).

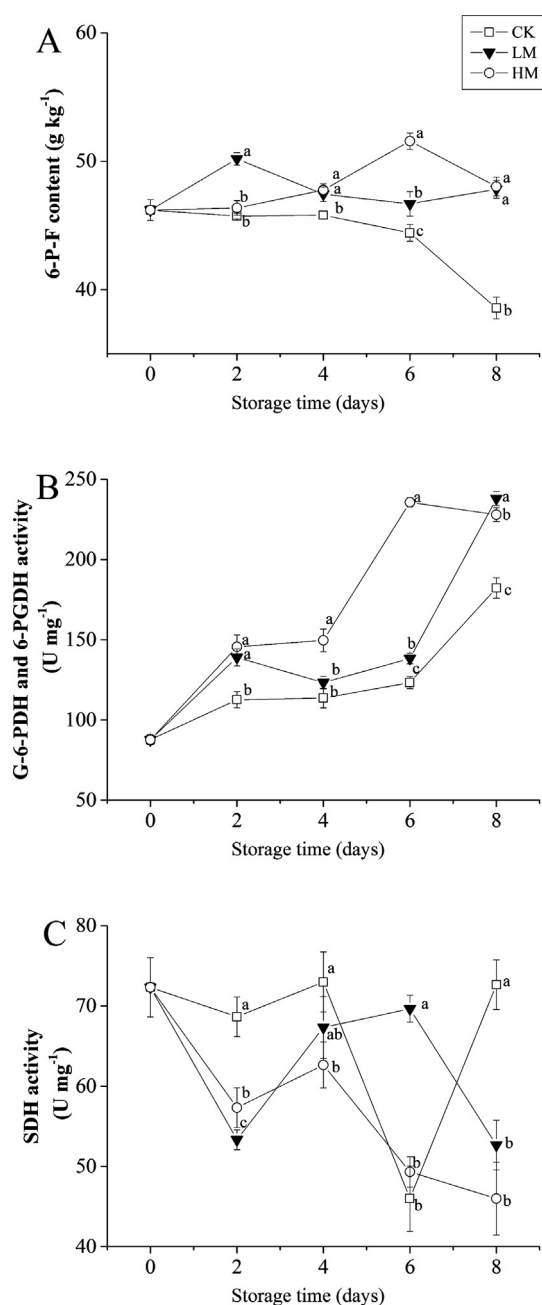
As shown in Fig. 5C and D, in comparison with the control fruit, the FRAP assay and determination of the  $\text{Cu}^{2+}$ -reducing power in fruit treated with different molecular weights chitosan were all notably promoted from during storage from 4 to 8 days. On days 6 and 8 separately, the FRAP value in LM was 1.39 and 1.15 times of that of the control, and that in the HM was 1.19 and 1.29 times of that of the control (Fig. 5C). Similarly, on day 8, the value of  $\text{Cu}^{2+}$ -reducing power in LM was 1.14 times of that of the control, and that in the HM was 1.32 times of that of the control (Fig. 5D).

#### 4. Discussion

Recently, researchers have shown that the postharvest application of chitosan coating can improve the quality and enhance antioxidant capacity of guava and mango (Batista Silva et al., 2018; Jongsri et al., 2016), which agreed with our finding. This study found that different molecular weights chitosan treatment delayed the senescence and enhanced antioxidant capacity by stimulating ASA-GSH cycle of

postharvest nectarine. Besides, the improvement effects in high molecular weight chitosan (HM: 120 kDa) were better than those of low molecular weight chitosan (LM: 30 kDa). In addition, it was shown here that HM chitosan treatment could change the respiratory pathway metabolism of postharvest nectarine. Since respiratory pathway metabolism significantly affects the energy state and redox state, and the changes in energy state and redox state directly determine the postharvest senescence of the fruit (Chumyarn et al., 2017; Zhang et al., 2017). Note that the present study showed the impacts of chitosan coating on respiratory pathway metabolism could be one of the potential reasons why chitosan coating can delay senescence.

As a typical climacteric fruit, nectarine fruit showed a rapid senescence in the postharvest storage period with the appearance of respiratory peaks (Xi et al., 2017). Through film formation, chitosan coating can inhibit respiration rate by affecting the exchange of gas components (e.g.,  $\text{H}_2\text{O}$ ,  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$ ) on the fruit surface, thus delaying the response of the fruit to changes in the external environment (Batista Silva et al., 2018; Kerch, 2015). This study showed that LM and HM chitosan coating both significantly reduced the respiration rate in postharvest nectarine fruit, while HM chitosan coating also suppressed respiration peak. Similar result have also observed in mango in that HM chitosan coating had a higher suppression of respiration rate, which resulted from the fact that HM can form a denser and thicker film,



**Fig. 3.** Effect of LM and HM chitosan on 6-P-F content (A), G-6-PDH and 6-PGDH activity (B), and SDH activity (C) of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates, and vertical bars indicate the standard deviations for each treatment ( $n = 3$ ). Bars with different letters represent statistical differences ( $P < 0.05$ ) within the same day. Untreated fruits were used as control.

blocked the pores on the mango surface (Jongsri et al., 2016). SSC and TA are important fruit quality parameters, with the respiratory rate increased, the SSC increased because of the hydrolysis of polysaccharides and the TA decreased due to the consumption of organic acids (Wannabussapawich & Seraypheap, 2018). In this study, the higher level of TA and lower level of SSC in HM chitosan coating nectarine fruit were primarily caused by the reduced rate of respiration.

With the senescence of nectarine fruit, the color of the pulp turned red from white. In this study, fruit coated with HM chitosan was observed with a greater impact on reduction change in the pulp color than the LM chitosan and control fruit. Similar result was reported by Cosme Silva et al. (2017) who had observed that mango fruit treated with 3.0%

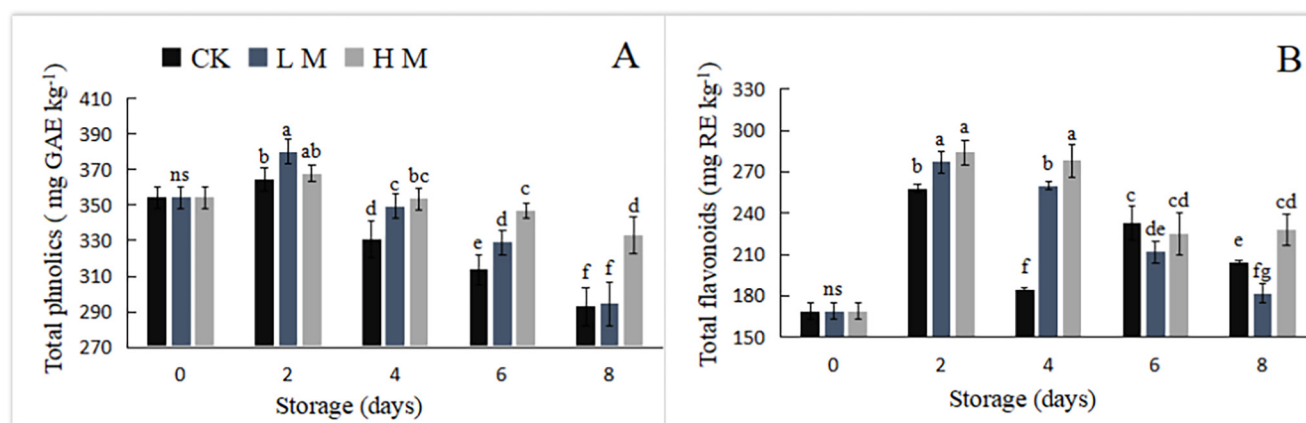
chitosan exhibited slower changes than control in peel color. Besides, the present study showed that the chitosan coating significantly suppressed the increase in weight loss and the decrease in firmness in postharvest nectarine fruit during storage time, and the suppression effect of HM chitosan coating was more significant. Likewise, fruits such as papaya (Ali et al., 2011), guava (Hong, Xie, Zhang, Sun, & Gong, 2012) and kiwifruit (Kaya, Cesoniene, Daubaras, Leskauskaitė, & Zabulione, 2016) have also been observed to be firmer and less weight loss when were covered with chitosan.

It has been known that postharvest fruit senescence involved the burst of ROS, with cell damage such as lipid peroxidation resulting in the accumulation of MDA (Mittler, 2016). Recently, more studies have reported that chitosan treatment could increase enzymatic and non-enzymatic antioxidative systems to reduce ROS accumulation, thereby improving the quality and delayed the senescence in postharvest fruits (Batista Silva et al., 2018; Jiang et al., 2018). So far, the enhancement of antioxidant capacity induced by chitosan in postharvest fruits has been attributed to the mechanism of induced resistance as exogenous elicitor (Romanazzi, Feliziani, Baños, & Sivakumar, 2017). The data here were consistent with above view, showing that HM chitosan coating not only significantly stimulated the ASA-GSH cycle, but increased the content of total phenolics and flavonoids, and suppressed the accumulation of H<sub>2</sub>O<sub>2</sub> and MDA in postharvest nectarine fruit. The same trend with respect to H<sub>2</sub>O<sub>2</sub> and MDA content in chitosan-coated plum fruit were reported (Kumar, Sethi, Sharma, Srivastav, & Varghese, 2017). Moreover, the present study shown that HM chitosan coating and LM chitosan coating both increased total antioxidant capacity including DPPH, ABTS, FRAP and Gu<sup>2+</sup> reducing power and modified the redox status in postharvest nectarine fruit during storage period. Consistent with our results, Candir, Ozdemir, and Aksoy (2018) reported higher total phenolic (TP) contents and antioxidant capacity assayed by the ferric reducing antioxidant power (FRAP) and the trolox equivalent antioxidant capacity (TEAC) in chitosan coating pomegranate fruits compared to control fruit during cold storage.

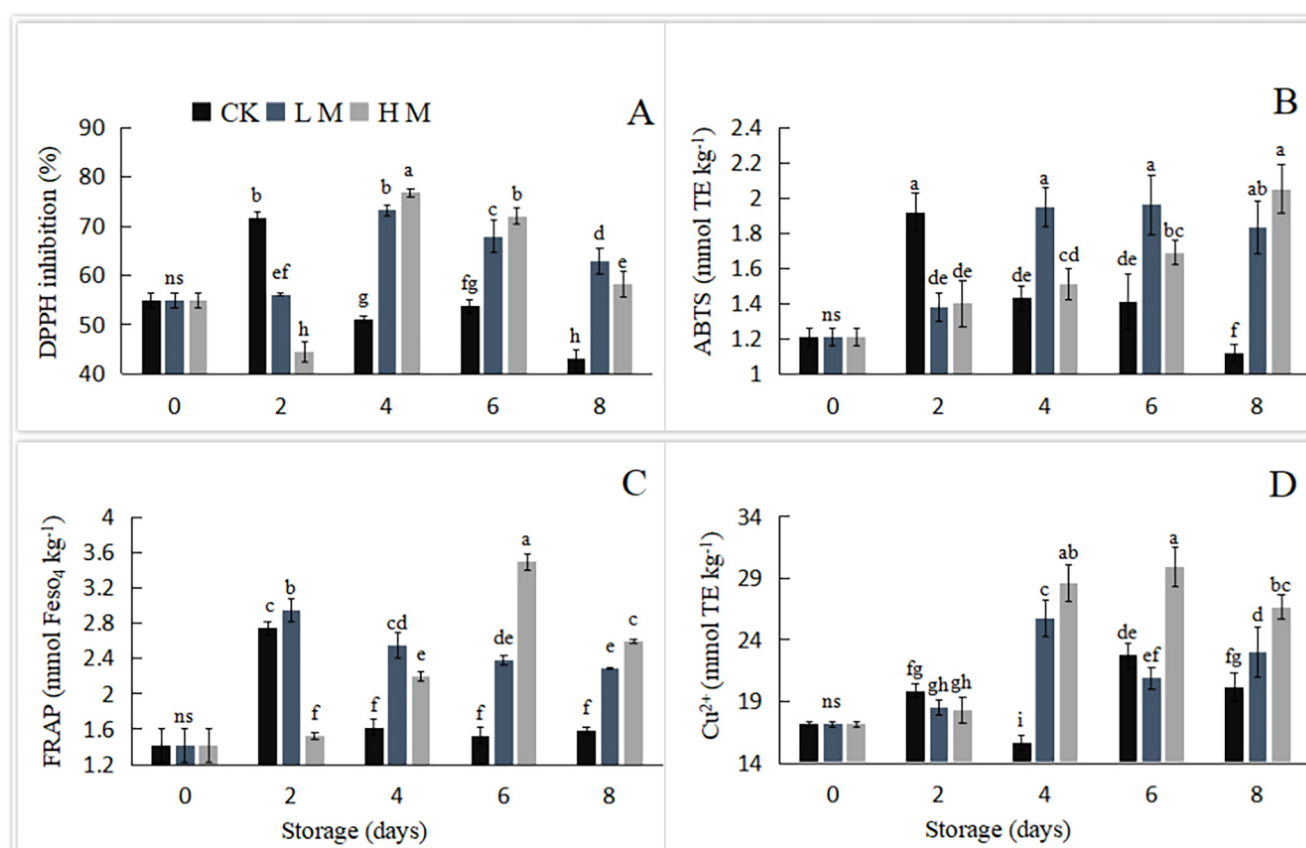
Cosme Silva et al. (2017) highlighted that chitosan coating severely affected the carbon metabolism of mango after storage and speculated that impact of the modified atmosphere of chitosan coating might reduce the EMP-TCA pathway and stimulate anaerobic pathway resulting in the delay of senescence process of mangoes. Moreover, several works had definitely documented that the chitosan coating caused a significant change in the internal gas composition of the postharvest fruit, there by leading to the increase in CO<sub>2</sub> and decrease in O<sub>2</sub> concentration (Ali et al., 2011; Dong, Cheng, Tan, Zheng, & Jiang, 2004). It is well-known that changes in CO<sub>2</sub>/O<sub>2</sub> can lead to changes in the respiratory pathways of plant cells. Note that the results here supported the above speculation, showing that HM chitosan coating significantly suppressed the activity of SDH enzyme and increased the total activity of G-6-PDH and 6-PGDH in postharvest nectarine fruit during storage. This suggested that the HM chitosan coating reduced respiratory pathway of TCA and increased HMP. NADPH, primarily produced by HMP, can be used to produce GSH to enhance the antioxidant capacity of the plant system (Sgherri & Navari-Izzo, 1995). Embden-meyerhof-paranas (EMP)-tricarboxylic-acid-cycle (TCA) serves as a major respiration and energy-generating pathway in plants, playing key roles in regulating senescence of postharvest fruit (Yang et al., 2014). Work by Yang et al. (2014) showed that the senescence retardation in peach fruit was linked to the decreased SDH activity. Thus, except for the mechanism of induced resistance as exogenous elicitor, this study suggested that the enhancement of total antioxidant capacity induced by chitosan in postharvest fruits had a correlation with changes of respiratory pathway metabolism.

## 5. Conclusion

In summary, the present study showed that the treatments of 30 KDa LM and 120 KDa HM chitosan both delayed senescence and



**Fig. 4.** Effect of LM and HM chitosan on total phenolics content (A), and total flavonoids content (B) of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates, and vertical bars indicate the standard deviations for each treatment (n = 3). Bars with different letters represent statistical differences (P < 0.05). Untreated fruits were used as control.



**Fig. 5.** Effect of LM and HM chitosan on DPPH inhibition (A), ABTS (B), FRAP (C) and Cu<sup>2+</sup> reducing power (D) of postharvest nectarine fruit stored at 25 °C for 8 days. Each value represents the mean for three replicates, and vertical bars indicate the standard deviations for each treatment (n = 3). Bars with different letters represent statistical differences (P < 0.05). Untreated fruits were used as control.

improved total antioxidant capacity in postharvest nectarine fruit during the storage period. Moreover, fruit treated with HM chitosan manifested better quality and redox state than LM. It is noteworthy that, our results proved that the changes in respiratory pathway metabolism by HM chitosan were involved in senescence retardation and redox status modification in postharvest nectarine fruit.

#### Conflict of interest

None declared.

#### Acknowledgement

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