



Interactions among chilling tolerance, sucrose degradation and organic acid metabolism in UV-C-irradiated peach fruit during postharvest cold storage

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Abstract

UV-C irradiation treatment has been demonstrated to be able to enhance chilling tolerance in peach (*Prunus persica* L. Batsch) during postharvest cold storage. Sugar and organic acid play central roles in plant metabolism. However, little is known about the relationships among chilling injury, soluble sugar and organic acid in peaches subjected to UV-C. In this study, peaches were irradiated with UV-C (1.5 kJ/m²) and then stored at 1 °C for 35 days. The content of sugar and organic acid, activities of enzymes, and the expression of enzyme genes that catalyze the metabolism of sugar and acid were evaluated. Results showed that UV-C significantly alleviated chilling injury and maintained the quality of peaches during storage. For sugar metabolism, UV-C suppressed sucrose degradation and glucose production mostly by inhibiting the enzyme activity and mRNA transcription of invertase (β -D-fructofuranoside fructohydrolase; EC 3.2.1.26) during the cold storage. For organic acid metabolism, UV-C irradiation downregulated the enzyme activities and gene expressions of aconitase (citrate hydrolyase; EC 4.2.1.3), and NADP-malic enzyme (*S*-malate: NADP-oxidoreductase; EC 1.1.1.40), but upregulated the enzyme activities and gene expressions of citrate synthase (acetyl-CoA: oxaloacetate *C*-acetyltransferase; EC 2.3.3.1) and NAD-malate dehydrogenase (*L*-malate: NAD-oxidoreductase; EC 1.1.1.37), leading to the low degradation of citric and malic acids during the whole storage periods. These results suggest that UV-C enhanced chilling tolerance in peach fruit during cold storage by suppressing the degradations of sucrose, citric, and malic acids.

Keywords Citric acid · Malic acid · Peach · Sucrose · UV-C

Introduction

Peach fruit (*Prunus persica* L. Batsch) is an economically important crop in China and favored by consumers because of its pleasant flavor and sweet taste. However, the fruit is susceptible to postharvest decay caused by several pathogenic fungi during the harvest at the ambient condition.

Refrigeration is the most common technology to extend the storage shelf-life for the fruit (Li et al. 2014; Lin et al. 2016; Zhang et al. 2011). Peach is one of the cold-sensitive fruits and usually susceptible to chilling stress especially at the range of 0–8 °C, causing tissue browning, losing quality and fading flavor (Crisosto et al. 1999; Lurie and Crisosto 2005).

Technologies to suppress fruit chilling injury include the treatment with hot air (Lurie 1998; Shao and Tu, 2014), low temperature conditioning (Sajid et al. 2013), controlled atmosphere (Kim et al. 2007), jasmonic acid (Gornik et al. 2014), and salicylic acid (Sayyari et al. 2017). All of these techniques have been verified to enhance the cell membrane integrity and inhibit the production of reactive oxidative species (ROS). Concerning the various diseases and stresses occurred in peach fruit during postharvest storage, ultraviolet (UV-C) irradiation exhibited the latent capacity to avoid osmotic stress, and reduced postharvest fungi and food-borne pathogens in different food matrices (Vicente et al. 2005; Bal and Celik 2008; Pongprasert et al. 2011; Kalaras

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et al. 2012). Besides, it is widely used as an alternative to chemical sterilization, which overcomes the residual chemical issues in the fruit. The application of UV-C in button mushroom has been confirmed to suppress the process of tissue browning by regulating the enzyme activity and mRNA transcript level of polyphenoloxidase (Kalaras et al. 2012). UV-C irradiation of the peach fruit at different doses has been verified to improve the quality of the fruit and inhibit chilling injury (Gonzalez-Aguilar et al. 2004).

Soluble sugar is responsible for the sweet taste in many fruits. In peaches, glucose, fructose, and sucrose are the three main soluble sugars, of which sucrose accounts for 75% of the total sugar (Aubert et al. 2014). Soluble sugars play a predominant role in plant structure and metabolism at the cellular and whole organism levels. Sugars participate in various metabolic pathways, contribute to flavor emission, nutritional values, and alleviate osmotic stress (Couée et al. 2006; Aubert et al. 2014). The relationships among ROS production, antioxidant capacity, and sugar metabolism have been verified by various studies, but the results are controversial. Yu et al. (2017) demonstrated that peaches treated with 1-MCP exhibited higher chilling tolerance than that of the untreated controls, which was mainly due to that 1-MCP suppressed the degradation of sucrose, leading to alleviate the production of ROS. While, in the loquat fruit, the high contents of glucose and fructose enhance chilling tolerance during cold storage (Cao et al. 2013; Shao and Tu 2014).

The organic acid is another important factor composing the qualities and flavors of the fruit. Malic acid and citric acid are two predominant organic acids in most of fruit species, contributing to sour taste (Silva et al. 2004). Organic acids also determine the maturity of the most of fruit species. They can convert into sugars and the respiratory chain components during the fruit ripening, resulting in significant reduced sour taste. Sheng et al. (2017) suggested that organic acids could maintain fruit quality and extend the shelf-life; a negative correlation has been found between the weight loss and organic acid in the pumelo during post-harvest storage. Besides, organic acids are natural substrates that participate in the tricarboxylic acid (TCA) cycle, releasing energy charge and adenosine triphosphate (ATP). Many environmental factors regulate the metabolisms of organic acid during the maturation of fruit. Among different influence factors, the temperature is a main regulatory factor. High temperature decreased titratable acid and promoted the degradations of citric acid and malic acid (Guo et al. 2016). Besides, Lin et al. (2016) demonstrated that Pokan fruit grown at low temperature exhibited higher citric acid to avoid cold stress.

Many researchers have suggested that the role of UV-C irradiation in alleviating chilling injury is by stabilizing cell membrane integrity and activating ROS-scavenging enzymes. To the best of our knowledge, no study has been

carried out to evaluate the effect of UV-C irradiation on soluble sugars and organic acids in peach fruit, and the interactions among chilling tolerance, sucrose degradation, and organic acid metabolism in response to UV-C irradiation. Therefore, the objective of this study was to verify the effect of UV-C irradiation on sugar and organic acid in peaches during cold storage and explore the possible mechanisms of UV-C enhancing chilling tolerance via sugar and organic acid metabolic pathways.

Materials and methods

Plant materials and treatments

Non-melting, clingstone, and white fleshed peach fruit (*Prunus persica* L. Batsch cv. Xiahui 6) was cultivated in an experimental orchard of agriculture science academy (Jiangsu Province, China). An unblemished fruit in uniform size and color, free of visible disease and mechanical wound, was selected for the present experiments. We collected 720 peaches at the mature stage (about 110 days after full blossom). Peaches were randomly divided into two groups, and each group contained three biological replications. For the UV-C-treated group, the peaches were irradiated with UV-C according to Gonzalez-Aguilar et al. (2004). The fruit was placed in an incubator equipped with two UV-C lamps (254 nm, 30 W, Benbang light Inc., Guangdong, China) and the light source was kept 50 cm away from the samples. To achieve the total dose (1.5 kJ/m²), the peaches were kept under the light source for 20 min and rotated at 180° for an additional 20 min of irradiation. For the control group, the fruit was maintained in the incubator equipped with fluorescent lamps (30 W, Benbang light Inc., Guangdong, China) for 20 min of irradiation for each side. After the treatments, all fruits were stored at 1 °C for 5 weeks. Twenty fruits from each replicate were randomly chosen, and the flesh was cut and mixed together for further analysis at every 7 days during the cold storage.

Quality analyses

Fruit firmness was measured on four opposite sides of peaches with skin removed at the equatorial region using a digital penetrometer (GY-4, Zhejiang Top Co. Ltd, China) equipped with a 7-mm-diameter probe. The unit was expressed as Newton (N). Soluble solid contents (SSC) were measured by juice obtained from peach samples using portable Brix meter (PAL-1, Atago Co. Ltd, Tokyo, Japan) and expressed as % of the original weight. Titratable acid (TA) was measured by acid–base titration according to the method of Jiang and Li (2001) and expressed as mg g⁻¹. Ascorbic acid was determined according to the method of Akgün

and Ünlütürk (2017) and expressed as mg g^{-1} . The internal browning (IB) index of peaches was measured according to Cao et al. (2013). 0: represents none internal browning in peaches, 1: the browning area is 0–25% of the original volume, 2: browning area is 25–50%, 3: browning area is 50–75%, and 4: represents the browning area is more than 75%. The IB index was calculated by the following formula: $\text{IB index} = \Sigma (\text{IB level} \times \text{number of fruit at the IB level}) / (4 \times \text{total number of fruit})$. Electrolyte leakage of peach fruit was determined according to the method of Zeng et al. (2015). Briefly, the fruit was cut into a ground piece at 6 mm in diameter and at 2 mm thickness. A total of 5 g peach slice was soaked with 30 mL distilled water. The electric conductivity of fresh cultures and boiled cultures was determined by conductometer (DDS-11A, Precision Scientific Instrument Co., Ltd. Shanghai, China). Electric conductivity was determined according to the equation: electrolyte leakage (%) = $[(T_v - C) / (T_b - C)] \times 100$, where T_v is the conductivity of vacuumed samples, T_b the conductivity of boiled samples, and C is the conductivity of distilled water. For juice yield, a total of 5 g peach slices was placed in the centrifuge tube (50 mL) with skimmed cotton in the bottom and centrifuged at 1500g for 10 min. Juice yield was calculated by the rate of weight loss in samples after centrifugation. All experiments were repeated three times.

HPLC analysis of soluble sugar and organic acid contents

Soluble sugars and organic acids were extracted according to the method of Yu et al. (2016) and Tang et al. (2010) with slight modification. Briefly, 5 g of frozen fruit samples was ground into powder in a liquid nitrogen mortar (A11, IKA Co., Ltd. Germany), and then thoroughly homogenized in 30 mL distilled water. The homogenate was extracted in the water bath with ultrasonic at 80 °C for 1 h, and then the homogenate centrifuged at 8000g for 10 min. After centrifugation, the supernatant was diluted using 100 mL of distilled water and filtered using a 0.45- μm water syringe filter. The filtered solution was used for the analysis of soluble sugars and organic acids. Glucose, fructose, sucrose, malate, citrate, quinate and shikimate were used as the standard substance in the tests.

Soluble sugars were determined using a high-performance liquid chromatography (HPLC) system (Model 1260, Agilent, USA) equipped with an evaporative light scattering detector. A total of 20 μL aliquot was used for HPLC analysis, and the column temperature was 40 °C. Carbohydrate column (250 mm \times 4.6 mm, 5 μm , Agilent corporation, USA) was used to perform the chromatographic separation with an isometric elution. Acetonitrile:water (75:25, v/v) was used as the mobile phase at a flow rate of 1.0 mL min^{-1} .

Organic acids were analyzed using an HPLC system (Model e2695, Waters, USA). A total of 20 μL aliquot was separated by the Atlantis T3 column (150 mm \times 4.6 mm, 5 μm , Waters Corporation, USA) using the mobile phase of potassium dihydrogen phosphate (20 mM, pH 2.5) at the flow rate of 0.6 mL min^{-1} . The detection of the separation was performed at a wavelength of 210 nm according to the scanning mode of the photodiode array detector (Model 2998, Waters, USA).

Identification and quantification of sugars and organic acids were made by comparing the retention times with the corresponding standards, and the unit of each compound was expressed as mg g^{-1} . Triplicate samples were analyzed in the experiments.

Extraction and activities of enzymes that catalyze sugar metabolism

The enzymes that catalyze sugar metabolism were extracted according to the previous method of Yu et al. (2016) with some modifications. The frozen peach tissue (2 g) was homogenized in 5 mL of homogenization buffer, including 100 mM Tris-HCl (pH 7.0), 5 mM MgCl_2 , 5 mM dithiothreitol (DTT), 2 mM ethylene diamine tetraacetic acid disodium salt (EDTA-Na_2), 2% glycol, 0.2% bovine serum protein and 2% polyvinylpyrrolidone. The homogenate was centrifuged at 10,000g for 30 min, and then the supernatant was used for the measurement of enzyme activities.

The crude enzymes were mixed with 100 mM sodium acetate buffer (pH 5.5) and 10 g L^{-1} sucrose for the acid invertase (AI) assay, and mixed with 100 mM sodium phosphate buffer (pH 7.5) and 10 g L^{-1} sucrose for the neutral invertase (NI) assay. All of the solutions were incubated at 37 °C for 30 min and added with 3,5-dinitrosalicylic acid in the boiled water for 5 min. Absorbance was measured at 540 nm, and the enzyme activity was expressed as a unit mg^{-1} protein (U mg^{-1} pro). One unit of the enzyme activity was defined as the amount of enzyme protein that catalyzes 1 μmol of glucose in 1 h.

4 mM uridine diphosphate glucose (UDPG), 60 mM fructose-6-phosphate, 15 mM MgCl_2 and 0.1 mM Tris-HCl (pH 7.0) were used for the sucrose phosphate synthase (SPS) assay. 4 mM UDPG, 60 mM fructose, 15 mM MgCl_2 and 0.1 mM Tris-HCl (pH 7.0) were used for the sucrose synthase (SS) activity assay. All the mixtures were incubated at 37 °C for 30 min and boiled for 5 min. After cooling, 30% HCl and 0.1% resorcinol were added into the mixture and incubated at 80 °C for 10 min to terminate the enzyme reactions. Absorbance was measured at 480 nm and the enzyme activities were expressed as a U mg^{-1} pro.

The total proteins were measured according to the method of Bradford (1976).

Extraction and activities of enzymes that catalyze organic acid metabolism

The extraction of enzymes that catalyze the organic acid metabolism was determined according to the method of Tang et al. (2010). The frozen peach tissue (6 g) was extracted using 4 mL buffer [0.2 M Tris–HCl (pH 8.0), 0.6 M sucrose, 10 mM isoascorbic acid] and centrifuged at 4000g for 20 min. The supernatant was diluted to the 10 mL of the final volume using the buffer containing 0.2 M Tris–HCl (pH 8.2), 10 mM isoascorbic acid and 0.1% TritonX-100. A total of 4 mL crude extraction was centrifuged at 12,000g for 15 min, then the supernatant was diluted to the 8 mL of the final volume for the cytosolic aconitase (cytACO) analysis, and the precipitate was resolved using the same buffer for the mitochondrial aconitase (mitACO) and NAD-isocitric dehydrogenase (NAD-IDH) analyses. The remaining extraction (6 mL) was diluted into 12 mL using the buffer and 4 mL extraction was used for NAD-malate dehydrogenase (NAD-MDH) and NADP-malic enzyme (NADP-ME) assays, while the remaining 8 mL of the extraction underwent dialysis with the buffer at 4 °C overnight and used for phosphoenolpyruvate carboxylase (PEPC) and citrate synthase (CS) analyses.

PEPC was determined using 800 mM Tris–HCl (pH 8.2), 40 mM MgCl₂, 200 mM KHCO₃, 10 mM glutathione (GSH), 3 mM nicotinamide adenine dinucleotide phosphate (NADP) and 4 mM phosphoenolpyruvic acid (PEP). CS activity was assayed using the reagent solution containing 800 mM Tris–HCl (pH 9), 0.8 mM DTNB, 0.8 mM acetyl-CoA and 80 mM oxalic acid (OAA). ACO (cytACO and mitACO) was determined using 2 mM GSH, 800 mM Tris–HCl (pH 7.5), 2 mM NaCl and 4 mM cis-aconitate. NAD-IDH was assayed using the buffer containing HEPES (pH 8.2), 16 mM

nicotinamide adenine dinucleotide (NAD), 4 mM MnSO₄ and 40 mM isocitrate. A total of 3 mL enzyme extract solution, containing 800 mM Tris–HCl (pH 7.5), 200 mM KHCO₃, 40 mM MgCl₂, 10 mM GSH, 3 mM NADH and 4 mM OAA, was used for the NAD-MDH assay. NADP-ME activity was determined using the enzyme extract solution, containing 800 mM Tris–HCl (pH 7.4), 3.4 mM NADP, 4 mM MnSO₄, and 4 mM malate.

The absorption was determined at 412 nm for the CS enzyme or 340 nm for the other enzymes for 120 s, and the values of absorbance were recorded every 10 s. One unit (U) represents the variation of absorbance per minute. The specific activities of enzymes were defined as a U mg⁻¹ pro.

Real-time quantitative PCR analysis of enzyme mRNA

Total RNA was extracted from the frozen tissue using RNA-plant Plus Reagent (Tiangen, Beijing, China) and synthesized into cDNA using HisScript™ Q RT SuperMix kit (Vazyme, Nanjing, China) according to manufacturer. Two-fold diluted cDNA was used for real-time quantitative PCR (qPCR) analysis. qPCR was performed on the Quantstudio 6 flex system (Applied Biosystems, Foster City, CA, USA) using the AceQ qPCR SYBR® Green Master Mix (Vazyme, Nanjing, China). qPCR reactions were set as the followings: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The sequences of all primers used for the qPCR reactions are shown in Table 1. Translation elongation factor 2 (TEF2) was used as the internal control gene. The relative quantification was measured according to the method of 2^{-ΔΔCt}. At least three different cDNAs were used for qPCR analysis, and the experiments were repeated three times for each cDNA.

Table 1 Primers used in the real-time qPCR analysis for the expressions of enzyme genes that catalyze the sugar and organic acid metabolism

Gene name	GeneBank ID	Forward primers (5'–3')	Reverse primers (5'–3')
TEF2	JQ732180.1	GGTGTGACGATGAAGAGTGATG	TGAAGGAGAGGGAAGGTGAAAG
AI	XM_007210252.2	CCCGAGAAATTGAGGCCAGT	AAGGCAGTTCTCTGCCATGA
NI	XM_020555550.1	ATCCTGCTGACAAGACACCG	TGGCAGTCAACCGTCTTCTC
SS1	XM_007204587.2	TTCCTGGGCTTTACCGTGTC	GGGTGGAATGATGTCAGCCT
SS2	XM_007225195.2	CGAACTCGTTGCCCTTCTCT	CTGTTTGGCTTCATCACCGC
SPS1	XM_007204234.2	CAAGGGAGTTGCGAGCAATG	GAAACCCGGATATCGTCGCT
SPS2	XM_007225340.2	GCGTTGGTTTGTAGCAGTGG	GCCGCAGAATTTTCGATCACC
CS	XM_020557490.1	TTCAGGAGTGTAATGCGCT	AGATCTGTGGAGGTTTTCGCT
cytACO	XM_007217082.2	TGGTGTGCTGAGATTGTT	AGTGGCCCTGTCAGCTAATG
mitACO	XM_007203178.2	GTGATTGGGCTGCTAAGGGT	TGGTGTAACGTTCTCACCG
NADP-ME	XM_007222000.2	CCCAACGTCCAGTCTGAAT	CACACGGATTGCTCCAGAGA
NAD-MDH	XM_007227409.2	CCAATGATCGCAAGGGGAGT	AGAGGGAAAGCAGCATCGAC
NAD-IDH	XM_020562633.1	TGCCAGACGGAAAGGATGAG	GCAAAAGCACGGATGGACTC
PEPC	XM_020554552.1	CATGGTCGAGGTGGAACAGT	GTGTAGCTGCTGTGAAACGC

Statistical analysis

All data were calculated using Microsoft Excel 2016 and represented as the mean \pm standard deviation (SD) of at least three independent experiments. Data were analyzed by one-way analysis of variance (ANOVA) and compared by Duncan multiple comparisons at $P < 0.05$ using SAS Software (Version 9.2; SAS Institute; Cary, 2006; USA).

Results

Effects of UV-C on peach quality during the storage

Table 2 shows the changes of peach quality during the storage. Flesh firmness sharply decreased during the storage period, ranging from 21 to 37 N. UV-C irradiation postponed the significant decrease of firmness only at 28 d. UV-C-irradiated peaches showed significant higher SSC than that of untreated controls during the storage period. Non-irradiated peach fruit showed higher TA values than that of irradiated peaches at 14, 21 and 28 days, however, lower than that of irradiated fruit at other time points. A reduction of ascorbic acid was shown during the storage. UV-C treatment could significantly inhibit the decomposition of ascorbic acid compared with the untreated controls for the storage period of 21–35 days. Chilling injury was characterized by low juice yield, high IB index, and electrolyte leakage.

Peaches presented chilling injury in the control group at 14 days with IB index being 0.142 compared with a postponed chilling injury occurred in the UV-C-irradiated group at 21 days with a significantly low value of IB index being 0.042. UV-C irradiation significantly prevented the increment of IB index during the storage period. Electrolyte leakage increased with the increasing storage time, and lower values were founded in UV-C-treated groups compared with the untreated controls during the storage, showing 9.53% lower than that of the untreated ones at the end of storage. Juice yield in the UV-C-treated group was significant higher than that of the control group at 14, 21 and 28 days. The higher juice yield, lower IB index and electrolyte leakage proved that UV-C inhibited chilling injury in peaches.

Effects of UV-C on soluble sugar content

Figure 1 shows the changes of soluble sugars in peach fruit during the storage. Fructose, glucose, and sucrose were the principal soluble sugars in 'Xiahui 6' peach fruit. Fructose increased in both groups during the storage, exhibiting 4.01- and 3.57-fold higher fructose contents in control and UV-C-treated group, respectively, at the end of storage than that of the fruit at harvest (Fig. 1a). Fruit without any treatment exhibited significant higher fructose contents compared with UV-C-treated fruit at 28 and 35 days. A similar change was observed in glucose (Fig. 1b). UV-C significantly suppressed the increment of glucose compared with untreated control

Table 2 Changes of firmness, SSC, TA, ascorbic acid, IB index, electrolyte leakage and juice yield in peach fruit during cold storage at 1 °C

	Firmness (N)	SSC (%)	TA (mg. g ⁻¹)	Ascorbic acid (mg. g ⁻¹)	IB index	Electrolyte leakage (%)	Juice yield (%)
0 day	37.154 \pm 0.791	11.156 \pm 0.786	0.192 \pm 0.005	0.100 \pm 0.003	ND	42.233 \pm 0.597	5.600 \pm 1.221
7 days							
CK	34.831 \pm 1.628a	10.827 \pm 0.636b	0.231 \pm 0.009b	0.094 \pm 0.003a	ND	55.651 \pm 1.592a	8.080 \pm 0.535a
UV-C	31.322 \pm 1.814a	12.561 \pm 0.407a	0.245 \pm 0.002a	0.092 \pm 0.005a	ND	45.794 \pm 0.527b	7.604 \pm 0.415a
14 days							
CK	32.170 \pm 0.802a	11.738 \pm 0.273b	0.248 \pm 0.006a	0.087 \pm 0.001a	0.142 \pm 0.029a	62.272 \pm 1.386a	8.909 \pm 0.199b
UV-C	30.541 \pm 0.360b	12.727 \pm 0.253a	0.238 \pm 0.001b	0.089 \pm 0.003a	ND	50.370 \pm 0.661b	10.740 \pm 0.088a
21 days							
CK	29.214 \pm 0.452a	12.390 \pm 0.149b	0.259 \pm 0.005a	0.075 \pm 0.002b	0.292 \pm 0.029a	66.299 \pm 1.319a	9.504 \pm 0.061b
UV-C	26.018 \pm 1.239a	12.924 \pm 0.130a	0.225 \pm 0.001b	0.083 \pm 0.002a	0.042 \pm 0.014b	58.087 \pm 1.923b	10.949 \pm 0.168a
28 days							
CK	21.700 \pm 0.821b	11.365 \pm 0.575b	0.224 \pm 0.003a	0.065 \pm 0.001b	0.583 \pm 0.052a	75.033 \pm 0.970a	9.321 \pm 0.106b
UV-C	25.256 \pm 0.428a	13.086 \pm 0.730a	0.212 \pm 0.002b	0.074 \pm 0.001a	0.117 \pm 0.014b	67.150 \pm 2.122b	10.978 \pm 0.185a
35 days							
CK	21.053 \pm 0.765a	11.338 \pm 0.167b	0.195 \pm 0.002b	0.058 \pm 0.001b	0.683 \pm 0.014a	77.522 \pm 0.968a	9.331 \pm 0.402b
UV-C	22.748 \pm 0.428a	12.402 \pm 0.335a	0.224 \pm 0.009a	0.065 \pm 0.002a	0.383 \pm 0.029b	67.989 \pm 2.122b	10.215 \pm 0.692b

Data were expressed as the mean \pm standard deviation of at least four experiments. Values with different letters in the same column at the same day indicate significant difference at $P < 0.05$. ND represents IB index being not detected

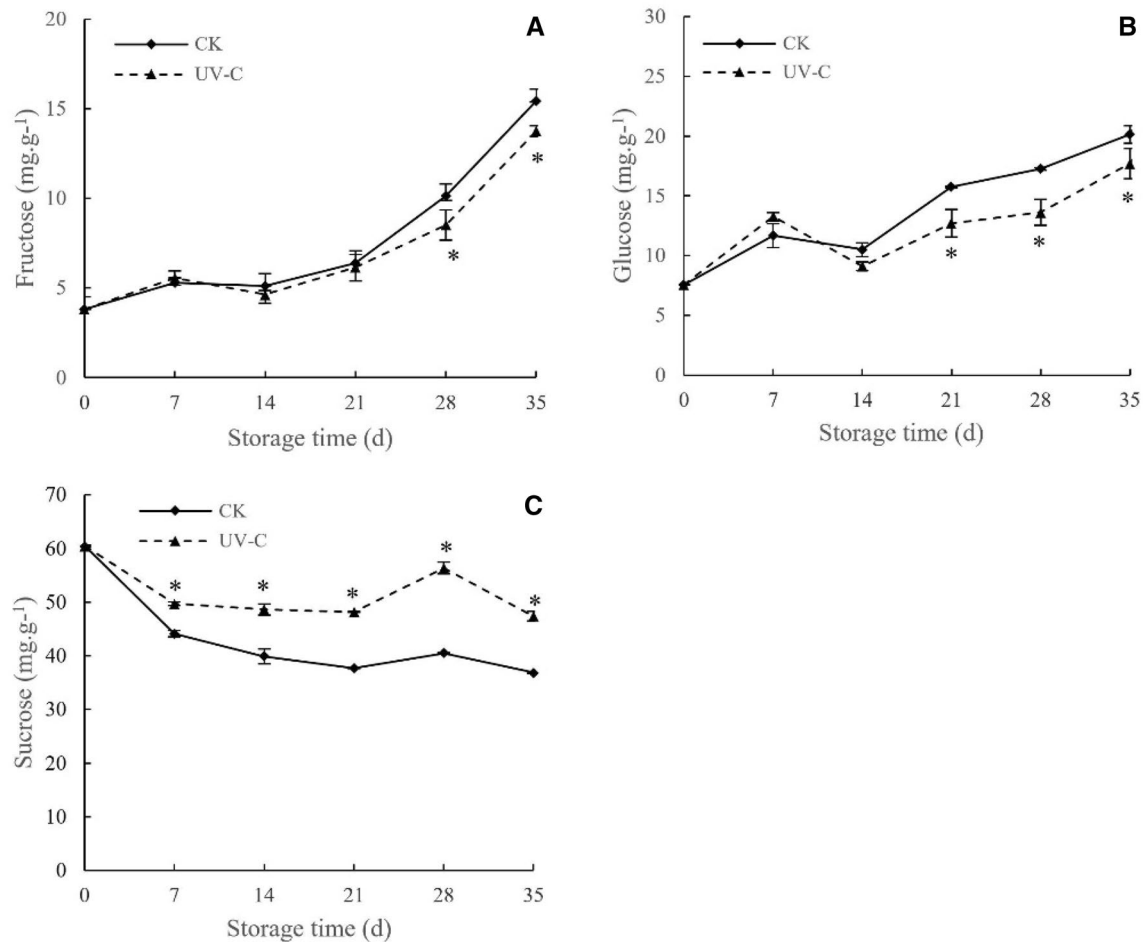


Fig. 1 Effects of UV-C irradiation on soluble sugars in the peach fruit stored at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments. Fructose (a), glucose (b) and sucrose (c)

at 21, 28 and 35 days. Unlike the changes in fructose and glucose, sucrose in both groups decreased during storage time (Fig. 1c). UV-C significantly delayed the degradation of sucrose during the storage. The sucrose was higher in the UV-C-treated group compared with the control group at every time point.

Effects of UV-C on organic acid content

Citric acid, malic acid, shikimic acid, and quinic acid were four main organic acids tested in ‘Xiahui 6’ peach fruit, of which citric and malic acid was the predominant organic acids (Fig. 2). Citric and malic acids showed similar changes during the storage, which significantly declined during the cold storage. UV-C irradiation significantly prevented the degradations of citric and malic acids from the 14 to 35 days of the storage. Peaches irradiated with UV-C showed a significant slowdown in the decline of citric acid compared with the control peaches (Fig. 2a), being 21.7 and 37.8% decrease compared with fruit at harvest, respectively. Malic

acid decreased 18.69% in UV-C-irradiated group compared with 26.82% in the untreated group at the end of storage (Fig. 2b). The shikimic acid in peaches irradiated with UV-C was significant higher than untreated control at the storage days 28 and 35 (Fig. 2c). Quinic acid showed a decreased trend in both control and UV-C-irradiated groups (Fig. 2d). UV-C-irradiated group showed significantly lower levels of quinic acid at 7 and 14 days, and higher levels of quinic acid at 28 and 35 days compared with untreated control.

Enzyme activity changes in AI, NI, SS-synthesis, and SPS

Figure 3a shows the changes of AI activity. AI activity reached a peak in both groups of fruit at 14 days, being 44.19 and 39.74 U mg⁻¹ pro for the control and UV-C group, respectively. Significantly lower levels of AI activity were observed in UV-C-irradiated fruit compared with untreated control from 14 to 28 days of the storage. NI activities increased to the peak value at 21 days in both groups

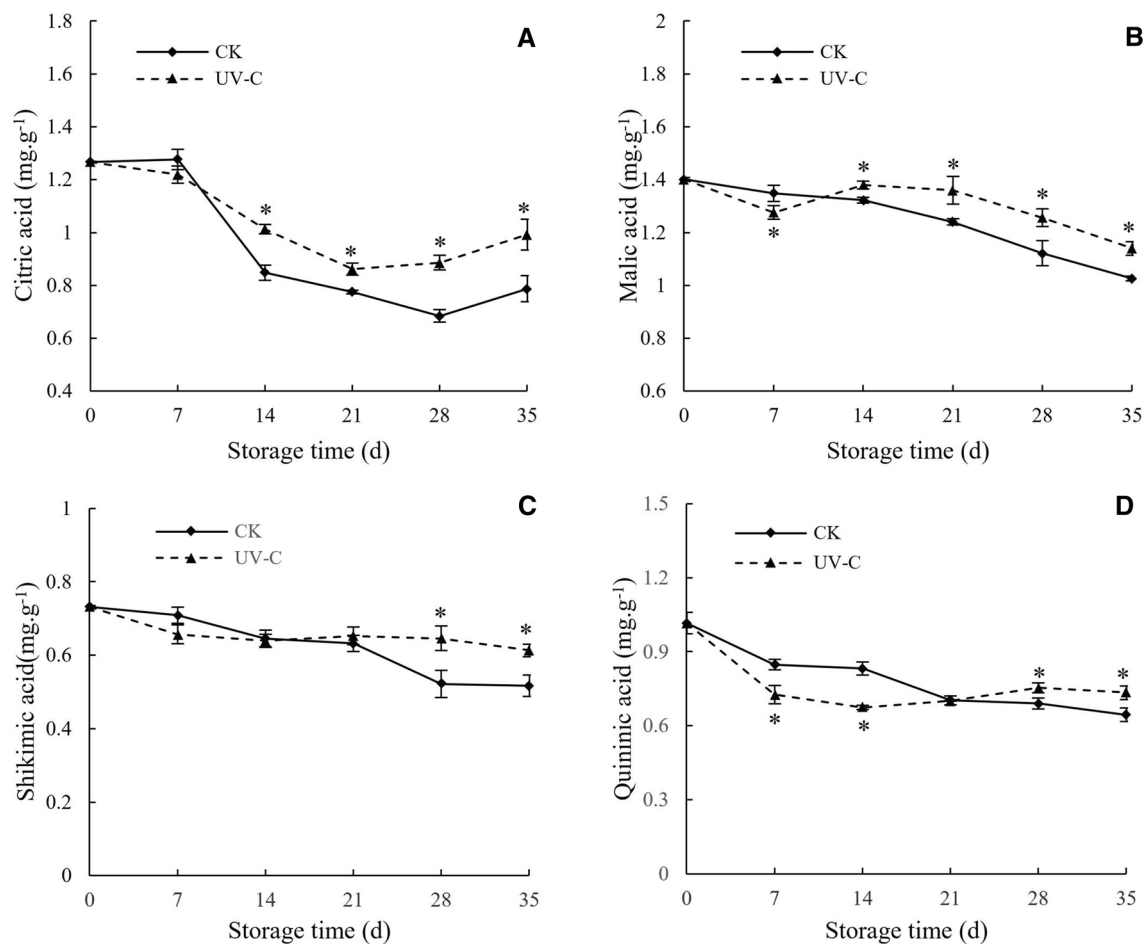


Fig. 2 Effects of UV-C irradiation on organic acids in the peach fruit stored at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments. Citric acid (a), malic acid (b), shikimic acid (c) and quinic acid (d)

(Fig. 3b). UV-C irradiation significantly suppressed the activity during the whole storage, except 28 days. Enzymes of SS-synthesis and SPS contributed to the synthesis of sucrose. SS-synthesis activity was affected by postharvest UV-C irradiation, showing significant higher values compared with untreated control from 7 to 28 days (Fig. 3c). UV-C irradiation showed the lower levels of SPS activity at the days 7 and 14 of the storage and higher levels of SPS activity at 21 and 35 days of the storage compared with the untreated controls (Fig. 3d).

Enzyme activity changes in PEPC, CS, ACO, IDH, MDH and ME

Figure 4 shows the activity changes of the enzymes that catalyze organic acid metabolism. PEPC activities increased to the peak in the non-irradiated controls at day 7 and the UV-C-irradiated peaches at day 14, respectively (Fig. 4a). Peaches without any treatment exhibited higher PEPC activity than that of the treated group at 7 days, but

UV-C-irradiated peaches showed significant higher PEPC activity than that of un-irradiated controls from 14 to 35 days of the storage. Changes of CS activity concerning different treatments are illustrated in Fig. 4b. CS activity declined in both irradiated and non-irradiated controls during the storage. Peaches irradiated with UV-C exhibited significant higher CS activity than that of untreated ones from 14 to 35 days of the storage. Figure 4c shows that cytACO activity declined in both groups of peach fruit during the storage. UV-C-treated peaches demonstrated significantly lower cytACO activity than that of the untreated controls during the storage. Unlike the changes of cytACO, the mitACO activity of the UV-C irradiated peaches was significantly higher at 7 days and lower at 14 days than that of untreated controls (Fig. 4d). NAD-IDH activity was higher in the UV-C-irradiated peaches than that of untreated control fruit at days 7, 14 and 21 of the storage but lower than that of untreated control fruit at days 28 and 35 (Fig. 4e). The enzyme activity of NAD-MDH was significant higher in the UV-C-irradiated peaches than that of untreated ones

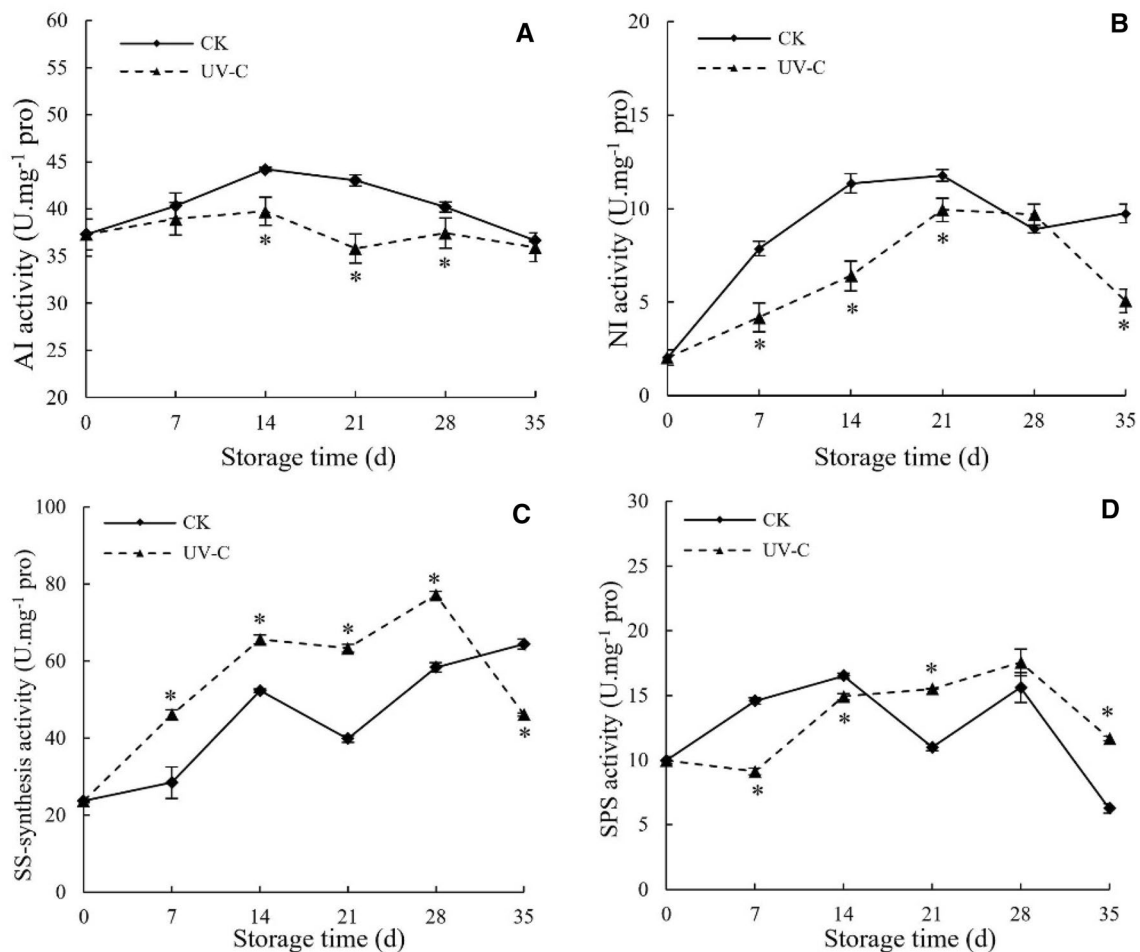


Fig. 3 Changes of AI (a), NI (b), SS-synthesis (c) and SPS (d) in the peach fruit irradiated with UV-C during cold storage at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments

at days 14, 21, and 28 of the storage (Fig. 4f). In contrast, the enzyme activity of NADP-ME was significantly higher in non-irradiated peaches than that of UV-C-irradiated fruit during the storage (Fig. 4g).

Relative gene expression of enzymes that catalyze sucrose metabolism

The mRNA transcript level of *PpAI* reached the peak at days 14 and 28 in the UV-C-irradiated group and untreated group, respectively (Fig. 5a). At day 28, the transcript level in the untreated peaches was 1800-fold higher more than that of the fruit at harvest. UV-C treatment significantly suppressed the expression of *PpAI* from 14 to 35 days of the storage. The transcript level of *PpNI* peaked at day 14 in the untreated control peaches and was 1800-fold greater than that of the fruit at harvest (Fig. 5b). The relative expression of *PpNI* in UV-C-irradiated groups was significantly lower than that of untreated ones from 14 to 28 days of the storage. The transcript profiles of *PpSS* families are shown in Fig. 5c, d. The

transcript level of *PpSS1* peaked at day 14 in the untreated fruit and postponed for a week after the UV-C irradiation. The transcript level of *PpSS1* was significantly lower in the UV-C-irradiated peaches than that of the untreated fruit at days 14 and 28 of the storage. The transcript profile of *PpSS2* was similar in the control group and UV-C-irradiated group. The UV-C irradiation significantly upregulated *PpSS2* gene expression at day 14 of the storage and significantly downregulated this gene expression at days 21 and 28. The transcript level of *PpSPS1* peaked at day 14 in the untreated control peaches and postponed for a week in the UV-C-treated group (Fig. 5e). UV-C irradiation significantly suppressed the relative gene expression of *PpSPS1* at days 14 and 21. The highest transcript level of *PpSPS1* achieved in the control group at day 21 was 400-fold higher than that of *PpSPS1* mRNA in the fruit at harvest. The relative gene expression profile of *PpSPS2* exhibited similar changes in both control and UV-C-irradiated peaches, which peaked at day 14 (Fig. 5f). The expression of *PpSPS2* was significantly

upregulated in the UV-C-irradiated fruit at days 14 and 21 of the storage.

Relative gene expression of enzymes that catalyze the organic acid metabolism

As shown in Fig. 6a, *PpPEPC* transcript level peaked at day 7 of the storage in the untreated control group and postponed for a week in the UV-C-irradiated group. Peaches irradiated with UV-C exhibited significant higher gene expression of *PpPEPC* than that of *PpPEPC* in the untreated ones at days 14, 21 and 28. The relative expressions of *PpCS* decreased in the untreated control peaches during the cold storage (Fig. 6b). UV-C treatment significantly upregulated *PpCS* gene expression compared with untreated control at days 7, 14 and 35. The gene expression profile of *PpPcytACO* was similar in both groups, which peaked at day 14 (Fig. 6c). UV-C irradiation significantly suppressed the gene expression at days 7, 28 and 35. The gene expression of *PpmitACO* peaked at day 14, which was fourfold higher in the untreated control peaches than that of *PpmitACO* in the UV-C-irradiated fruit (Fig. 6d). The UV-C irradiation significantly downregulated the *PpmitACO* gene expression at days 14, 21 and 28 of the storage. The changes of *PpNAD-IDH* gene expression are shown in Fig. 6e. The relative expression of *PpNAD-IDH* peaked at days 14 and 28 in the UV-C-irradiated fruit and the untreated control peaches, respectively. UV-C irradiation significantly upregulated *PpNAD-IDH* during the first 21 days of storage. The relative gene expression profile of *PpNAD-MDH* was similar in both the UV-C-irradiated and non-irradiated groups as shown in Fig. 6f. The gene expression peaked at day 14 in both groups, and the UV-C significantly upregulated this gene expression at days 7 and 14. The transcript profile changes of *PpNADP-ME* are shown in Fig. 6g. The transcript levels of *PpNADP-ME* were significantly downregulated by UV-C irradiation from 21 to 35 days of the storage.

Discussion

Our results have confirmed that UV-C irradiation maintains the qualities of peaches and prevents the loss of firmness during cold storage, exhibiting higher SSC and ascorbic acid content, reducing TA. The previous study by Gonzalez-Aguilar et al. (2004) has verified that peaches irradiated with different UV-C dose showed high chilling tolerance at 5 °C storage. In agreement with the findings, we have demonstrated that peaches irradiated with UV-C (1.5 kJ/m²) present high juice yield, low IB index, and electrolyte leakage. These physiological responses of the peaches have indicated that UV-C irradiation prevents chilling injury of peaches. Other researches have suggested that UV-C irradiation alleviates

chilling injury by maintaining the integrity of cell membrane (Gonzalez-Aguilar et al. 2004; Guan et al. 2016).

In this study, electrolyte leakage in the UV-C-treated peaches has been lower than that of un-irradiated controls, confirming that UV-C irradiation has maintained the integrity of the cell membrane. Similar evidence has been unveiled in banana (Pongprasert et al. 2011), tomato (Liu et al. 2012), and plum (Bal and Celik 2008), which UV-C treatment has inhibited chilling injury of fruits by enhancing the integrity of cell membrane. Oxidative stress is the principal factor causing chilling injury of fruits and in consequence, the responsible factor of cell wall disassembly. Antioxidant capacities, including enzymatic and non-enzymatic antioxidant activities, and alternative oxidase (AOX), are the powerful means to neutralize ROS in fruits (Møller 2001). The relationships between UV-C irradiation and ROS content have been widely verified in bamboo (Zeng et al. 2015), pepper (Vicente et al. 2005) and peach (Gonzalez-Aguilar et al. 2004).

Soluble sugars play an obvious central role in plant glycolysis pathway, which could generate energy charge and respond to numerous stresses. Sucrose contributes to forming the cell membrane, enhancing its integrity to resist chilling stress (Couée et al. 2006). In the present study, we have found that UV-C irradiation significantly inhibits chilling injury of peaches and the degradation of sucrose. The high sucrose content positively protects the integrity of cell membrane in peach fruit. Sugar-regulated enzyme gene expression offers the control of oxidative stress, which eliminates more ROS (Couée et al. 2006). While glucose increases the production of ROS, stimulating the leakage of the cell membrane (Van den Bogart et al. 2007; Lara et al. 2009), in this study, we have found high sucrose and low glucose in the UV-C irradiated peaches which exhibit a high tolerance to the chilling injury of the fruit. Similar findings have been reported in peaches treated with methyl jasmonate and hot air, in which MeJA and hot air have enhanced chilling tolerance by suppressing the degradation of sucrose during cold storage (Yu et al. 2016). Our findings have illustrated that UV-C irradiation has significantly alleviated chilling injury in the peaches during cold storage, likely by suppressing the degradation of sucrose, suggesting that high sucrose and low glucose promote chilling tolerance in the UV-C irradiated peaches.

Organic acids, which participate in various metabolic pathways, are responsible for the production of energy charge and maintain life activities in the plant (Borsani et al. 2009). Citric acid, malic acid, shikimic acid, and quinic acid are four important organic acids in “Xiahui 6” peaches. Shikimic and quinic acid both participate in the shikimate pathway and can be converted into chorismic acid. Chorismic acid plays a pivotal role in the biosynthesis of aromatic amino acids and is involved in phenylalanine and

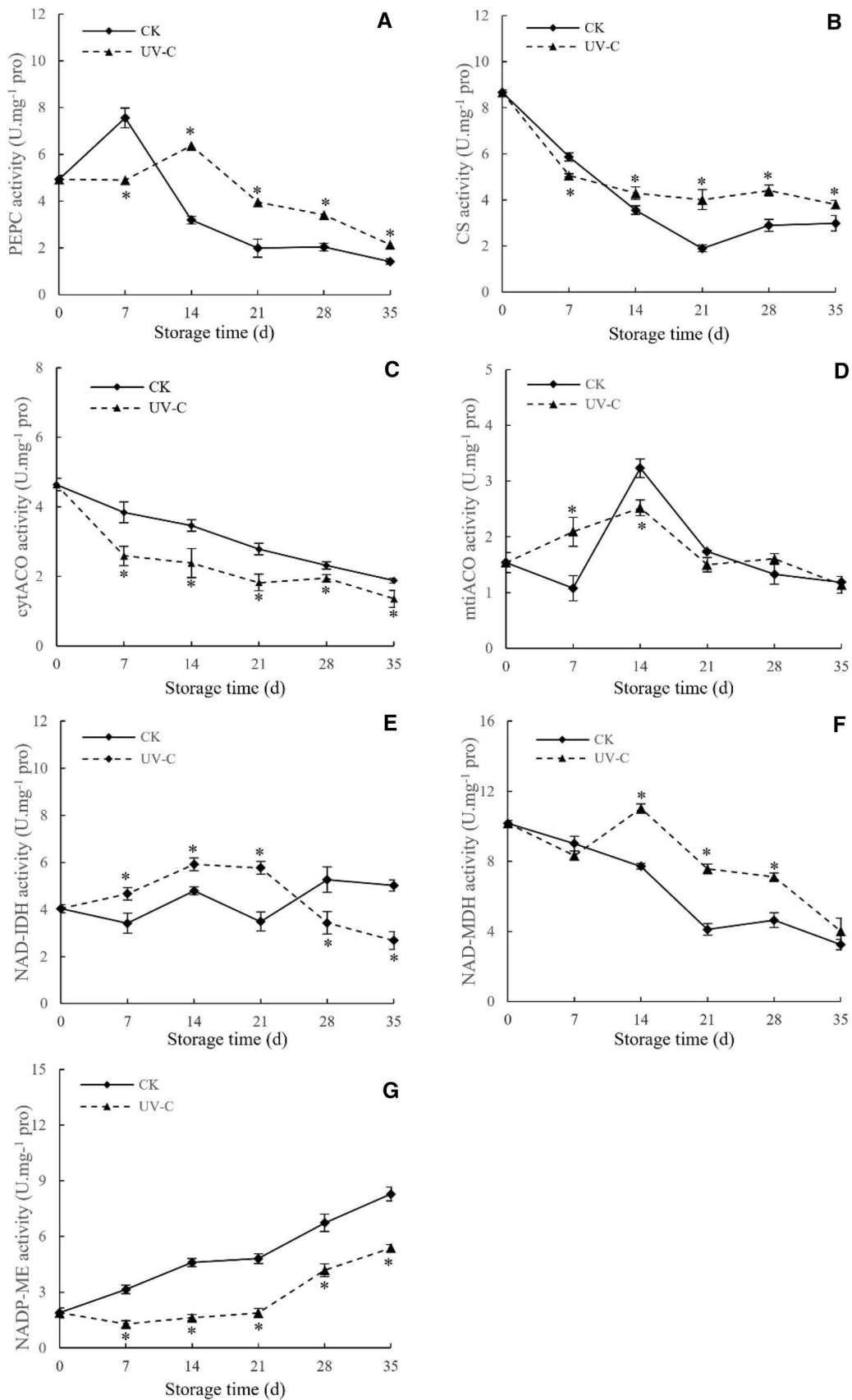


Fig. 4 Enzyme activity changes of PEPC (a), CS (b), cytACO (c), mitACO (d), NAD-IDH (e), NAD-MDH (f) and NADP-ME (g) in the peach fruit irradiated with UV-C during cold storage at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments

phenylpropanoid pathways (Herrmann and Weaver 1999). In this study, UV-C irradiation increases the content of shikimic and quinic acids at late storage time points, and the two organic acids may accelerate phenylpropanoid metabolism, offering a defense response to the chilling injury of peaches. Chen et al. (2012) demonstrated that citrus fruit

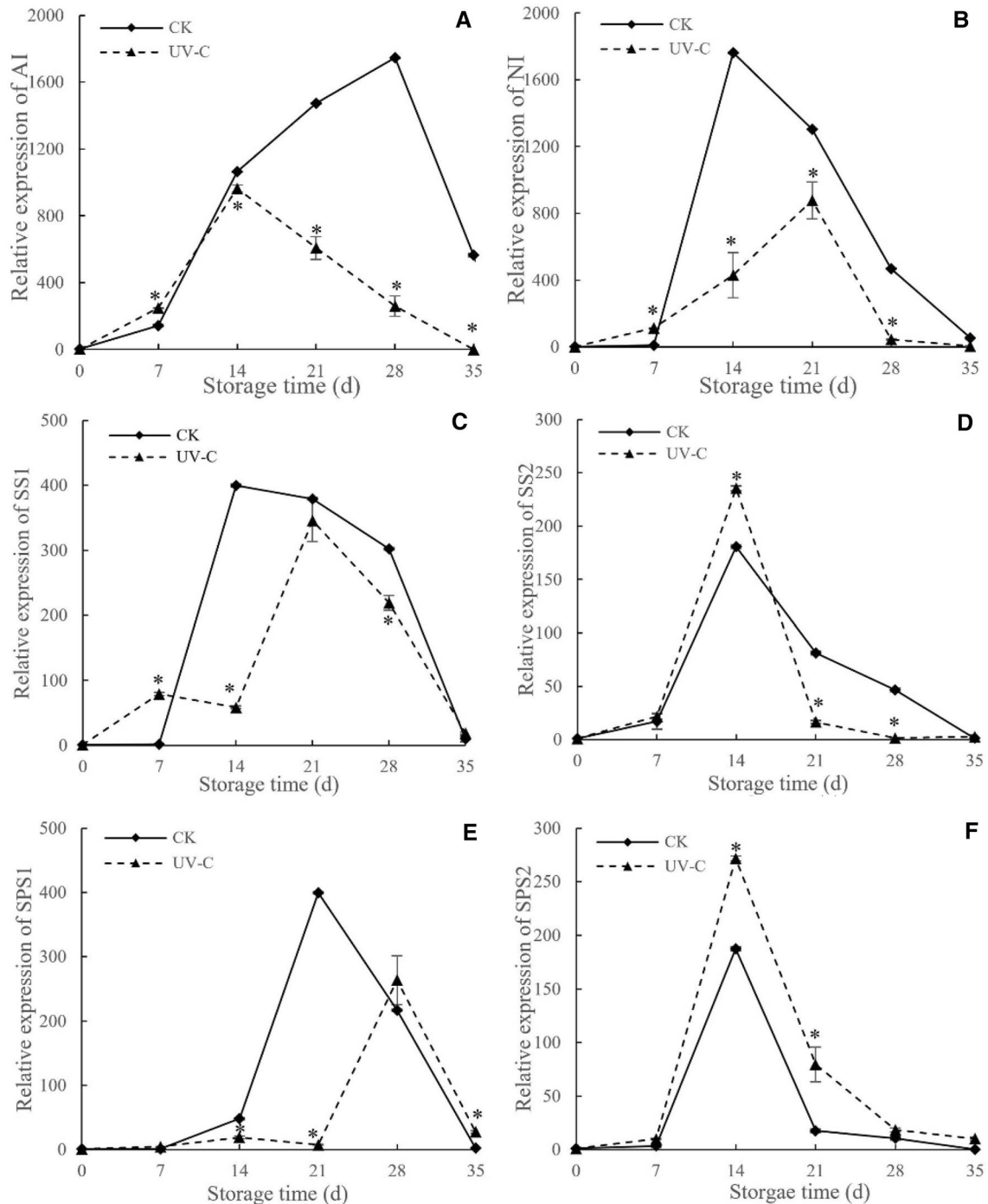


Fig. 5 The relative gene expression of *PpAI* (a), *PpNI* (b), *PpSS1* (c), *PpSS2* (d), *PpSPS1* (e) and *PpSPS2* (f) in the peach fruit irradiated with UV-C during cold storage at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments

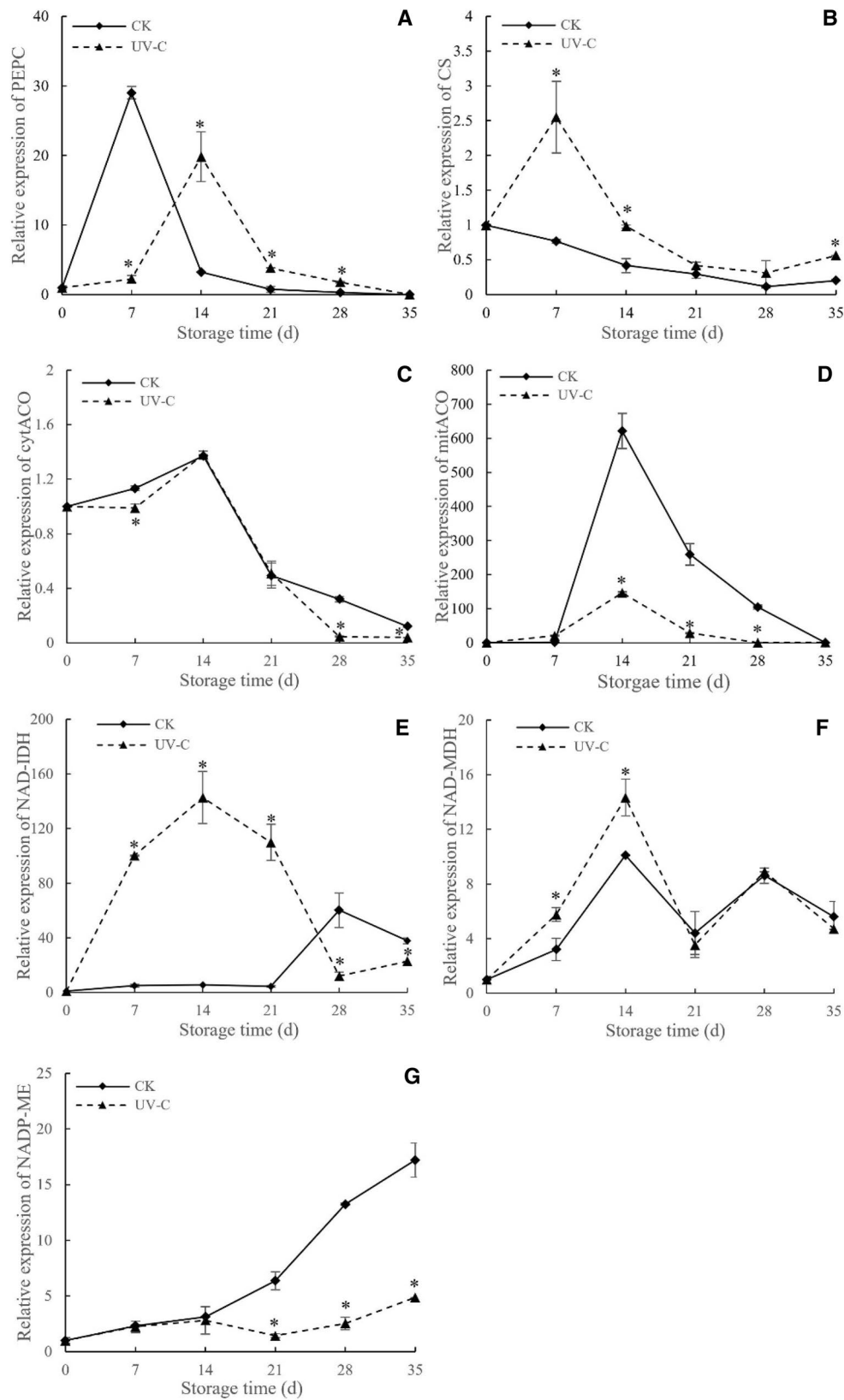


Fig. 6 The relative gene expressions of *Pp*PEPC (a), CS (b), *Pp*cytACO (c), *Pp*mitACO (d), *Pp* NAD-IDH (e), *Pp*NAD-MDH (f) and *Pp*NADH-ME (g) in the peach fruit irradiated with UV-C

during cold storage at 1 °C for 35 days. Data were expressed as the mean \pm SD of four experiments

treated with hot air exhibited reduced levels of malic and citric acids during the storage, and hot air significantly promoted the degradation of citric acid but no significantly effect on malic acid. Lara et al. (2009) demonstrated that peach fruit treated with hot air resulting in an increased level of citric acid and declined level of malic acid during ambient storage. Unlike the above findings, in this study, the four main organic acids all decreased during the cold storage, and the UV-C treatment significantly suppressed the degradation of malic and citric acids. To date, little investigation has been carried out to explore the relationships between organic acids and the chilling injury. In the cultured tobacco cells, Gray et al. (2004) found that exogenous malic and citric acids upregulated the gene expression of *AOXI*, which can enhance the resistance of the chilling injury by scavenging the production of ROS. Bustamante et al. (2016) found that peaches with higher citric and malic acid exhibited higher chilling tolerance during cold storage. The above results suggest that high levels of citric and malic acids might suppress chilling injury. Our results have demonstrated that UV-C-treated peaches have a high level of citric and malic acids which enhances chilling tolerance in peach fruit.

In sucrose metabolism, invertase (AI and NI) is responsible for sucrose decomposition reaction, and sucrose can be synthesized by enzymes of SS and SPS using glucose and fructose (Borsani et al. 2009). Yu et al. (2015) demonstrated that peach fruit with high activities of AI and NI, and low activities of SS and SPS exhibited a significant low level of sucrose. In other cultivars, such as grape and loquat, the accumulation of hexose was due to the high activities of invertase, while the decrease of hexose was mainly due to the notable increase of SPS (Tian et al. 2012; Cao et al. 2013). Consistent with the above results, we found that low activities of AI and NI, and high activities of SS and SPS suppress the degradation of sucrose and the generation of fructose and glucose. The transcript expression of AI, NI, SPS, and SS is in charge of sugar metabolism. Yu et al. (2017) demonstrated that *PpAI* was the vital encoding gene in sugar metabolism of “Zajiao” and “Yulu” peaches, the increment of *PpAI* enhanced AI activities leading to the degradation of sucrose in the peaches during cold storage. While, in “Dixieland” peach, Lara et al. (2009) found that *PpNI3* was the most important encoding gene and responsible for sucrose degradation. In our present study, the transcript levels of *PpAI* and *PpNI* increased to the highest values with a relative expression of more than 1500 times during the cold storage. The UV-C irradiation significantly suppressed the transcript levels of the two invertases. This means that the two invertases probably play a vital role in regulating sucrose decomposition in peach fruit during postharvest storage. In the present report, the enzyme activities and their relative gene expression suggest that UV-C treatment suppressed the degradation of sucrose by inhibiting

the activities and gene expression of AI and NI, rather than SS-synthesis and SPS.

PEPC and CS contributed to the synthesis of citric acids. Citric acid can be decomposed to ketoglutaric acid by ACO and NAD-IDH (Borsani et al. 2009). NADP-ME is responsible for the degradation of malic acid. In contrast, NAD-MDH catalyzes the reversible reaction of malic acid into oxalic acid and the synthesis of malate (Sweetman et al. 2009; Martín-Ezesteso et al. 2011; Yao et al. 2009; Etienne et al. 2013). Li et al. (2018) reported that waxing could postpone the degradations of malic and citric acids in pineapple by regulating the activities of CS, NADP-ME, and NAD-MDH. Chen et al. (2012) demonstrated that HA inhibited the degradation of citric acid by enhancing PEPC and CS, as well as suppressing NAD-IDH and ACO in citrus fruit. In this study, the level of citric acid and the activities of PEPC and CS decreased in both groups. UV-C treatment significantly suppressed the reduction of citric acid by enhancing the activities of PEPC and CS and inhibiting the activity of *cytACO*. The high NAD-MDH activity and low NADP-ME activity both contributed to a high level of malic acid in the UV-C-irradiated peaches during the storage. The transcript levels of *PpmitACO* were relatively higher than those of *PpPEPC* and *PpCS* in this study, which indicated that degradation of citric acid was prior to the synthesis of citric acid during postharvest storage and this was by the decrease of citric acid. *PpNADP-ME* and *PpNAD-MDH* were the major factors to regulate malic acid during postharvest storage (Li et al. 2018). In this study, the transcript level of *PpNADP-ME* sharply increased but *PpNAD-MDH* exhibited a sustainable level during the storage. The increment of *PpNADP-ME*, rather than *PpNAD-MDH*, contributed to malic acid degradation. Therefore, we propose that UV-C irradiation suppressed the degradation of citric and malic acids by mainly enhancing the activities and gene expressions of PEPC, CS, NAD-MDH, as well as inhibiting the activities and gene expressions of *cytACO* and NADP-ME.

In conclusion, UV-C irradiation significantly suppressed the degradation of sucrose, citric and malic acids, resulting in high levels of them in the UV-C-irradiated peaches and further enhancing chilling tolerance in the peaches during cold storage. The present study presents a new relationship among chilling tolerance, sugar and acid metabolism in the UV-C-irradiated peaches, showing that UV-C treatment postponed chilling injury of the fruit by regulating soluble sugar and organic acid metabolism. A proposed regulation among sucrose degradation, organic acid and chilling tolerance in peaches by UV-C irradiation is shown in Fig. 7. ROS metabolism plays a vital role to control chilling injury in peaches. A previous study by Liu et al. (2012) proposed that UV-C irradiation enhanced the antioxidant capacity to eliminate ROS and further maintained the integrity of cell membrane to avoid chilling injury of the fruit during the cold

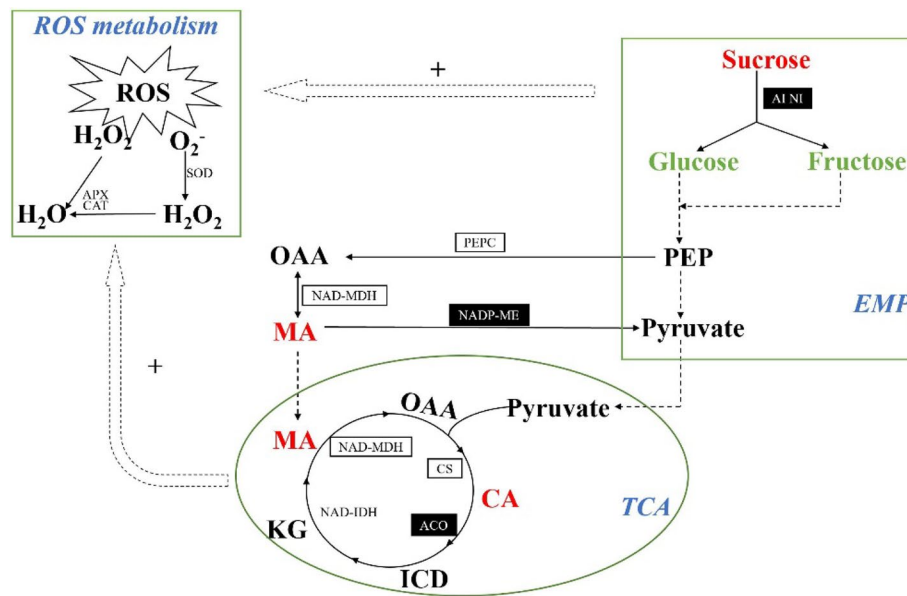


Fig. 7 A proposed model: UV-C suppressed chilling injury by regulating pathways of soluble sugars and organic acids in peach fruits stored at 1 °C. The principal components marked with red color mean that UV-C irradiation suppressed the degradation of these metabolites, while the components inhibited by UV-C irradiation are marked with green color. Enzymes in black with white background indicated

that UV-C irradiation enhanced gene expression and activities of these enzymes, while the ones in white with black background inhibited by UV-C irradiation. “+” means that sucrose, citric and malic acids contribute to the elimination of ROS. *PEP* phosphoenolpyruvic acid, *CA* citric acid, *ICD* isocitric acid, *KG* α -ketoglutaric acid, *MA* malic acid, *OAA* oxalic acid

storage. Sucrose activates antioxidant enzymes to eliminate ROS and glucose promotes the production of H_2O_2 . UV-C radiation inhibits the activities and transcript levels of AI and NI, leading to a relatively high level of sucrose and low level of glucose. Therefore, UV-C treatment enhances the chilling tolerance of the peaches by suppressing the degradation of sucrose. Citric and malic acids contribute to eliminating ROS. The enhanced chilling tolerance of the fruit by the UV-C irradiation might be due to the high contents of citric and malic acids, which is a consequence of induced activities and gene expressions of PEPC, CS, and NAD-MDH as well as inhibition of ACO, and NADP-ME. The cascade of events in multiple metabolic pathways could overlap and occur at the same time point.

Author contribution statement DZ performed the experiments, analyzed the samples and drafted the manuscript; SC contributed to analyze the data; RX contributed to perform the experiments; ST helped revise the article; KT conceived the original research plans, supervised the research and revised the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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