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Effects of postharvest UV-C irradiation on phenolic acids, flavonoids, and key phenylpropanoid pathway genes in tomato fruit

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ABSTRACT

The possibility of increasing the content of phenolic acids and flavonoids, and inducing the key phenylpropanoid pathway genes in tomato fruit by postharvest UV-C irradiation was assessed. Mature-green tomato fruit were irradiated with UV-C at 4 kJ m^{-2} and stored in the dark at 13 °C and 95% RH. UV-C irradiation was effective in increasing the total phenolics content and individual phenolic acids and flavonoids, including cafferic acid, *p*-coumaric acid, trans-ferulic acid, chlorogenic acid, gallic acid, protocatechuic acid, rutin and quercetin. UV-C treatment also induced expression of genes coding for key enzymes in the phenylpropanoid pathway, including *PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H* and *FLS*, and enhanced the activities of PAL, C4H, 4CL, CHS and CHI during storage, which in agreement with a corresponding increase in phenolic compounds content. UV-C irradiation resulted in an increase of the content of phenolic acids and flavonoids in tomato fruit during storage.

1. Introduction

There is convincing epidemiological evidence that diets rich in vegetables and fruits is associated with a lower risk of chronic diseases such as neurodegeneration diseases, cardiovascular diseases, and cancer. These protective effects are partly attributed to phenylpropanoid secondary metabolites, mainly phenolic acids and flavonoids (Crozier et al., 2009; Rio et al., 2013). Several papers describe their attributes as natural and potent antioxidant, anticarcinogenic and other bioactivities (Hertog et al., 1993; Fernández-Panchon et al., 2008). Therefore, increasing the content of phenolic acids and flavonoids in vegetables and fruits by postharvest treatments has potential for enhancing protection of human health and is of great interest to the food industry.

Tomato (*Solanum lycopersicum*) is one of the most widely consumed fresh and processed vegetables worldwide and it could be considered as an important dietary source of antioxidants such as ascorbic acid, phenolic compounds and carotenoids (Martínez-Valverde et al., 2002; Canene-Adams et al., 2005; Periago et al., 2009). Its consumption is a source of several classes of phenolic compounds, mainly phenolic acids and flavonoids (Slimestad et al., 2008; Slimestad and Verheul, 2009). UV-C irradiation, as a postharvest treatment, can not only improve storage potential, but also induce the biosynthesis of phenolic compounds in vegetables and fruits (Shama and Alderson, 2005; Charles and Arul, 2007; Urban et al., 2018). Previous studies have reported that postharvest UV-C irradiation induced phenolic compounds in tomatoes (Jagadeesh et al., 2011; Bravo et al., 2012; Liu et al., 2012; Severo et al., 2015b), grapes (Cantos et al., 2000; Pinto et al., 2016), strawberries (Severo et al., 2015a), blueberries (Wang et al., 2009), and mangos (González-Aguilar et al., 2007).

Despite the evidence supporting the utility of UV-C irradiation as an effective postharvest treatment for increasing phenolic compounds, a comprehensive and systematic study of phenylpropanoid metabolism has not yet been conducted. To the best of our knowledge, only Pinto et al. (2016) studied the effects of postharvest UV-C irradiation on the expression of the phenolic compounds biosynthetic genes in postharvest grapes. Relatively little is known about the underlying physiological and molecular mechanisms inducing the responses, and how gene expression influences the regulation of the phenylpropanoid pathway in tomato fruit after UV-C irradiation. Therefore, the current study was aimed to further explore the physiological and molecular mechanisms of the effects of postharvest UV-C irradiation on accumulation of the predominant phenolic acids and flavonoids in tomato fruit through analysis of the gene expression and enzymatic activities involved in phenylpropanoid pathway.

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2. Materials and methods

2.1. Chemicals

Protocatechuic acid, *p*-coumaric acids, caffeic acid, trans-ferulic acid, chlorogenic acid, gallic acid, rutin, naringenin, quercetin, Folin-Ciocalteu reagent, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

2.2. Sample preparation, treatment and storage

Mature-green tomato fruit (*Solanum lycopersicum*, cv. Wanza 15) were harvested from a commercial greenhouse in Hefei, China. After harvest, fruit of uniform color and size and free from apparent defects and blemishes were selected, and immediately transported to our laboratory. The tomato fruit were rinsed in tap water, air-dried at ambient temperature, and then irradiated immediately.

UV-C irradiation was provided by two UV-C lamp tubes (30 W/G30T8 with spectral peak at 254 nm, Philips). Tomato fruit were subjected to postharvest UV-C irradiation at 4 kJ m^{-2}, which was found to be the most effective in maintaining postharvest tomato fruit quality and increasing total phenolic contents in preliminary investigation (Liu et al., 2012). To achieve the total dose, 6 min of exposure were required on each of the two sides of the fruit, totaling 12 min of treatment. The untreated tomato fruit were used as the control. Following treatments, both untreated and UV-C treated fruit were stored in the dark at 13 °C and 95% RH for 35 days. Eight fruit samples were randomly taken from the control and UV-C treated groups at 0, 7, 14, 21, 28, or 35 days after treatment. The pericarp of the sampled fruit was cut into small pieces, frozen in liquid nitrogen immediately, and then stored at −80 °C.

2.3. Determination of total phenolics content

The total phenolic content of tomato fruit was determined by the Folin-Ciocalteu's method according to Toor and Savage (2005). An aliquot (40 μL) of fruit extracts was added to 1.0 mL of Folin-Ciocalteu reagent (diluted in 1:10 ratio). Subsequently, the mixture was shaken vigorously and left to stand for 6 min, before addition of 0.8 mL of 7.5% Na_2CO_3 solution. After incubation for 30 min in the dark at 25 °C, the absorbance was recorded at 765 nm. The results were expressed as gallic acid equivalents (GAE) on a fresh weight basis (mg kg^{-1}).

2.4. Quantification of phenolic acids and flavonoids

Individual phenolic acids and flavonoids were analyzed using HPLC-ESI-MS (Thermo Fisher, HPLC-LTQ Orbitrap XL ETD) according to the method of Vallverdú-Queralt et al. (2011) with slight modifications. The optimal operation parameters were experimentally determined as follows: source voltage, 4 kV; scanning range, 50–1000 *m/z*; sheath gas, 30 (arbitrary units); auxiliary gas, 10 (arbitrary units); and capillary temperature, 275 °C. The liquid chromatograph was equipped with a photodiode array detector (PDA), a quaternary pump and a thermostated autosampler. The separation was performed on a Hypersil Gold C18 column (150 mm × 2.1 mm, 3 μm particle size) at a flow rate of 0.3 mL min^{-1} and injection volume was 10 μL. The mobile phase A (water/0.1% formic acid) and mobile phase B (acetonitrile) using a linear gradient program as follows: from 5 to 18% B (10 min), from 18 to 98% B (5 min), 98% isocratic (15 min), from 98 to 5% (1 min), 5% B (9 min). The column was equilibrated for 5 min prior to each analysis.

2.5. Determination of enzymatic activities

Phenylalanine ammonia lyase (PAL) activity was measured by using the method described by Løvdal et al. (2010). The reaction mixture was 3 mL of 100 mmol L^{-1} Tris-HCl buffer (pH 8.8), 0.5 mL of 100 mmol L^{-1} L-phenylalanine and 0.5 mL of enzyme extract. The reaction mixture was incubated at 37 °C for 1 h, and then 50 μL of 5 mol L^{-1} HCl was added to the mixture to terminate the reaction. After that, the absorbance of the sample was measured at 290 nm. The enzyme activity was expressed as unit mg^{-1} of protein, where 1 unit was expressed as the increase rate of absorbency per mass of protein per hour. Cinnamic acid 4-hydroxylase (C4H) activity was measured by a modified method of Sánchez-Rodríguez et al. (2011). The enzyme extract was added to 2.97 mL of reaction buffer (50 mmol L^{-1} phosphate buffer containing 2 mmol L^{-1} trans-cinnamic acid, 0.5 mmol L^{-1} NADPH, and 2 mmol L^{-1} 2-mercaptoethanol), which was incubated at 37 °C for 1 h. The reaction was terminated with addition of 6 mol L^{-1} HCl and then the absorbance of the sample was measured at 340 nm. 4-coumarate-CoA ligase (4CL) activity was determined using caffeic acid as the preferred phenolic substrate (Sánchez-Rodríguez et al., 2011). The reaction mixture was 5 μmol L^{-1} *p*-coumaric acid, 1 mmol L^{-1} CoA-SH, 50 μmol L^{-1} ATP, and 15 mmol L^{-1} $MgSO_4$. The reaction mixture was incubated for 30 min at 40 °C and then the absorbance of the sample was measured at 333 nm. Chalcone synthase (CHS) activity was measured by an enzyme-linked immunoassay (ELISA) using the CHS assay kit (GE Healthcare) according to the manufacturer instructions. Chalcone isomerase (CHI) was measured using the method described by Lister et al. (1996). The CHI activity was measured using the initial rate of disappearance of the chalcone (ΔA_{381}) in the presence of enzyme. The protein content was determined by the Lowry method using bovine serum albumin as the standard (Bradford, 1976).

2.6. RNA extraction and real-time quantitative PCR

The tomato pericarp samples stored at −80 °C were milled using liquid nitrogen, and total mRNA was extracted using RNAiso Plus (TaKaRa Biotechnology Co., Ltd) according to the instruction of the manufacturer. About 100 ng of mRNA was used for cDNA synthesis using PrimerScript RT reagents Kit with gDNA Eraser (TaKaRa Biotechnology Co., Ltd) according to the instruction of manufacturer. Real-time quantitative PCR was performed using the cDNA and the primers used were presented in Table 1. Real-time quantitative PCR was performed using SYBR® Premix Ex Taq™ (Tli RNaseH Plus; TaKaRa Biotechnology Co., Ltd) in a LightCycler® 480 Real-time PCR system (Roche Diagnostics, Switzerland). The PCR conditions were as follows: 95 °C for 3 min followed by 40 cycles of 95 °C for 7 s, 57 °C for 10 s, and 72 °C for 15 s. The relative expression level was calculated using the $2^{-\Delta\Delta C}$T method (Schmittgen and Livak, 2008), using β-actin (Pirrello et al., 2006; Severo et al., 2015b) as an internal standard (not affected by UV-C, fruit growth and development) and control fruit for calibration.

Table 1
Primers used in real-time quantitative PCR reactions.

Genes	Primer sequences (5' → 3')
Phenylalanine ammonia lyase (PAL5)	F: TTTCTCCATTACAAATCAAACCA
	R: TTCACTTCATCCAAATGACTCC
Cinnamic acid 4-hydroxylase (C4H)	F: CAGGGAAGGGTCAAGATA
	R: CAATCCCATTTGTAGCAG
4-coumarate-CoA ligase (4CL)	F: TCGGAGTTTGAGGAGAGGACA
	R: CTGGCAGATGAGATACGTTTCG
Chalcone synthase (CHS2)	F: GTGGGACCGTTATCCGACTG
	R: CATTGCGGCTGCCCTATC
Chalcone isomerase (CHI)	F: TACTCTGCAGGGCCTTCATC
	R: GGATGTCCCGAACTTCTCCT
Flavanone 3-hydroxylase (F3H)	F: CCATTCGTTTAACTGGGCTT
	R: GGATCAGCCCGTTGTAAAGT
Flavonol synthase (FLS)	F: TAAGATTTGGCCTCCTCCTG
	R: ACCAAGCCCAAGTGATAAGC
β-actin	F: TGGTCGGAATGGGACAGAAG
	R: CTCAGTCAGGAGAACAGGGT