Postharvest Biology and Technology 129 (2017) 90–96

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Increased temperature elicits higher phenolic accumulation in fresh-cut pitaya fruit

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ARTICLE INFO

Keywords:
Fresh-cut pitaya fruit
Storage temperature
Phenolics
Reactive oxygen species
Antioxidant activity

ABSTRACT

The effect of temperature on phenolic accumulation in fresh-cut pitaya fruit was reported during storage at 5, 10 and 15 °C. Fresh-cut pitaya fruit were treated with ROS production inhibitor (diphenyliodonium iodide, DPI) or elicitor (glucose/glucose oxidase, G/GO) and the total soluble phenolics (TSP) content, antioxidant activity (AOX), phenylalanine ammonia lyase (PAL) activity, as well as ROS production and antioxidant enzymes activity were determined. Results showed that higher storage temperature promoted the production of ROS, enhanced the accumulation of TSP and improved the AOX of fresh-cut pitaya fruit. Moreover, treatment with DPI dramatically inhibited ROS generation, PAL activity and TSP accumulation, while G/GO treatment significantly increased ROS level, PAL activity and TSP accumulation. These results demonstrate that phenolics accumulate with increasing temperature and confirm previous report of ROS effects on wound induced phenolic accumulation in pitaya fruit.

1. Introduction

In the process of fresh-cut fruits and vegetables production, the tissue is inevitably subjected to wounding stress, which will elicit wounding responses to produce more secondary metabolites at the injured site or site adjacent to defend and heal the wounding damage (Ryan, 2000; Cisneros-Zevallos, 2003; Rakwal and Agrawal, 2003). Many of the secondary metabolites including phenolics have high antioxidant activity which would help reduce chronic diseases such as obesity (Thom, 2007). Many studies reported significant increases in phenolics content and antioxidant activity after cut processing in various fresh-cut fruits and vegetables including broccoli (Martinez-Hernandez et al., 2013), carrot (Surjadinata and Cisneros-Zevallos, 2012; Torres-Contreras et al., 2014; Han et al., 2017), celery (Vina and Chaves, 2006), lettuce (Zhan et al., 2012), mangoes (Robles-Sanchez et al., 2013), mushroom (Oms-Oliu et al., 2010) and Welsh onion (Han et al., 2016). Therefore, the accumulation of phenolic antioxidants may improve the functional value of these fresh-cut products.

Previous studies suggested that reactive oxygen species (ROS) may act as signaling molecules in plant response to wounding stress and play an important role in the wound-induced phenolic accumulation in fresh-cut carrots, whereas ethylene and jasmonic acid are essential to modulate ROS levels (Jacobo-Velazquez et al., 2011, 2015). More recently, Han et al. (2017) found that both increased wounding intensity and higher storage temperature promoted the production of ROS, enhanced the accumulation of phenolic compounds and improved the antioxidant capacity of wounded carrots, confirming that ROS are essential for mediating wound-induced phenolic accumulation in carrots. However, if ROS are involved in wound-induced phenolic accumulation in other fresh-cut fruits and vegetables is still unknown.

For many years, the demand for fresh-cut products has been increasing steadily due to their convenient, freshness and healthy characteristics. Fresh-cut pitaya fruit have appeared in supermarket in recent years and the consumption of this product has been increasing, mainly because of its unique flavor and healthy benefits owing to its high levels of minerals, vitamins, dietary fiber and phenolic antioxidants (Beltran-Orozco et al., 2009). Our previous study (Li et al., 2017) showed that wounding stress caused by cutting could promote the production of ROS, activate the phenylpropanoid pathway, induce the accumulation of phenolics and enhance the antioxidant activity of fresh-cut pitaya fruit. Moreover, we found that the production of ROS and the accumulation of phenolics increased with the increase of cutting wound intensity, which suggested that ROS may also play a role in the wound-induced phenolic accumulation in fresh-cut pitaya fruit. In this study the physiological role of ROS in wound-induced phenolic accumulation is confirmed and novel generated information on phenolic antioxidant accumulation with increasing temperature is presented for fresh-cut pitaya fruit.

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http://dx.doi.org/10.1016/j.postharvbio.2017.03.014
Received 24 November 2016; Received in revised form 22 March 2017; Accepted 22 March 2017

2. Materials and methods

2.1. Sample processing and storage studies

Pitaya fruit (*Hylocereus undatus* cv. Shuijing) were obtained from a local wholesale market in Nanjing, China. All pitayas were selected and sterilized with chlorinated water (200 μL L^{-1}, pH 6.5). Whole pitaya fruit were peeled manually, cut into quarter-slice (1/4 section from a slice of 1 cm thickness) with a sharp stainless steel knife as described previously (Li et al., 2017).

There were two independent experiments in the present study. In the first experiment, quarter-sliced pitaya fruit were stored for 7 d at 5 °C, 4 d at 10 °C and 2 d at 15 °C to ensure the visual and safety quality of the produce. Fruit quality parameters including total soluble solids (TSS), titratable acidity (TA), Vitamin C and flesh color, total soluble phenolics (TSP) content, antioxidant activity (AOX), ROS production as well as the activity of such antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were evaluated during the storage period. Each treatment was replicated three times, and the experiment was conducted twice.

In the second experiment, ROS production elicitor or inhibitor was applied to evaluate the role of ROS on phenolic accumulation in fresh-cut pitaya fruit. The quarter-sliced pitaya fruit were dipped in the following solutions for 3 min. (1) glucose/glucose oxidase (G/GO, ROS elicitor): 0.5 U mL^{-1} of glucose oxidase in 100 μM glucose solution; (2) 100 μM of glucose as control to G/GO treatment; (3) 300 μM of diphenyliodonium iodide (DPI, ROS inhibitor); (4) water as control to DPI treatment. All the treated samples were air dried for about 30 min and stored at 15 °C for 48 h. Samples were taken every 12 h during storage, frozen in liquid nitrogen and stored at −80 °C for measurements of ROS levels, TSP content and PAL activity. Each treatment was replicated three times, and the experiment was conducted twice.

2.2. Fruit quality parameters analysis

TSS, expressed as percentage (%), was determined by an Abbe refractometer (14081 S/N, USA). TA, expressed as the percentage of malic acid, was analyzed by titrating 20 mL of pitaya juice to pH 8.2 with 0.1 M NaOH. The Vitamin C content, expressed as g kg^{-1} based on fresh weight, was measured by the method of Arakawa et al. (1981). Flesh color parameter values of L^* (lightness), a^* (red-green) and b^* (yellow-blue) were measured with a colorimeter (Konica minolta, Japan). These values were used to calculate the browning index (BI) using the following equations according to Cliffe-Byrnes and O'Beirne (2008).

$$BI = 100\ (x - 0.31)/0.17$$

$$x = (a^* - 1.75 \times L^*)/(5.645 \times L^*) + [a^* - (3.012 \times b^*)]$$

2.3. Total soluble phenolics (TSP) content determination

The content of TSP was analyzed based on the Folin-Phenol method of Swain and Hillis (1959) with minor changes. Frozen samples (2 g) were extracted in methanol (5 mL). The homogenates were centrifuged at 12,000 × *g* for 20 min after 12 h of extract in darkness at 4 °C. The supernatants were prepared for the TSP analysis. Mixtures of 0.1 mL extract supernatant, 0.1 mL of distilled water, 1 mL of Folin-Ciocalteu and 0.8 mL 7.5% (w/v) of Na_2CO_3 were incubated in darkness at 30 °C for 2 h and then the absorbance at 765 nm was determined. The content of TSP was expressed as g kg^{-1} of GAE on a fresh weight basis.

2.4. Antioxidant activity (AOX) measurement

The antioxidant activity was evaluated by measuring the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity according to the procedure of Brand-Williams et al. (1995). The methanol extract supernatant (0.15 mL) was added into 0.12 mM of DPPH solution (2.85 mL) and the mixture was incubated in darkness at 25 °C for 30 min. Methanol instead of supernatant was used as control and the absorbance at 515 nm was measured. Results were calculated with the following formula according to Gorinstein et al. (2004):

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 and A_1 are the absorbance of the control and the sample, respectively.

2.5. Phenylalanine ammonia lyase (PAL) enzyme activity assay

PAL activity was analyzed using the method of Ke and Saltveit (1986). Frozen samples (1 g) were extracted in 5 mL of ice-cold borate buffer (50 mM, pH 8.5), which contained 40 g L^{-1} of PVPP, 5 mM of β-mercaptoethanol and 2 mM of EDTA. The obtained extract supernatant was used for enzyme analysis. A mixture of 50 mM of borate buffer (2.8 mL) and 20 mM of L-phenylalanine (0.5 mL) was incubated at 37 °C for 10 min, and 0.7 mL of the enzyme extraction was added into the reaction system and the absorbance at 290 nm was determined promptly (OD_0). The mixture was incubated for another 1 h and absorbance was measured (OD_1). A unit of PAL activity was equivalent to a variation of 0.1 at 290 nm per second and was expressed as U kg^{-1} based on protein content.

2.6. O_2^- and H_2O_2 measurements

The production of O_2^- was measured according to the method of Elstner (1976). Samples (1 g) were extracted with 5 mL of phosphate buffer (100 mM, pH 7.8), the obtained homogenates were centrifuged at 12,000 × *g* at 4 °C for 20 min. Extract supernatants were prepared for O_2^- determination. The O_2^- production rate was calculated based on a standard curve and expressed as μmol kg^{-1} s^{-1} NO_2 based on protein content. For H_2O_2, frozen tissue (2 g) was extracted in ice cold acetone (5 mL), the measurement was based on the method of Patterson et al. (1984). The level of H_2O_2 was indicated as mmol kg^{-1} based on fresh weight.

2.7. Antioxidant enzymes measurements

The extraction of antioxidant enzymes was carried out according to Kang and Saltveit (2001) and the extract supernatant was used for measurements of enzyme activity.

SOD activity was analyzed according to the method of Rao et al. (1996). A unit of SOD activity was calculated as the quantity of enzyme which causes 50% of nitroblue tetrazolium reduction per second and expressed as U kg^{-1} based on protein content.

CAT activity was determined using the method of Maehly and Chance (1954). A unit of CAT activity was calculated as the quantity of enzyme that deoxidized 1 μmol of H_2O_2 per second and expressed as U kg^{-1} based on protein content.

APX activity was measured according to the method of Chen and Asada (1989). A unit of APX activity was calculated as the quantity of enzyme which oxidized 1 μmol of ascorbate per second and expressed as U kg^{-1} based on protein content.

The protein contents in enzyme extracts were measured according to the method of Bradford (1976), bovine serum albumin was used as the standard.

2.8. Statistical analysis

Experiments were performed using a completely randomized design and each treatment was replicated three times. All data were expressed