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The effect of temperature on phenolic content in wounded carrots

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ABSTRACT

Reactive oxygen species (ROS) have been shown to play important roles in biosynthesis of phenolic antioxidants in wounded carrots. This study has gone further to understand the effects of storage temperature on phenolics accumulation in wounded carrots. The results indicated that both increased wounding intensity and higher storage temperature promoted the generation of ROS and enhanced phenolics accumulation in wounded carrots. Moreover, treatment with ROS inhibitor inhibited ROS generation, suppressed the activities of key enzymes in phenylpropanoid pathway (phenylalanine ammonia lyase, PAL; cinnamate-4-hydroxylase, C4H; 4-coumarate coenzyme A ligase, 4CL) and restrained phenolics accumulation in shredded carrots confirming previous reports. In contrast, treatment with ROS elicitor promoted ROS generation, enhanced the activities of PAL, C4H and 4CL, and induced phenolics accumulation. Thus, our results confirmed that ROS are essential for mediating wound-induced phenolics accumulation in carrots and suggested that increase temperature enhanced the accumulation of phenolics through inducing ROS generation.

1. Introduction

Wounding is one of the primary stresses experienced by fresh-cut produce, which will cause some physiological and biochemical changes to the tissue (Hodges & Toivonen, 2008). When wounding stress occurs, plants can adjust their metabolism to heal the damaged tissues and activate defense mechanisms that prevent further damage (León, Rojo, & Sánchez-Serrano, 2001). As part of defense-related responses, many phenylpropanoid compounds are synthesized by triggering phenylalanine ammonium lyase (PAL) activity. The phenomena of wound-induced accumulation of phenolic compounds have been reported in many fruits and vegetables, including lettuce (Campos-Vargas & Saltveit, 2002), lemon (Artés-Hernández, Rivera-Cabrera, & Kader, 2007), carrot (Surjadinata & Cisneros-Zevallos, 2012), and potato (Torres-Contreras, Nair, Cisneros-Zevallos, & Jacobo-Velázquez, 2014). Thus, wounding has been suggested as an innovative and simple tool to produce more phenolic compounds in horticultural crops (Jacobo-Velázquez & Cisneros-Zevallos, 2012). Among these products, carrot is receiving considerable attention because of its remarkable accumulation of phenolic compounds after wounding and has been used as an ideal material to study the effect of additional stresses on wound-induced production of phenolic compounds and to elucidate the possible regulation mechanisms (Becerra-Moreno et al., 2015; Jacobo-Velázquez & Cisneros-Zevallos, 2012).

Since Babic, Amiot, Nguyen-The, and Aubert (1993) first reported that chlorogenic acid accumulated in shredded carrots during storage, studies on the regulation of phenolic content in carrot tissues have been continued in the past two decades. For instance, Surjadinata and Cisneros-Zevallos (2012) quantitatively compared the total soluble phenolic (TSP) content of slices, pies, and shreds, and found that increasing wounding intensity was a feasible method to enhance the biosynthesis of phenolic antioxidants in carrots. Besides, wounding in combination with other postharvest abiotic stresses, such as hormone, controlled atmospheres, heat shock, hyperoxia stress, UV radiation, herbicide and water stress, can synergistically increase the accumulation of phenolic antioxidants in carrot (Alegria et al., 2012; Becerra-Moreno, Benavides, Cisneros-Zevallos, & Jacobo-Velázquez, 2012; Becerra-Moreno et al., 2015; Du, Avena-Bustillos, Breksa, & McHugh, 2012; Heredia & Cisneros-Zevallos, 2009; Jacobo-Velázquez &

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Cisneros-Zevallos, 2012; Jacobo-Velázquez, Martinez-Hernandez, Rodriguez, Cao, & Cisneros-Zevallos, 2011; Simões, Allende, Tudela, Puschmann, & Gil, 2011). However, little is known about the effect of storage temperature on phenolics accumulation in wounded carrots.

Reactive oxygen species (ROS), mainly superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$), are constantly generated in plant tissues in response to wounding and have been suggested to play important roles in mediating defense-related responses (Orozco-Cárdenas & Ryan, 1999). It has been shown that a wide range of environmental stresses such as high and low temperature, drought, salinity, UV or ozone stress and pathogen infections, can all contribute to enhancing ROS generation in plants (Breusegem, Vranová, Dat, & Inzé, 2001). Jacobo-Velázquez et al. (2011) found that ROS can act as signaling-molecules for the wound-induced accumulation of phenolic compounds in carrot. More recently they demonstrated that ROS play the central role on the wound-induced accumulation of phenolic compounds in carrot, whereas ethylene and jasmonic acid are essential to modulate ROS levels (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015). However, the effect of temperature on ROS levels and their relation to phenolics accumulation in wounded carrots is still unclear. The major objective of this study was to understand the effect of storage temperature on phenolics accumulation in wounded carrots. Therefore, we applied three different cutting styles and three storage temperatures to investigate the influences of wounding and temperature on ROS production, TSP content and antioxidant activity of wounded carrots.

2. Materials and methods

2.1. Chemical reagent

Methanol was purchased from Guangdong Guanghua Sci-Tech CO., Ltd. (Guangdong, China). Acetone was purchased from Shanghai Lingfeng Chemical Regent CO., Ltd. (Shanghai, China). Diphenyliodonium iodide (DPI) was purchased from Tokyo Chemical Industry CO., Ltd. (Tokyo, JAPAN). Nitrotetrazolium blue chloride (NBT) and L-Phenylalanine were purchased from Shanghai Ryon Biological Technology CO., Ltd. (Shanghai, China). Folin–Ciocalteu reagent, ascorbic acid and glucose oxidase were purchased from Beijing Solarbio Science & Technology CO., Ltd. (Beijing, China). Glucose, hydroxyammonium chloride, p-aminophenylsulfonic acid, and α-naphthylamine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used in this study were obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals were of reagent grade.

2.2. Plant material, treatment and storage

Fresh carrots (*Daucus carota* L. cv. Sanhongliucun) were purchased from a local wholesale market in Nanjing, P.R. China. After transferred to the laboratory, all carrots were washed and selected for uniform size (20–24 cm in length, 2.5–3 cm in diameter of equator), color, firmness, shape and free from blemishes. The selected carrots were conditioned at 10 °C overnight prior to two independent experiments.

In the first experiment, wounding treatment was carried out according to the method of Surjadinata and Cisneros-Zevallos (2012). Carrots from each group were cut into slices, pies and shreds to create different wounding intensities. The wounding intensity of each cut type was defined by the ratio of the new surface area (A) created by wounding in cm^2 over the tissue weight (W) in g. The calculated wounding intensities (A/W) were 4.9, 6.4 and 18.5 cm^2/g, respectively, for slices, pies, and shreds. Whole carrots with 0.00 cm^2/g of A/W were used as controls. After wounding treatment, slices, pies, and shreds were placed into 15 cm × 10 cm × 4 cm rigid polypropylene containers and stored at 4, 10 and 20 °C with 85–90% relative humidity for 7, 4 and 2 days, respectively. During storage, samples were taken and frozen in liquid nitrogen, and stored at −80 °C until use.

In the second experiment, the impact of ROS on wound-induced accumulation of phenolic compounds in carrots was conducted. Shredded carrots were selected and immersed in the following solutions for 3 min: water, 300 μM diphenyliodonium iodide (DPI, ROS inhibitor), 50 μM glucose, 50 μM glucose/glucose oxidase (G/GO, ROS elicitor). Each treatment was applied to three replications. The concentrations of the chemicals were chosen based on the literatures (Jacobo-Velázquez et al., 2011; Orozco-Cárdenas, Narváez-Vásquez, & Ryan, 2001) and our preliminary experiments. After treatment, all samples were then air-dried for approximately 15 min and stored at 20 °C with 85–90% RH for 48 h. During storage, samples were taken every 12 h, frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Measurements of $O_2^{\cdot-}$ and H_2O_2

$O_2^{\cdot-}$ production was measured following the method of Elstner (1976) with some modifications. Five grams of fresh tissue was ground in 20 mL of 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 10,000*g* for 20 min at 4 °C. A sample of the crude extract (1 mL) was added to 1 mL of 1 M hydroxyammonium chloride and incubated at 25 °C for 1 h. Then 2 mL ether was added to the incubation mixture in order to prevent the interference of pigment and the mixture was centrifuged at 10,000*g* for 5 min. After that 1 mL mixture from the water layer, 1 mL of 17 mM p-aminophenylsulfonic acid and 7 mM α-naphthylamine (dissolved in glacial acetic acid: H_2O = 3:1) were added and the mixture incubated at 25 °C for a further 20 min, the absorbance was measured at 530 nm. $O_2^{\cdot-}$ production was calculated against the standard curve using sodium nitrite as a standard and expressed as nM [NO_2] g^{-1} min^{-1}.

For analysis of H_2O_2 content, three grams of fresh tissue was ground in 10 mL of chilled acetone. The homogenate was centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis using the method of Patterson, Mackae, and Ferguson (1984). The content of H_2O_2 was expressed as μM g^{-1}.

2.4. Total soluble phenolics (TSP) content assay

TSP content was assayed following the method of Slinkard and Singleton (1977) with some modifications. Frozen tissue samples (5 g) were extracted using a mortar and pestle with 25 mL of cold methanol. The homogenate was centrifuged at 13,000*g* for 15 min at 4 °C and the supernatant was used for the TSP content assay. A sample of the crude extract (500 μL) was added to 1.5 mL of distilled water and 1 mL of Folin−Ciocalteu reagent, after that 1 mL 7.5% (w/v) Na_2CO_3 solution was added. The mixtures were incubated at 25 °C for 2 h before reading at 765 nm. The TSP content was expressed as milligrams of gallic acid (GAE) per kilogram.

2.5. Measurement of antioxidant capacity

Antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity as reported by De Ancos, Sgroppo, Plaza, and Cano (2002) with some modifications. The same extracts prepared for TSP assay were used for determination of antioxidant capacity. An aliquot of 0.2 mL of the TSP extract was added to 2.8 mL of 0.12 mM DPPH solution (prepared with ethanol). After incubated at 25 °C for 30 min, the absorbance was measured at 525 nm, and 0.2 mL of 80% ethanol