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Hydrogen sulfide inhibits enzymatic browning of fresh-cut lotus root slices by regulating phenolic metabolism

Ying Sun, Wei Zhang, Tao Zeng, Qixing Nie, Fengying Zhang, Liqin Zhu *

College of Food Science and Engineering, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, People's Republic of China

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ABSTRACT

The effect of fumigation with hydrogen sulfide (H_2S) gas on inhibiting enzymatic browning of fresh-cut lotus root slices was investigated. Browning degree, changes in color, total phenol content, superoxide anion production rate ($O_2^{\cdot-}$), H_2O_2 content, antioxidant capacities (DPPH radical scavenging ability, ABTS radical scavenging activity and the reducing power) and activities of the phenol metabolism-associated enzymes including phenylalanine ammonialyase (PAL), catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO) were evaluated. The results showed that treatment with 15 μl L^{-1} H_2S significantly inhibited the browning of fresh-cut lotus root slices ($P < 0.05$), reduced significantly $O_2^{\cdot-}$ production rate and H_2O_2 content, and enhanced antioxidant capacities ($P < 0.05$). PPO and POD activities in the fresh-cut lotus root slices were also significantly inhibited by treatment with H_2S ($P < 0.05$). This study suggested that treatment with exogenous H_2S could inhibit the browning of fresh-cut lotus root slices by enhancing antioxidant capacities to alleviate the oxidative damage.

1. Introduction

Lotus root (*Nelumbo nucifera* Gaertn. ssp. nucifera) is widely planted in China and contains a lot of nutrition such as starch, sugars, proteins, lecithin, alkaloids, flavonoids, carotene, riboflavin, niacin, vitamin C, vitamin B_6, thiamine, copper, manganese, phosphorus, chlorogenic acid and other phenolic substances (Guo, 2008). Recently, fresh-cut lotus root slices have risen in popularity and received increasing attention as a novel minimally processed vegetable (Zhang, Yu, Xiao, Wang, & Tian, 2013). Enzymatic browning usually limits the shelf-life of lotus root slices and preventing enzymatic browning should be an important way to extend storage life of fresh-cut lotus root slices.

Enzymatic browning of fresh-cut produce is generally assumed to be a direct consequence of polyphenol oxidase (PPO) and peroxidase (POD) action on polyphenols to form quinones (Tomas-Barberan & Espin, 2001). The first step in the phenylpropanoid pathway is conversion of L-phenylalanine to trans-cinnamic acid by phenylalanine ammonia lyase (PAL), which is a key enzyme of polyphenol synthesis (Saltveit, 2000). Metabolism of free oxygen radical (ROS) was also considered to be very important for the browning reactions (Toivonen & Brummell, 2008).

Hydrogen sulfide (H_2S) is the third endogenous signaling gasotransmitter after nitric oxide (NO) and carbon monoxide (CO) (Gadalla & Snyder, 2010). Studies showed that treatments with NO and CO inhibited the development of browning of fresh-cut peach slices (Zhu, Zhou, Zhu, & Guo, 2009), apple slices (Huque, Wills, Pristijono, & Golding, 2013) and lotus root slices (Zhang et al., 2013). Interestingly, recent research has also demonstrated that H_2S treatments extend the postharvest life of strawberries (Hu et al., 2012), peaches (Wang et al., 2014), kiwifruits (Zhu et al., 2014) and fresh-cut pears (Hu et al., 2014). However, there are few reports on effects of H_2S on enzymatic browning of fresh-cut fruits and vegetables. It is reported that treatments with H_2S inhibits browning of fresh-cut pears with lower PAL and PPO activity (Hu et al., 2014). The objective of this study was to investigate the effects of H_2S fumigation on enzymatic browning and phenol metabolism of fresh-cut lotus root slices during storage.

2. Materials and methods

2.1. Plant material

Lotus roots (*N. nucifera* Gaertn. cv. Taikong Lian III) were purchased from a local wholesale market at Nanchang, China. They were selected for uniformity of size and ground color, and freedom from defects and mechanical damage. Before treatment, lotus roots were stored at 4 °C for 24 h and then washed with tap water,

* Corresponding author. Fax: +86 791 83813420.
E-mail address: zhuliqin07@126.com (L. Zhu).

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peeled and cut into about 4 mm slices with a stainless steel knife. The slices were put into a glass container sealed with rubber plug. H_2S gas with purity of 99.99% (Tianjin Saiteer Special Gases Co., Ltd, Tianjin, China) was injected into the glass container with an injector through the rubber plug to the final concentrations of 0, 10, 15, 20 μl L^{-1}. The lotus root slices were fumigated with H_2S gas at 25 °C for 30 min. The slices not fumigated with H_2S gas was regarded as the control (CK). After fumigation, all slices were packed in 0.02 mm thick polyethylene bags and stored at 4 °C, RH 95%. Each treatment contained 1000 g fresh-cut lotus root slices, and was replicated three times. Six slices were picked out randomly for color analysis, and each slice was measured twice (each side), then some wedged-shaped slices from six lotus root slices per replicate were mixed and used for the further measurements. Changes in color, browning degree, $O_2^{\cdot-}$ production rate, contents of H_2O_2 and total phenol, antioxidant capacity and enzymes activities (PAL, CAT, PPO, POD) were measured every 2 days during storage of 10 days.

2.2. Changes in color

Color was determined using a color difference meter CR-10 (Minolta, Japan). CIE-Lab color parameters were recorded as L^*, a^*, and b^*. The chromameter was calibrated on a standard white tile ($L^* = 97.06$, $a^* = 0.04$ and $b^* = 2.01$) before each series of measurements.

2.3. Measurement of browning degree (BD)

Browning degree of the sample was evaluated according to Zhang, Tan, McKay, and Yan (2005) with some modifications. About 1 g tissue was homogenized in 8 mL of cold sodium borate buffer (0.1 mol L^{-1}, pH 6.8) containing 5% polyvinylpyrrolidone (PVP) at 4 °C. After centrifuged at 10,000*g* for 15 min at 4 °C, the supernatant was collected. The absorbance was measured using a spectrophotometer (Analytik Jena, Germany) at 410 nm and browning degree was expressed as $10 \times A_{410}$.

2.4. Extraction and determination of total phenols

The phenols in slices were extracted using a procedure described by Zhu et al. (2009). Total phenol content was determined with a spectrophotometer (Analytik Jena, Germany) at 765 nm according to the Folin–Ciocalteu method (Ainsworth & Gillespie, 2007). Gallic acid was employed as calibration standard and the results were expressed as gallic acid equivalents (GAE) per gram of fresh weight (μg GAE g^{-1} FW).

2.5. Determination of $O_2^{\cdot-}$ production rate and H_2O_2 content

$O_2^{\cdot-}$ production rate was determined with the method of Zhu et al. (2014). Lotus root slices (2 g) was homogenized in 5 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8). After filtering the homogenate through four layers of cheesecloth, the filtrate was centrifuged at 10,000*g* for 10 min. The supernatant (1 mL) was mixed with 0.9 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8) and 0.1 mL of 10 mmol L^{-1} hydroxylammonium chloride and then incubated for 20 min at 25 °C. The incubation solution (0.5 mL) was then mixed with 0.5 mL of 17 mmol L^{-1} 4-aminobenzenesulfonic acid and 0.5 mL of 7 mmol L^{-1} α-naphthylamine and further incubated for 20 min at 25 °C. Following incubation, the mixture was collected in a separating funnel and mixed with 1.5 mL of *n*-butanol. The upper phase was collected and the absorbance at 530 nm was determined with phosphate buffer as a blank. $O_2^{\cdot-}$ production rate was expressed as nmo1 g^{-1} FW min^{-1}. For H_2O_2 determination, 3 g of fresh tissue was homogenized with 5 mL of cold 100% acetone and then centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis according to the method of (Patterson, MacRae, & Ferguson , 1984). H_2O_2 content was expressed as μmol g^{-1} FW.

2.6. Evaluation of antioxidant capacity

Lotus root slices (2 g) were ground in liquid nitrogen. The powder was mixed with 70% ethanol (50 mL), and then sonicated for 40 min. After centrifuged at 10,000*g* for 15 min at 4 °C, the supernatant was used to evaluate the antioxidant capacities. The DPPH (2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl) radical scavenging capacity was determined according to the method of Williams, Cuvelier, and Berset (1995) with some modifications. Extracts (0.2 mL) were added to 0.2 mmol L^{-1} DPPH in methanolic solution. The mixture was reaction in the dark for 30 min and then the decrease in absorbance at 517 nm was measured. Radical scavenging activity was calculated using the following formula: scavenging activity (%) = $[(A_0 - A_i)/A_0)] \times 100$, where A_0 was the absorbance of the control, and A_i was the absorbance of the sample. The ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging capacity was determined according to the method of Roberta et al. (1999). The stock solutions included $ABTS^{\cdot+}$ (7 mmol L^{-1}, 25 mL) and potassium persulfate solution (140 mmol L^{-1}, 440 μL). The working solution was prepared by allowing the mixture to react for 12 h at room temperature in the dark. The solution was then diluted in methanol to an absorbance of 0.7 (±0.02) at 734 nm. Extracts (0.1 mL) were allowed to react with the $ABTS^{\cdot+}$ solution (4.9 mL) for 10 min in a dark condition. The absorbance was measured at 734 nm. Trolox was employed as calibration standard and results were expressed as μmol g^{-1} FW. The reducing power was determined according to the method of Oyaizu (1986) with some modifications. Extracts (0.6 mL) were mixed with sodium phosphate (2.5 mL, 0.2 mol L^{-1}, pH 6.6), distilled water (0.4 mL) and 1% potassium ferricyanide (1 mL). The mixture was incubated at 50 °C for 20 min. After 10% trichloroacetic acid (1 mL) was added, the mixture was centrifuged at 3000*g* for 10 min. The upper layer (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.1%, 0.5 mL), and the absorbance at 700 nm was measured to indicate the increase of reducing power.

2.7. Extractions and assays of PAL, CAT, POD and PPO activities

PAL activity was assayed by the method of Lu et al. (2013) with some modifications. About 2 g of samples were homogenized with 5 mL of sodium borate buffer (0.05 mmol L^{-1}, pH 8.8, containing 0.5 g PVP, 5 mmol L^{-1} 2-mercaptoethanol and 2 mmol L^{-1} EDTA) in an external ice bath. The homogenates were centrifuged at 10000*g* for 15 min at 4 °C. The supernatants were used as the crude enzyme extract. The reaction mixture contained 2 mL sodium borate buffer (0.1 mol L^{-1}, pH8.8), 1 mL 20 mmol L^{-1} L-phenylalanine and 0.1 mL enzyme extract. The mixture was incubated for 30 min at 37 °C. PAL activity was measured by change in absorbance at 290 nm. One unit of enzyme activity was defined as the amount that caused an increase of 0.001 absorbance units per hour.

CAT activity was measured following Havir and McHale (1987) with some modifications. About 2 g of samples was homogenized in 5 mL sodium phosphate buffer (50 mmol L^{-1}, pH 7.0). The homogenate was centrifuged at 10,000*g* for 15 min at 4 °C, and the supernatant was used as crude enzyme solution. As substrates,