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Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid

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ABSTRACT

The effect of 0.2% ascorbic acid (AA), 5 μM nitric oxide (NO), and the simultaneous use of 0.2% AA and 5 μM NO solutions on inhibiting surface browning of fresh-cut peach slices stored at 10 °C and RH 95% was investigated. The browning index, relative leakage rate, microstructure, total phenol content, and activity of the phenol metabolism-associated enzymes phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) were evaluated. The results indicate that treatment with 0.2% AA, 5 μM NO and simultaneous use of 0.2% AA and 5 μM NO resulted in higher total phenol content, inhibition of PPO and POD activity, reduced membrane permeability and protection of cell microstructure to maintain compartmentation between enzymes and their substrates. In addition, NO increased PAL activity. The causes of inhibition in the browning of peach slices by NO are discussed.

1. Introduction

There has been a steady increase in consumer demand for fresh-cut fruits and vegetables. However, enzymatic browning is a particular problem in fruit with a white flesh such as apples, pears and peaches. Browning reactions are generally assumed to be a direct consequence of polyphenol oxidase (PPO) and peroxidase (POD) action on polyphenols to form quinones, which ultimately polymerize to produce the browning appearance of fresh-cut fruit and vegetable products. Once the compartmentalisation of the cells begins to fail, PPO and/or POD can act on phenol substrates (Degl'Innocenti, Guidi, Pardossi, & Tognoni, 2005). 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 and is generally induced by wounding (Saltveit, 2000; Tomás-Barberán, Gil, Castañer, Artés, & Saltveit, 1997). Most polyphenols (about 97%) exist in vacuoles and apoplast/cell wall compartments, while smaller quantities of phenolic compounds are in chromoplast, the cytoplasm and mitochondria (Toivonen & Brummell, 2008). The initial event in the oxidative browning process is the breakdown of membranes within the cells of plant tissues, and browning is associated with the loss of membrane integrity, which occurs during tissue deterioration and senescence. Membrane integrity is negatively correlated with membrane permeability and can be expressed as relative cell leakage rate (You et al., 2007). So membrane stability is potentially a major factor controlling the browning of fruit and vegetable slices.

Ascorbic acid, hydrogen peroxide, edible coatings and modified atmosphere packaging (MAP), together with low temperature storage are used to inhibit enzymatic browning and increase shelf life of fresh-cut fruits and vegetables (Lamikanra & Watson, 2001; Rojas-Graü, Tapia, & Martín-Belloso, 2008). Heat treatment of peaches before cutting also controls browning of peach slices and retains their firmness during storage (Koukounaras, Diamantidis, & Sfakiotakis, 2008).

Nitric oxide (NO) is a highly reactive free radical gas known to be involved in resisting vegetative stress and senescence of horticultural products. Short-term exposure to a low concentration of NO gas or its donor compounds has been shown to extend the postharvest life of various intact fresh fruits and vegetables (Wills, Ku, & Leshem, 2000; Wills, Soegiarto, & Bowyer, 2007; Zhu & Zhou, 2007). It has also been shown that they delayed the onset of browning of apple slices (Pristijono, Wills, & Golding, 2006) and cut lettuce (Wills, Pristijono, & Golding, 2008). However, there have been no reports on the effect of NO on fresh-cut peach slices during storage. The objective of this study was to investigate the effect of NO on the surface browning of fresh-cut peach slices and the effect on cell microstructure and phenolic metabolism.

2. Materials and methods

2.1. Plant material

Peaches (*Prunus* persica (L.) Batsch, cv. Zhonghuashoutao) were purchased from a local supermarket (Taian, China) in October 2007 at a pre-climacteric, but physiologically mature stage. They were

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selected for uniformity of size and ground colour, freedom from defects and mechanical damage. Before treatment, the peaches were stored at 4 °C for 24 h. Each peach was peeled and cut into about 1 × 3 cm size slices with a stainless steel knife. The peach slices were immersed in a solution containing 0.2% AA, 0.5 μM NO, or 0.5 μM NO plus 0.2% AA (NO/AA) for 10 min with immersion in distilled water used as a control. The peach slices were then drained in the air for another 5 min and placed into trays over wrapped with plastic film and stored at 10 °C, RH 95%. Each treatment unit consisted of six slices from six different peaches and experiments were replicated three times.

2.2. Measurement of browning index

Evaluation of surface browning on slices was subjectively based on a numeral scoring index (González-Aguilar, Ruiz-Cruza, Cruz-Valenzuela, Rodrıguez-Felixa, & Wang, 2004) using a scale of 1–5, where 1 = none, 2 = slight (up to 5% surface affected), 3 = moderate (5–20% surface affected), 4 = moderately severe (20–50% surface affected) and 5 = extreme (>50% surface affected) for individual slices. A browning index was then calculated using the following formula: Σ (browning scale × percentage of corresponding slices within each class).

2.3. Measurement of relative leakage rate

Relative leakage rate was determined according to Song, Ding, Zhao, Sun, and Zhang (2006) with some modifications. Fifteen pieces of peach discs (1 mm) from six slices were rinsed and incubated in 40 ml distilled water and the initial electrolyte leakage (P_o) was monitored with a conductivity meter (DDS-11A, China). Electrolyte leakage was also monitored after 10 min as P_1. Each sample was boiled for 10 min, and cooled to room temperature (20 °C) when the final electrolyte leakage (P_2) was monitored (the solution was diluted to 40 ml with distilled water to offset loss of the evaporation). Relative leakage rate was calculated according to the following equation: Relative leakage rate (%) = $(P_1–P_0)/(P_2–P_0) \times 100$.

2.4. Extraction and determination of total phenols

The phenols in slices were extracted using a procedure described by Chiou et al. (2007). A weighed quantity of each homogenized sample (approximately 1.0 g) was mixed with 75% methanol (5 ml), placed in a sonicator bath for 15 min and stirred for 12 h at 20 °C. The mixture was then centrifuged at 10,000*g* for 10 min and the methanol extract was collected. The remaining residue was extracted two additional times with methanol (10 ml). For each of the latter two extractions, the mixture was placed in a sonicator bath for 15 min and stirred for 10 min. All extracts were combined and the methanol was evaporated under reduced pressure leaving a residue which was dissolved in doubly distilled water (10 ml). Aliquots of these solutions were stored at 4 °C for determination of total phenol content.

Total phenol content was determined according to the Folin–ciocaulteu method (Ainsworth & Gillespie, 2007). Gallic acid was employed as a calibration standard and the results were expressed as gallic acid equivalents (GAE) (μg GAE/g peach). All spectrophotometric data were acquired using a UV-2450 spectrophotometer (Shimadzu, Japan) at 765 nm. Each experiment was conducted with three replicates.

2.5. Extractions and assays of PAL, PPO and POD activity

For the PAL activity assay, peach tissue (2 g) from six peach slices was homogenized in a cold 10 ml solution of 0.05 M sodium borate buffer (pH 8.0) containing 0.5 g polyvinylpyrrolidone (insoluble), 5 mM 2-mercaptoethanol and 2 mM EDTA at 4 °C. The homogenate was centrifuged at 19,000*g* for 20 min at 4 °C, and the supernatant was used as the crude enzyme extract and stored at 4 °C for the determination.

PAL activity was assayed by the method of Zheng et al. (2005). The reaction mixture contained 2 ml of 100 mM Tris-Hcl buffer (pH 8.5, containing 1 mM 2-mercaptoethanol), and 2 ml 15 mM L-phenylalanine and 1 ml of enzyme extract. The reaction mixture was incubated for 30 min at 37 °C. The increase in absorbance at 290 nm, due to the formation of trans-cinnamate, was measured using a UV-2450 spectrophotometer (Shimadzu, Japan). One unit of enzyme activity was defined as the amount that caused an increase of 0.001 absorbance units per hour.

Crude enzyme fractions for PPO and POD activity analysis were prepared as reported by Ayaz, Demir, Torun, Kolcuoglu, and Colak (2008). Frozen fruit (2 g), in triplicate, was homogenized in a cold 10 ml solution of 50 mM phosphate buffer (pH 7.0), containing 2 mM EDTA, 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1% (w/v) PVPP and 0.6% (w/v) Triton X-114 for 2 min at 4 °C. The homogenate was filtered using three layers of cheese cloth before centrifugation at 17,000*g* for 15 min at 4 °C. The supernatant was used as the crude enzyme extract and stored at 4 °C for the determination.

PPO activity was determined using resorcinol as substrates by measuring the increase in absorbance at 500 nm at pH 7.0 and 25 °C. Resorcinol was assayed with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in 50 mM potassium phosphate buffer (pH 7.0). Enzyme activity was assayed using a 1 ml cuvette containing 100 μl of substrate (stock 100 mM), an equal volume of MBTH (stock 10 mM), and 20 μl of dimethylformamide (DMF). The solution was diluted to 950 μl with buffer and 50 μl enzyme extract was added to start the reaction. One unit of enzyme activity was defined as an increase of 0.01 in absorbance per minute per mg protein.

POD activity was measured according to the procedure of Sun (1988). The reaction mixture consisted of 2 ml acetic acid buffer containing 5 mM benzidine and 1 ml enzyme solution. This mixture was incubated for 5 min at 37 °C, then 1 ml 0.3% H_2O_2 was added to the solution to start the reaction. The increase in absorbance at 470 nm was recorded for 2 min. One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.01 in absorbance per minute per mg protein.

Soluble protein content in the crude enzyme of triplicate extractions was determined with bovine serum albumin as standard (Bradford, 1976). The absorbance obtained was evaluated by graphic interpolation on a calibration curve at 595 nm.

2.6. Transmission electron microscopy

Samples were fixed for 12 h with a 3.5% glutaraldehyde solution, washed for 2 h with 0.1 M phosphate buffer (pH 7.2), post-fixed for 6 h with 1% OsO_4 solution, washed for 2 h with 0.1 M phosphate buffer (pH 7.2), dehydrated using a graded ethanol series (45%, 50%, 70%, 95%, and 100%), and embedded in spur epoxy resin. The samples were cut using a LKB chip cutter (LKB, Sweden). Ultrathin sections (0.7 μm) were stained with uranyl acetate–citrate and then observed in JEM-1200EX transmission electronic microscope.

2.7. Statistical analysis

Each experiment was repeated three times and the data was processed by analysis of variance (ANOVA), comparing treatments at $P = 0.05$ according to least significant difference (LSD) test, indicating the LSD value in each case. The data values were expressed as mean ± SE ($n = 3$).