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Effect of nitric oxide on pericarp browning of harvested longan fruit in relation to phenolic metabolism

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Abstract

The effects of nitric oxide (NO) on enzymatic browning of harvested longan fruit in relation to phenolic metabolisms were investigated. Fruits were dipped for 5 min in 1 mM sodium nitroprusside (SNP), a nitric oxide donor, then packed in 0.03 mm thick polyethylene bags, and finally stored for 6 days at 28 °C. Changes in pericarp browning and pulp breakdown were evaluated, while total phenol content, activities of phenolic-associated enzymes, polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL), and concentrations of total soluble solids, titratable acidity and ascorbic acid were measured. SNP treatment delayed pericarp browning, inhibited activities of PPO, POD and PAL and maintained a high total phenol content of longan fruit during storage. Furthermore, NO showed a significant inhibition of the *in vitro* activities of PPO and POD, indicating that the beneficial effect of NO was direct. Moreover, application of NO resulted in a lower pulp breakdown and maintained relatively high levels of total soluble solids and ascorbic acid.

Keywords: Longan; Nitric oxide; Browning; Phenolic metabolism; Quality

1. Introduction

Longan is a non-climacteric subtropical fruit with high value. However, the fruits deteriorate rapidly after harvest, due to pericarp browning and pulp breakdown, resulting in reduced market value (Jiang, Zhang, Joyce, & Ketsa, 2002). Postharvest browning of longan fruit has mainly been attributed to oxidation of phenolics by polyphenol oxidase (PPO) and peroxidase (POD) (Jiang, 1999; Jiang & Li, 2001). Therefore, delaying or reducing enzymatic browning should be an important way to extend storage life and maintain quality of longan fruit.

Nitric oxide (NO) is a relatively stable free racial gas. Initially, it has attracted attention as an environmental pollutant. However, recent research reveals that NO may act as a key signalling molecule in plants, which mediates various pathophysiological and developmental processes, including expression of defence-related genes and programmed cell death, stomatal closure, seed generation and root development (Lamattina, García-Mata, Graziano, & Pagnussat, 2003; Neil, Desikan, & Hancock, 2003). Additional evidence indicates that NO may have anti-senescence and ripening properties. Leshem and Haramaty (1996) found that application of a NO donor to pea leaves under senescence-promoting conditions inhibited ethylene production. NO treatment extends the postharvest life of fresh horticultural produce (Wills, Ku, & Leshem, 2000; Bowyer, Wills, Badiyan, & Ku, 2003; Badiyan, Wills, & Bowyer, 2004; Soegiarto & Wills, 2004). Furthermore, there may be an antagonistic effect of both NO and ethylene during fruit maturation and senescence (Leshem & Pinchasov, 2000). However, little information on the effect of NO on browning of fresh horticultural produce is available.

The objective of this study was to test the effects of exogenous sodium nitroprusside (SNP), a NO donor, on

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pericarp browning of harvested longan fruit in relation to phenolic metabolism.

2. Materials and methods

2.1. Plant materials and treatments

Fruits of longan (*Dimocarpus longan* Lour.) cv. Shixia, at the commercially mature stage, were harvested from a commercial orchard in Guangzhou. Fruits were selected for uniformity of shape, colour and size, while any blemished or diseased fruits were discarded. Preliminary study showed that, at a concentration range of 0.25–1.5 mM, treatment with SNP at 1 mM most effectively reduced pericarp browning of longan fruit held for 6 days at 28 °C. In this study, 1 mM SNP was therefore used. Fruits were dipped for 5 min in 1 mM SNP solution at 28 °C. After dipping, the fruits were air-dried for 30 min, packed in 0.03 mm thick polyethylene bags (20 fruits per bag) and then stored at 28 °C. The fruit dipped in water was used as control. Samples were taken initially and at 2 day intervals during storage for quality evaluation and following analyses.

2.2. Fruit quality evaluation

Fruit browning was assessed by measuring the extent of the total browned area of inner pericarp of 60 individual fruits, using the following visual appearance scale: 0: no browning (excellent quality), 1: <1/8 browning, 2: <1/4 browning, 3: 1/4 – 1/2 browning and 4: >1/2 browning (poor quality). The browning index was calculated as $\sum$(browning rating × percentage of fruit within each class). Pulp breakdown was subjectively assessed on a scale from 0 (none), 1 (slight), 2 (moderate) to 3 (severe). Results were expressed as pulp breakdown index and calculated using the following formula: $\sum$(pulp breakdown scale × percentage of fruit within each class). Fruits at higher than 2.0 browning index or 1.0 pulp breakdown index were considered unacceptable for marketing.

2.3. Determination of total phenols

The total phenol contents in longan pericarp tissues were determined by the method of Folin–Ciocalteu reaction (Singleton & Rossi, 1965), using gallic acid as standard. The phenol contents were expressed as gallic acid equivalents in milligrams on a fresh weight (FW) basis.

2.4. Extraction and assay of PPO, POD and PAL activities

Pericarp tissues (5 g) from 30 fruits were homogenized in 20 ml of 0.05 M phosphate buffer (pH 7.0) and 0.5 g of polyvinylpyrrolidone (insoluble) at 4 °C. After filtration of the homogenate through a cotton cloth, the filtrate was centrifuged for 20 min at 19,000*g* and 4 °C. The supernatant was then collected as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate, according to the method of Jiang (2000). One unit of enzyme activity was defined as the amount that caused a change of 0.001 in the absorbance per minute. POD activity, using guaiacol as a substrate, was assayed by the method of Zhang, Pang, Duan, Ji, and Jiang (2005) in a reaction mixture (3 ml) containing 25 μl of enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 1% H_2O_2 and 0.1 ml of 4% guaiacol. The increase in the absorbance at 470 nm, due to the guaiacol oxidation, was recorded for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per minute.

For PAL activity assay, pericarp tissues (5 g) from 30 fruits were homogenized in 20 ml of 0.1 M Na borate buffer (pH 8.0) containing 0.5 g of polyvinylpyrrolidone (insoluble), 5 mM β-mercaptoethanol and 2 mM EDTA at 4 °C (Jiang & Joyce, 2003). The homogenate was centrifuged for 20 min at 19,000*g* and 4 °C and then the supernatant was collected for enzyme assay. PAL activity was determined by incubating the mixture of 0.1 ml enzyme extract and 2.9 ml of 0.1 M Na borate buffer (pH 8.0) containing 3 mM L-phenylanine for 1 h at 37 °C. The increase in the absorbance at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hour.

Protein content was determined according to the method of Braford (1976), with bovine serum as the standard.

2.5. Effects of NO on in vitro PPO and POD activities

To analyse effects of NO on *in vitro* PPO and POD activities, 0.1 ml of SNP solutions at the final concentrations of 0, 0.05, 0.1, 0.5, 1, 5 and 10 mM was added to the reaction solution. PPO and POD activities were determined using the methods described previously and the relative enzymatic activities were expressed by considering the activity without SNP as 100.

2.6. Total soluble solids, titratable acidity and ascorbic acid

Pulp tissues (20 g) from 30 fruits were homogenized in a grinder and the supernatant phase was collected for analyses of total soluble solids, titratable acidity and ascorbic acid contents. Total soluble solids were assayed by using a hand-held refractometer (J1-3A, Guangdong Scientific Instruments). Titratable acidity and ascorbic acid were determined by titration with 0.1 M NaOH and 2,6-dichlorophenolindophenol, respectively.

2.7. Data handling

The experiments were arranged in a completely randomized design, and each was comprised of three replicates. Data were tested by analysis of variance, using SPSS version 7.5. Least significant differences (LSD) were calculated to compare significant effects at the 5% level.

3. Results

3.1. Skin browning and pulp breakdown

As shown in Fig. 1, the browning index of longan pericarp rapidly increased as storage time progressed, which indicated that the fruit pericarp gradually turned brown. The change in pulp breakdown index was similar to that of the browning index. NO treatment markedly delayed the increase in pericarp browning index and pulp breakdown index of longan fruit. After 6 days of storage, the pericarp browning index and pulp breakdown index in non-SNP-treated (control) fruits were 4.64 and 2.72, while they were 2.35 and 0.97 in SNP-treated fruits, respectively.

3.2. Total phenol content

As shown in Fig. 2, there was a significant decrease in total phenol content during storage for control and SNP-treated fruits. The fruits treated with SNP decreased more slowly than the control fruits.

3.3. Activities of PPO, POD and PAL

PPO, POD and PAL are associated with phenolic metabolism. PPO activity in control fruits first increased, reached the peak value at day 2, and then decreased (Fig. 3a). Treatment with SNP slowed the increase of PPO activity.

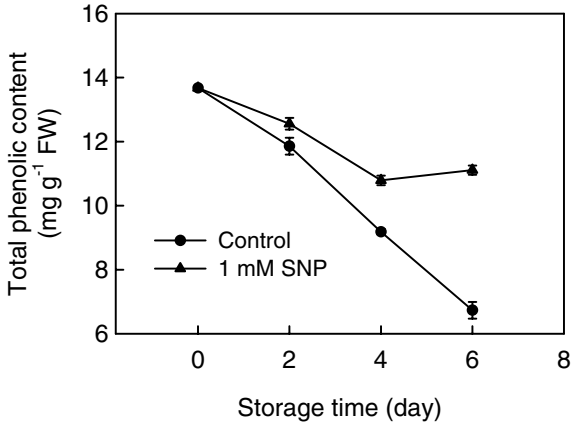

Fig. 2. Effects of NO on total phenol contents of longan fruit during storage at 28 °C. Each value is presented as the mean ± standard error ($n = 3$).

Differently from PPO, POD activity in control fruits slowly increased and then increased rapidly after 4 days of storage, which was similar to the change in browning index. SNP treatment resulted in a slight increase of POD activity throughout 6 days of storage, compared with the control fruits (Fig. 3b).

As shown in Fig. 4, treatment with SNP significantly inhibited PAL activity of longan fruit. After 4 and 6 days of storage, the PAL activities of the SNP-treated fruits were 36.8% and 44.0% of control fruits, respectively.

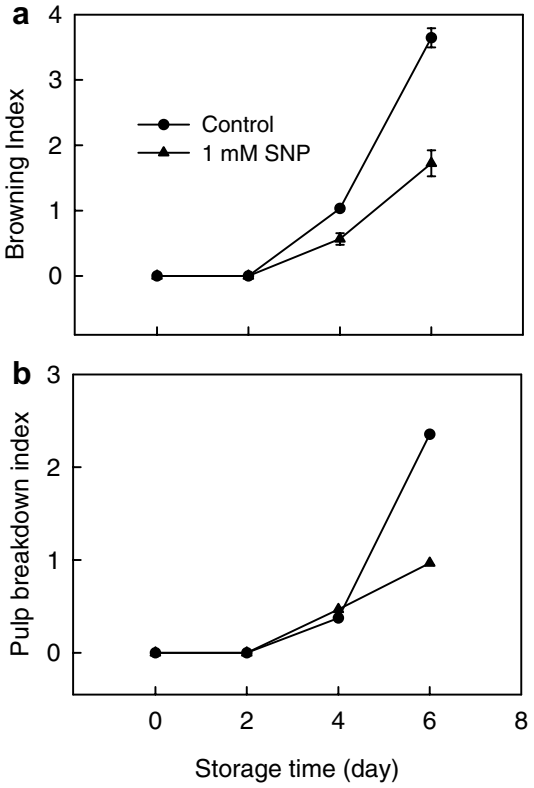

Fig. 1. Effects of NO on pericarp tissue browning (a) and pulp breakdown (b) of longan fruit during storage at 28 °C. Each value is presented as the mean ± standard error ($n = 3$).

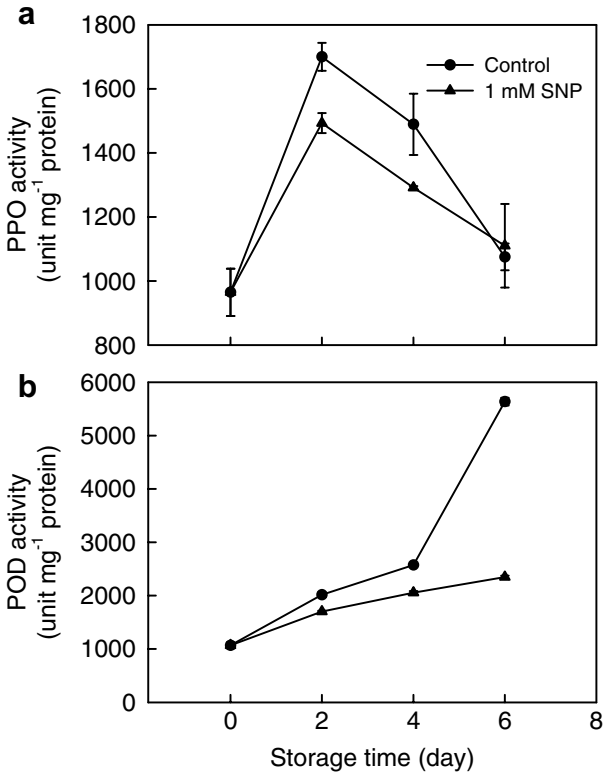

Fig. 3. Effects of NO on activities of PPO (a) and POD (b) of longan fruit during storage at 28 °C. Each value is presented as the mean ± standard error ($n = 3$).

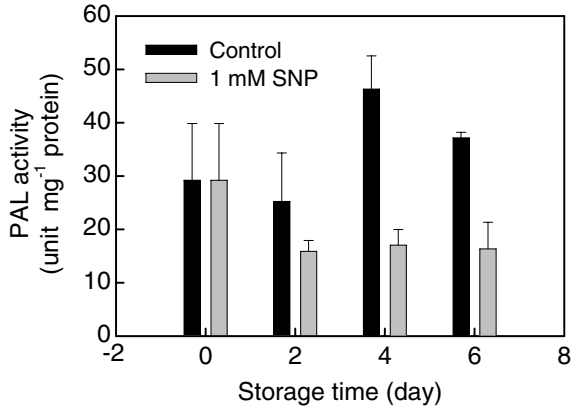

Fig. 4. Effects of NO on PAL activity of longan fruit during storage at 28 °C. Each value is presented as the mean ± standard error ($n = 3$).

3.4. Effect of SNP on in vitro PPO and POD activities

Fig. 5 shows effects of SNP on *in vitro* activities of PPO and POD pericarp tissues of longan fruit. Treatment with SNP at low concentrations exhibited a slight inhibitory effect on PPO activity while SNP at 0.5 mM, or higher concentrations, significantly inhibited the activity of the enzyme in a dose-dependent manner. POD activity was also inhibited by SNP. However, a lower inhibitory effect of SNP on POD activity was observed, compared with PPO.

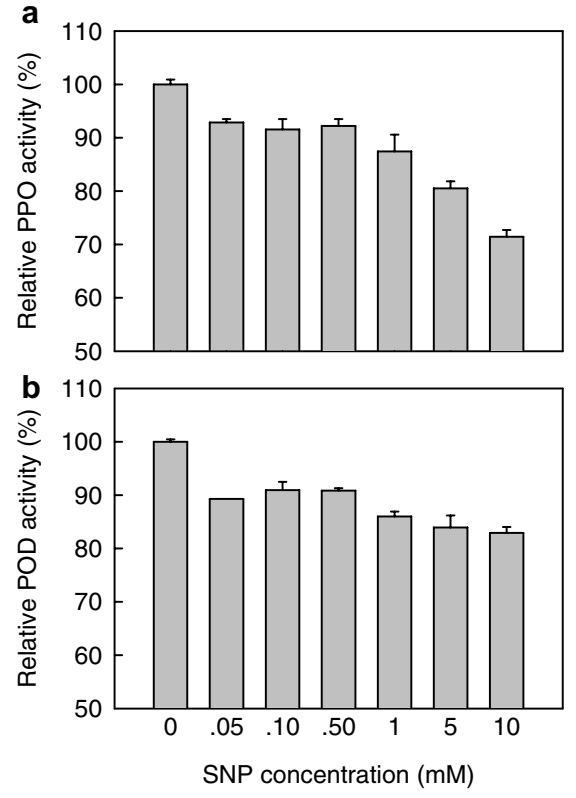

Fig. 5. Effects of NO on *in vitro* activities of PPO (a) and POD (b) of longan fruit during storage at 28 °C. Each value is presented as the mean ± standard error ($n = 3$).

3.5. Total soluble solids, titratable acidity and ascorbic acid

As shown in Table 1, the contents of total soluble solids, titratable acidity and ascorbic acid of flesh tissues of longan fruit decreased markedly after 6 days of storage. Treatment with SNP maintained higher levels of total soluble solids and ascorbic acid, but it had no significant effect on the titratable acidity content.

4. Discussion

Some evidence has shown an antagonistic effect between NO and ethylene (Leshem, Wills, & Ku, 1998; Leshem & Pinchasov, 2000), while ethylene plays an important role in regulating maturation and senescence of fruits. Application of exogenous NO was found to delay ripening and senescence of some horticultural crops by inhibiting ethylene production. For example, fumigation with NO extended the postharvest life of strawberry (Wills et al., 2000), pear (Sozzi, Trinchero, & Fraschina, 2003), broccoli, green bean, bok choy (Soegiarto & Wills, 2004), and carnations (Bowyer et al., 2003). Furthermore, Badiyan et al. (2004) reported that use of NO donor compounds was more efficient in extending the shelf life of snapdragon, delphinium, chrysanthemum, tulip, gerbera, oriental lily, rose and iris. Additionally, NO may act as an antioxidant, to delay senescence of plant tissues (Beligni, Fath, Bethke, Lamattina, & Jones, 2002; Cheng, Hsu, & Kao, 2002; Huang & Kao, 2005; Guo & Crawford, 2005). Senescence and deterioration of harvested longan fruit are characterized by increased pericarp browning and pulp breakdown, and reduced flavour and nutritive quality (Jiang et al., 2002). In this study, SNP treatment markedly slowed the increases in pericarp browning index (Fig. 1a) and pulp breakdown index (Fig. 1b), and maintained high levels of total soluble solids and ascorbic acid (Table 1), which indicated that NO treatment delayed the senescence of longan fruit during storage.

Involvement of PPO and POD in enzymatic browning of longan fruit has been widely reported (Jiang, 1999; Jiang & Li, 2001; Jiang et al., 2002). PPO and POD catalyze the oxidation of phenolic compounds to quinones and then condense tannins to brown polymers. At the late stage of storage, the rapid increase in activities of PPO and POD (Fig. 3) possibly accelerated the oxidation of

Table 1
Effects of SNP on contents of total soluble solids, titratable acidity and ascorbic acid of longan fruit after 6 days of storage at 28 °C

Treatment	Total soluble solids (%)	Total titratable acidity (%)	Ascorbic acid [mg/100 g pulp (FW)]
Control	15.9b	0.14a	85.5b
SNP	18.2a	0.14a	88.8a

Each value is the mean of three replicates. The contents of total soluble solids, titratable acidity and ascorbic acid of longan fruit at harvest were 20.1%, 0.17% and 99.3 mg/100 g pulp (FW), respectively. Means within a column followed by the same letter are not significantly different at the 5% level.

polyphenols (Fig. 2) and thus led to rapid pericarp browning of longan fruit (Fig. 1). Treatment with SNP inhibited activities of PPO and POD (Fig. 3) in association with high total phenolic content (Fig. 2), which may account for the inhibition of the pericarp browning (Fig. 1). Furthermore, NO had a direct inhibitory effect on *in vitro* activities of PPO and POD (Fig. 5). Bogdan (2001) suggested that NO can interact with transition metals (e.g. iron, copper and zinc) and thiol-containing proteins. Clark, Durner, Navarre, and Klessig (2000) and Barcelo et al. (2002) found that NO inhibited the haem-containing enzymes, catalase and peroxidases, with potential knock-on effects on levels of reactive oxygen species and xylem development.

PAL is the first key enzyme involved in the biosynthesis of phenols in fruits and it can be induced by various stress conditions (Dixon & Paira, 1995). Increase in PAL activity was related to tissue browning of fresh fruits and vegetables during storage (Loaiza-Velarde, Mangrich, Campos-Vargas, & Saltveit, 2003; Nguyen, Ketsa, & van Doorn, 2003; Fujita, Tanaka, & Murata, 2006). In this study, treatment with SNP significantly inhibited PAL activity of longan fruit during storage (Fig. 4), which could be associated with reduced browning index. Similar inhibitory effects of PAL activity by NO were reported for Taxus cells cultures (Wang & Wu, 2005).

In conclusion, treatment with SNP effectively inhibited pericarp browning and pulp breakdown and maintained high levels of total soluble solids and ascorbic acid of longan fruit during storage. The inhibition of the pericarp browning by NO was related to reduced activities of PPO, POD and PAL. Thus, it is suggested that application of NO may be a promising method for extending shelf life and maintaining quality of longan fruit.

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