Postharvest Biology and Technology 108 (2015) 21–27

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effect of nitric oxide on energy metabolism in postharvest banana fruit in response to chilling stress

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ARTICLE INFO

Article history:
Received 6 November 2014
Received in revised form 3 May 2015
Accepted 6 May 2015
Available online 22 May 2015

Keywords:
Nitric oxide
Chilling injury
Energy metabolism
Banana fruit

ABSTRACT

Effects of postharvest nitric oxide (NO) treatment on energy metabolism and chilling injury in cold-stored banana fruit were investigated. Banana fruit were treated with 0.05 mM NO donor sodium nitroprusside, and then stored at 7 °C for up to twenty days. NO treatment apparently inhibited the development of chilling injury. The contents of adenosine triphosphate (ATP) and energy charge in the NO-treated fruit were significantly higher than control fruit. Meanwhile, the activities of enzymes involved in energy metabolism, including H^+-ATPase, Ca^{2+}-ATPase, succinic dehydrogenase and cytochrome C oxidase were markedly enhanced by NO treatment. In addition, notably elevated activities of fructokinase, glucokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were observed in NO-treated banana fruit. These results indicated that NO could enhance chilling tolerance of banana fruit through maintaining high levels of energy status and inducing enzyme activities involved in energy metabolism during cold storage.

1. Introduction

Cold storage is used to control decay and retard ripening of fruit and vegetables. However, some tropical and subtropical fruit, like banana fruit, are highly susceptible to chilling injury (CI). CI symptoms in banana fruit include rapid peel browning, pitting, and failure of fruit softening, which considerably reduce commercial quality and consumer acceptance (Jiang et al., 2004). With the extensive application of cold storage, the study of more effective techniques for banana fruit storage under low temperature is in urgent need.

Accumulating evidence demonstrates that the reduction of cellular energy may account for the chilling injuries and physiological disorders in those cold-sensitive fruits in response to chilling stress (Liu et al., 2011; Zhou et al., 2014). It has been suggested that the enhanced energy status could contribute to chilling tolerance in various kinds of fruits such as cucumber, mango and peach (Chen and Yang, 2013; Jin et al., 2013, 2014; Li et al., 2014; Yang et al., 2011). Cellular energy may mediate the chilling resistance of cold-stored fruit through directly affecting the biosynthesis of membrane lipids and cell membrane restoration (Jin et al., 2014). Chen and Yang (2013) reported that a higher level of ATP content and energy charge may be involved in inducing activities of antioxidant enzymes, thus protecting postharvest cucumber fruit against oxidative damage at low temperature. In addition, glycolysis, the oxidative pentose phosphate (OPP) pathway, the mitochondrial tricarboxylic acid (TCA) cycle, and the electron transport system are metabolic pathways associated with energy metabolism, which provides the energy for plant biochemical processes (Vanlerberghe, 2013). It is assumed that energy metabolism may play important roles in the development of chilling resistance.

Nitric oxide (NO) is a key signaling molecule mediating multiple plant responses to biotic and abiotic stresses (Arasimowicz and Floryszak-Wieczorek, 2007). Some researchers suggested that NO may suppress ATP synthesis in plant mitochondria, which can be attributed to its inhibition effect on the cytochrome pathway (Yamasaki et al., 2001). However, Gupta et al. (2011, 2012),) found that mitochondrial NO could contribute to improving the energy status in root nodules, similarly as under hypoxic/anoxic conditions. The involvement of endogenous NO in cold response has been investigated in loquat fruit, in which chilling resistance was remarkably reduced by an NO scavenger (Xu et al., 2012). Exogenous NO treatment can alleviate chilling injuries in cold-stored banana fruit, which may be attributed to the enhancement of the antioxidant defense system and secondary metabolites accumulation (Wang et al., 2013b). However, it appears that there is little information on the effect of NO treatment on energy metabolism in cold-stored

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http://dx.doi.org/10.1016/j.postharvbio.2015.05.007
0925-5214/

banana fruit has been available. The objective of this study was to investigate the effect of NO treatment on energy metabolism in postharvest banana fruit in response to cold stress.

2. Material and methods

2.1. Fruit material and treatments

Banana fruit (*Musa* spp., AAA group cv. 'Brazil') were transported from Guangxi Province to the laboratory at Zhejiang University after being harvested at a commercially mature stage (70–80%). Upon arrival, fruit were separated into fingers, and selected for uniformity of size, color and absence of damage. The selected fingers were randomly divided into two groups of 240, comprising three replicates of 80. Fingers (80) in the first group were immersed in 0.05 mM sodium nitroprusside (SNP) solution in a 30 L sealed vacuum container, and vacuum infiltrated at low pressure (10 kPa) for 5 min (NO). As an NO donor that can alleviate the chilling injuries of cold stored banana fruit, SNP at 0.05 mM concentration was selected based on our preliminary research (data not shown). Fingers in the second group were soaked in sterile deionized water under the same conditions (Control). The banana fruit of the two groups were placed into unsealed polyethylene bags (0.04 mm) and stored at 7 °C for 20 day. A sample of nine fruit was randomly collected at a period of 5 day. The peels of the selected fruit were cut into pieces, frozen in liquid nitrogen and stored at −80 °C for the subsequent analysis of energy status and enzyme activities. The peel material was thoroughly homogenized to ensure that samples taken for analysis were representative. Three independent replicates were conducted.

2.2. CI index assessment

CI index was assessed using a five-stage scale based on the extent of chilling symptoms on the surface of twenty individual banana fruit (Nguyen et al., 2003); 0: no chilling injury; 1: mild injury; 2: moderate injury; 3: severe injury; 4: very severe injury. The CI index was calculated using the following formula:

$$\text{CI index} = \sum(\text{CI scale}) \times \frac{(\text{number of fruit at that scale})}{(\text{total number of fruit in the group})}$$

2.3. Measurement of electrolyte leakage

The rate of electrolyte leakage was determined according to the method described by Chen et al. (2008). Twenty discs of banana peel were excised with a 1 cm diameter stainless steel borer from six banana fruit. The discs were added into 20 mL of distilled water and maintained in a water bath shaker at 25 °C for 30 min. The electrical conductivity (L_0) was measured using a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). Then the mixture was heated to 100 °C for 20 min and quickly cooled down to room temperature. Another reading of the electrical conductivity was taken and recorded as L_1. The rate of electrolyte leakage was expressed using the following equation:

$$\text{Electrolyte leakage } (\%) = \left(\frac{L_0}{L_1}\right) \times 100\%$$

2.4. Extraction of mitochondria

Crude mitochondria were extracted from banana peel by the method of Zhou et al. (2014) with a slight modification. A frozen sample (20.0 g) was homogenized in 30 mL of 50 mM Tris–HCl buffer (pH 7.8), containing 0.25 M sucrose, 0.3 M mannitol, 1 mM EDTA, 0.1% (w/v) bovine serum albumin, 0.1% (w/v) cysteine and 5 g/L polyvinyl pyrrolidone at 4 °C. The homogenate was centrifuged at 4000 × *g* for 10 min at 4 °C, using a refrigerated centrifuge (Universal 320R, Hettich, Tuttlingen, Germany). The supernatant was collected and centrifuged at 12,000 × *g* for 10 min at 4 °C for sedimentation of mitochondria. The mitochondria pellet was then re-suspended in washing buffer (10 mM Tris–HCl buffer, containing 0.25 M sucrose, 0.3 M mannitol and 1 mM EDTA) and again centrifuged 12,000 × *g* for 10 min at 4 °C. The final sediment was dissolved with 4 mL washing buffer as crude mitochondria extract for the enzyme assays.

2.5. Measurement of mitochondrial ATPase

H^+-ATPase and Ca^{2+}-ATPase activities were determined by measuring the inorganic phosphorus liberated after catalytic hydrolysis of ATP to ADP (Jin et al., 2013).

For H^+-ATPase activity assay, 3 mL of total reaction mixture containing 0.5 mL of crude mitochondria extract, 30 mM Tris–HCl buffer (pH 8.0), 3 mM Mg_2SO_4, 0.1 mM Na_3VO_4, 50 mM $NaNO_3$, 50 mM KCl and 0.1 mM $(NH_4)_2MoO_4$.

For Ca^{2+}-ATPase activity assay, 3 mL of total reaction mixture containing 0.5 mL of crude mitochondria extract, 30 mM Tris–HCl buffer (pH 8.0), 0.1 mM Na_3VO_4, 50 mM $NaNO_3$, 50 mM KCl, 3 mM $Ca(NO_3)_2$ and 0.1 mM $(NH_4)_2MoO_4$.

The reaction was initiated by the addition of 100 μL of 30 mM ATP–Tris (pH 8.0). After incubating at 37 °C for 20 min, 30 mM trichloroacetic acid was added into the mixture to terminate the reaction. The absorbance at 660 nm was measured using a spectrophotometer (UV-1750, Shimadzu Corporation, Tokyo, Japan). One unit of H^+-ATPase and Ca^{2+}-ATPase activities were expressed as the release of 1 μmol of phosphorus per second.

2.6. Measurement of cytochrome C oxidase (CCO) activity

CCO activity was assayed by the method of Jin et al. (2013). The assay mixture contained 50 mM phosphate buffer (pH 7.5), 20 mM dimethyl phenylene diamine and 0.3 mM reduced cytochrome C. The reaction was initiated by adding 0.5 mL of crude mitochondria extract and absorbance change was recorded at 510 nm. One unit of CCO activity was defined as an increase of 0.01 in absorbance per second under the assay conditions.

2.7. Measurement of succinate dehydrogenase (SDH) activity

SDH activity was measured according to the method of Acevedo et al. (2013). The substrate solution contained 50 mM potassium phosphate buffer (pH 7.8), 0.08 mM DCPIP, 1 mM phenazine methosulphate (PMS), 4 mM sodium azide and 100 mM sodium succinate, incubating at 30 °C for 10 min. Blanks were performed by replacing the succinate with 100 mM sodium malonate. The activity was determined by adding 0.5 mL of crude mitochondria extract. The absorbance was measured at 600 nm. One unit of SDH activity was defined as an increase of 0.01 in absorbance per second.

Protein content in the crude mitochondria extract was determined according to the method of Bradford (1976), using bovine serum albumin as a standard. Specific activity of the enzymes was expressed as units per kilogram protein.

2.8. Measurement of ATP, ADP and AMP contents and energy charge

ATP, ADP and AMP were extracted and assayed according to Zhou et al. (2014) with a minor modification. Frozen sample (2.0 g) was ground in liquid nitrogen and extracted with 5 mL of 0.6 M

perchloric acid. The homogenate was centrifuged at 12,000 × *g* for 15 min at 4 °C. A 3 mL aliquot of the supernatant was quickly neutralized to pH 6.5–6.8 with 1 M KOH, then diluted to 5 mL and finally passed through a 0.45 μm filter. Measurements of ATP, ADP and AMP contents were conducted by a high-performance liquid chromatography (HPLC) system (LC-2010A, Shimadzu Corporation, Kyoto, Japan) equipped with a reverse-phase Luna 5u C18 column (4.6 mm × 250 mm, Phenomenex, Torrance, CA) and a ultraviolet detector. Mobile phase A consisted of 30 mM K_2HPO_4 and 20 mM KH_2PO_4 dissolved in deionized water and adjusted to pH 7.0 with 0.1 M KOH. Mobile phase B was methyl alcohol. Elution was conducted by a linear gradient program as follows: 0 min, 100% A; 7 min, 80:20 (A:B); 9 min, 75:25 (A:B); and 10 min, 100% A. The program took a further 5 min to return to the initial conditions and stabilise. The flow rate was 16.7 μL/s. Sample aliquots (20 μL) were injected into the HPLC, and peaks were detected at 254 nm. ATP, ADP, and AMP contents were determined according to the external standard program and expressed on a fresh weight basis. Energy charge was calculated as:

$$\frac{[\mathrm{ATP} + 1/2\ \mathrm{ADP}]}{[\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP}]} \times 100\%$$

2.9. Measurement of fructokinase and glucokinase activities

Fructokinase and glucokinase activities were measured according to a modification of the method of Schaffer and Petreikov (1997). A frozen sample (3.0 g) was homogenized in 6 mL extraction buffer consisting of 100 mM Tris–HCl (pH 8.0), 10% glycerol, 2 mM DTT, 10 mM KCl, 5 mM EDTA, 1 mM $MgCl_2$. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was collected for the enzyme assays. The assay mixture contained 100 mM Tris–HCl (pH 7.5), 1 mM $MgCl_2$, 10 mM KCl, 1 mM NAD, 1 mM ATP, 1 unit of NAD-dependent glucose-6-phosphate-dehydrogenase and 0.1 mL enzyme extract.

For the assay of glucose phosphorylation, the reaction was initiated with 2 mM glucose. For the assay of fructose phosphorylation, 2 U of phosphoglucoisomerase was added and the reaction was initiated with 2 mM fructose. One unit of enzyme activity was defined as an increase of 0.01 in absorbance at 340 nm per second under the assay conditions.

2.10. Measurement of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) activities

Samples (2.0 g) were ground in liquid nitrogen and extracted in 6 mL of 100 mM Tris–HCl buffer (pH 8.0) containing 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 1% PVP (w/v). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was used for assaying the enzyme activities. G6PDH and 6PGDH activities were assayed by the method of Sgherri et al. (2002) with modification. G6PDH activity was measured at 25 °C following the change in absorbance at 340 nm in an assay mixture containing 100 mM Tris–HCl buffer (pH 8.0), 10 mM $MgCl_2$, 1 mM $NADP^+$ and 0.1 mL enzyme extract. The reaction was initiated by the addition of 5 mM glucose-6-phosphate. For the determination of 6PGDH, the reaction mixture was similar to that described for G6PDH, but the substrate was 5 mM 6-phosphogluconate. One unit of enzyme activity was defined as an increase of 0.01 in absorbance per second under the assay conditions.

2.11. Statistical design

Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA). The overall least significant difference (LSD) at $P = 0.05$ was calculated and used to detect significant differences among treatments.

3. Results

3.1. Effect of NO on CI index and electrolyte leakage

Chilling symptoms were observed in both control and NO-treated fruit after 5 day under storage at 7 °C, and the CI index gradually increased. NO treatment significantly ($P < 0.05$) reduced CI index of cold-stored banana fruit. As shown in Fig. 1A, CI index was 37.5% and 26.9% lower in NO treated-fruit than in control fruit on day 5 and day 10, respectively. Chilling stress caused increases of electrolyte leakage in banana fruit during cold storage. Electrolyte leakage of control fruit increased slightly during storage while NO treatment remarkably inhibited the increases in electrolyte leakage (Fig. 1B).

3.2. Effect of NO on contents of ATP, ADP and AMP and energy charge

ATP content increased slightly within the first 5 day and decreased rapidly to the end of the storage (Fig. 2A). NO treatment maintained the significantly ($P < 0.05$) higher ATP content in comparison with control fruit after 10 day of storage. ADP content increased before day 15 and decreased afterwards (Fig. 2B). NO-treated banana fruit exhibited higher ADP content in comparison with control fruit during the storage period. AMP content gradually increased with the storage time, which was inhibited by NO treatment (Fig. 2C). Based on the changes of ATP, ADP and AMP, energy charge in banana fruit decreased during the whole storage (Fig. 2D). Compared with control fruit, the decrease of energy charge was retarded by NO treatment.

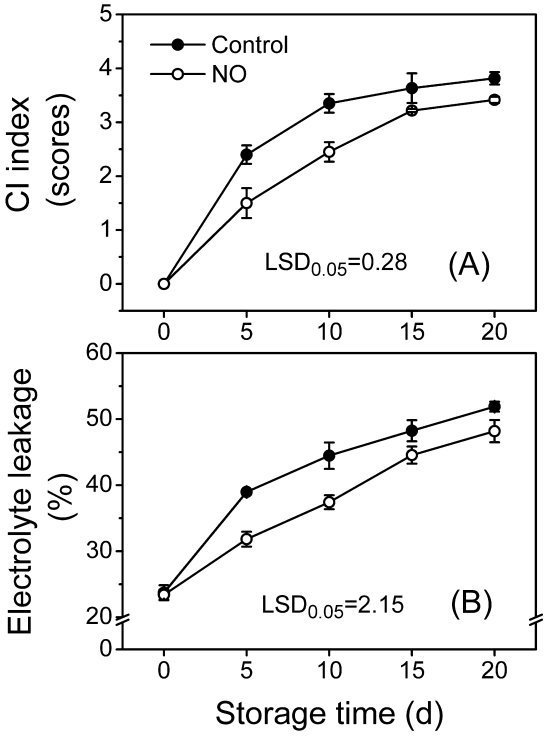

Fig. 1. Effect of NO treatment on CI index (A) and electrolyte leakage (B) of banana fruit during cold storage. Values are the means ± SD of triplicate assays.

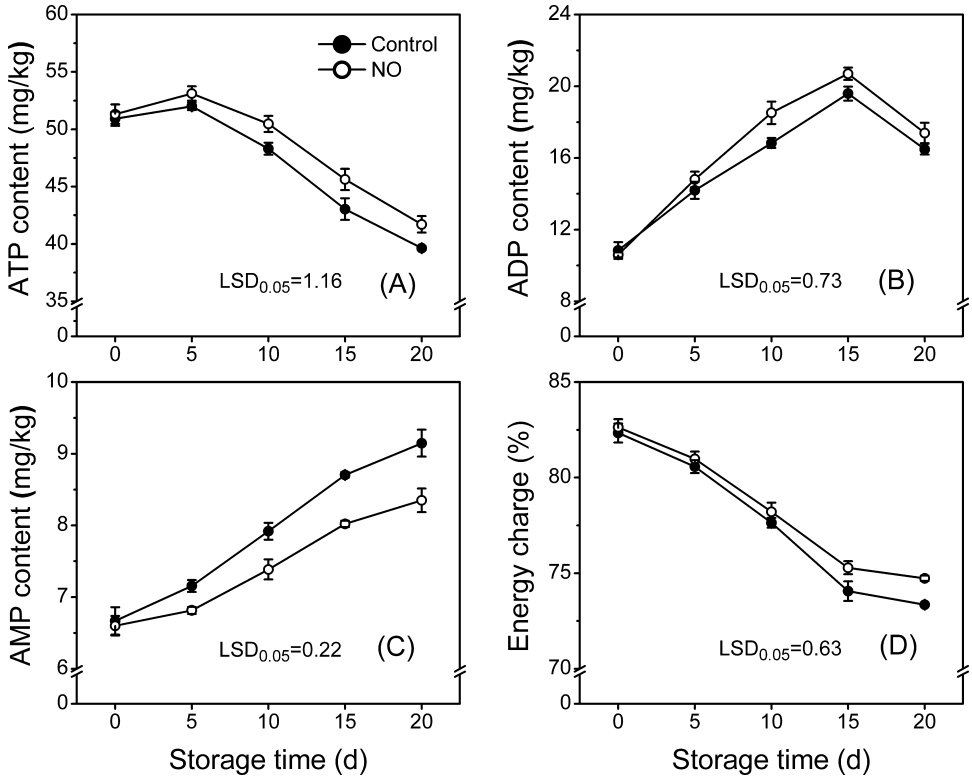

Fig. 2. Effect of NO treatment on contents of ATP (A), ADP (B), AMP (C) and energy charge (D) of banana fruit during cold storage. Values are the means ± SD of triplicate assays.

3.3. Effect of NO on activities of H^+-ATPase and Ca^{2+}-ATPase

H^+-ATPase activity in both control and NO-treated banana fruit decreased gradually with storage time (Fig. 3A). A significantly ($P < 0.05$) higher H^+-ATPase activity was observed in NO-treated fruit compared to control fruit. Ca^{2+}-ATPase activity decreased rapidly over the initial 10 day of storage and then declined steadily afterwards (Fig. 3B). Activities of H^+-ATPase and Ca^{2+}-ATPase in NO-treated fruit were 7.0% and 7.6% higher respectively than control fruit at the end of the storage.

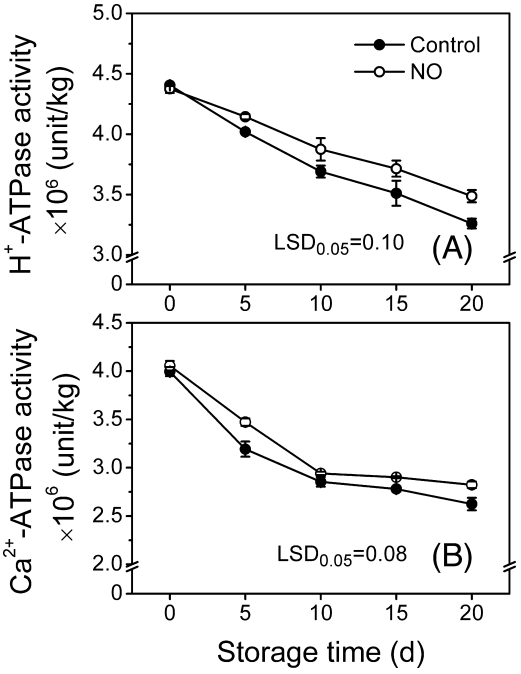

Fig. 3. Effect of NO treatment on activities of H^+-ATPase (A) and Ca^{2+}-ATPase (B) of banana fruit during cold storage. Specific activity of the enzymes was expressed as units per kilogram protein. Values are the means ± SD of triplicate assays.

3.4. Effect of NO on activities of CCO and SDH

As depicted in Fig. 4A, NO treatment significantly accelerated the increase of CCO activity. CCO activity in NO-treated fruit was 20.6% higher than that in control fruit on day 5. No major shift was observed in banana fruit during the whole storage. The activity of SDH declined all over the storage at 7 °C (Fig. 4B). NO-treated fruit exhibited significantly higher SDH activity when compared with control fruit ($P < 0.05$). Averaged over the cold storage period, SDH enzyme activity in NO-treated fruit was 31.2% higher than control fruit.

3.5. Effect of NO on activities of fructokinase and glucokinase

As shown in Fig. 5, changes of fructokinase and glucokinase activities exhibited a similar pattern. Significant differences were observed between the control and NO-treated banana fruit during the whole cold storage ($P < 0.05$). NO treatment retarded the decline of fructokinase and glucokinase activities. At the end of storage, activities of fructokinase and glucokinase in NO-treated fruit were 17.5% and 31.2% higher than that in control fruit, respectively.

3.6. Effect of NO on activities of G6PDH and 6PGDH

G6PDH activity in banana fruit showed an increase during the first 15 day of storage and then a slight decrease for the rest of storage period (Fig. 6A). The increase of G6PDH activity in response to NO treatment appeared sharp in view of a modest increase in the

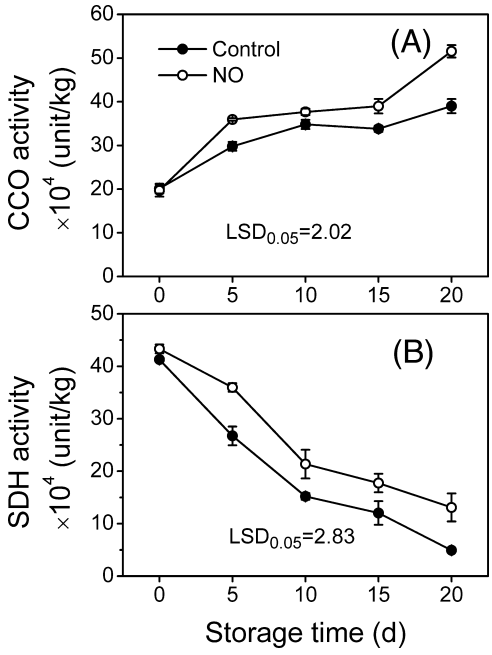

Fig. 4. Effect of NO treatment on activities of CCO (A) and SDH (B) of banana fruit during cold storage. Specific activity of the enzymes was expressed as units per kilogram protein. Values are the means ± SD of triplicate assays.

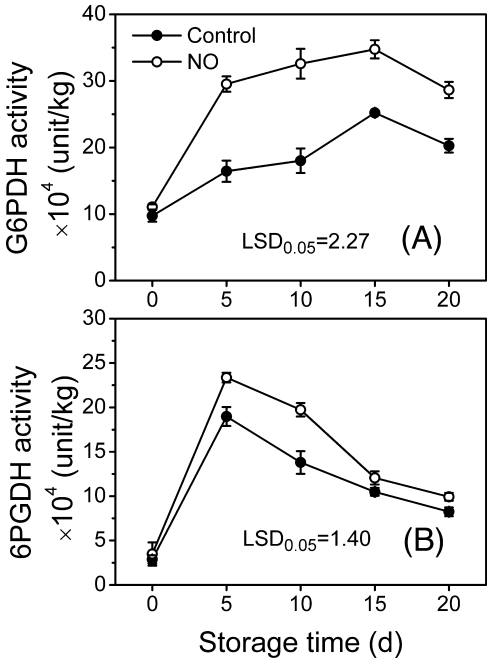

Fig. 6. Effect of NO treatment on activities of G6PDH (A) and 6PGDH (B) of banana fruit during cold storage. Specific activity of the enzymes was expressed as units per kilogram protein. Values are the means ± SD of triplicate assays.

control fruit by day 5. Compare to control fruit, G6PDH activity in banana fruit treated with NO was 1.8-fold on day 5. The patterns of change in 6PGDH activity among the control and NO-treated banana fruit were similar during the cold storage, in which 6PGDH activity increased quickly to a peak on day 5 and then decreased (Fig. 6B). Maximum 6PGDH activity in NO-treated fruit was recorded on day 5 with 23.2% higher than control fruit.

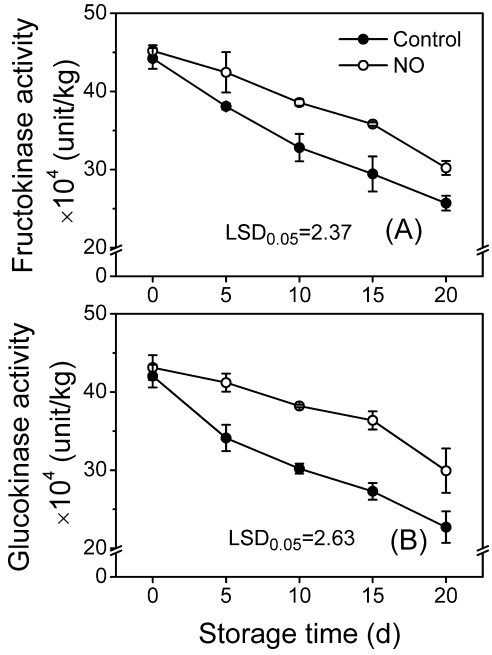

Fig. 5. Effect of NO treatment on activities of fructokinase (A) and glucokinase (B) of banana fruit during cold storage. Specific activity of the enzymes was expressed as units per kilogram protein. Values are the means ± SD of triplicate assays.

4. Discussion

Cellular energy is a crucial factor in controlling ripening and senescence of postharvest horticultural products (Wang et al., 2013a). Increasing evidence has demonstrated that in cold-sensitive fruits stored at low temperature, chilling injuries and physiological disorders may be related to the reduction of cellular energy levels (Liu et al., 2011; Zhou et al., 2014). Cellular energy may directly affect the biosynthesis of membrane lipids and cell membrane restoration, thus mediating the chilling resistance of fruit in response to cold stress (Jin et al., 2014). In the present study, alleviation of chilling injuries in NO-treated banana fruit, as reflected by the reduced CI index and electrolyte leakage (Fig. 1), could be a result of an elevated energy metabolism induced by NO treatment. Long-term cold storage could result in reduced ATP and energy charge in postharvest fruits, leading to disordered energy metabolism. As illustrated in Fig. 2, the ATP content and energy charge in both control and NO-treated banana fruit dropped significantly along with the cold storage. However, NO treatment effectively reduced the development of chilling injury, associated with a higher level of ATP and energy charge as compared to the control fruit. The results suggested that alleviation of chilling injuries in NO-treated banana fruit may be correlated with higher levels of ATP and energy charge. Sufficient available energy status in NO-treated banana fruit contribute to maintenance of membrane integrity, thereby alleviating CI symptoms of banana fruit in long-term cold storage. A similar correlation between energy metabolism and chilling resistance was established for mango, peach and cucumber fruit (Chen and Yang, 2013; Li et al., 2014; Yang et al., 2011).

ATPase is linked with a specific role in protecting chilling-sensitive fruit from the injury effects of low temperature (Ghasernnezhad et al., 2008). H^+-ATPase, a proton pumping ATPase in plants, couples ATP hydrolysis to proton transport out of the cell, thereby establishing an electrochemical proton gradient used for nutrient transport (Sondergaard et al., 2004). Ca^{2+}-ATPase is the primary calcium transporter energized by ATP hydrolysis. A sustained high level of cytosolic calcium could lead to metabolic dysfunction and structural damage in chilling-sensitive plants under cold stress, while Ca^{2+}-ATPase could maintain a calcium homeostasis, thus becoming involved in the development of chilling resistance (Jian et al., 1999). Higher activities of H^+-ATPase and Ca^{2+}-ATPase were observed in NO-treated banana fruit associated with elevated chilling tolerance (Fig. 3). The results suggest that NO-induced chilling tolerance is correlated with the increases of H^+-ATPase and Ca^{2+}-ATPase activities in banana fruit under cold stress. Through promoting the energy metabolism in banana fruit, NO treatment protected the integrity and selected permeability of membrane against chilling stress, and this may result in the higher activities in ATPase enzymes. Jin et al. (2013, 2014),) reported that enhanced activities of H^+-ATPase and Ca^{2+}-ATPase contributed to increasing chilling tolerance of peach fruit during cold storage. Higher levels of the ATPase transcript were observed in hot water-treated mandarins associated with less CI symptoms, indicated that the ATPase may be instrumental in protecting fruit against chilling damage by sustaining the membrane energization (Ghasernnezhad et al., 2008).

Glycolysis, the OPP pathway and the TCA cycle are the central metabolic pathways in plants. These pathways can either provide energy and carbon intermediates for biosynthesis, or couple carbon oxidation with the reduction of NAD(P) to NAD(P)H, which can be used to support biosynthetic reactions or be oxidized by the mitochondrial electron transport chain in the inner mitochondrial membrane (Vanlerberghe, 2013). In the present study, it is suggested that NO-treated banana fruit could maintain the energy metabolism of glycolysis, TCA and OPP to provide sufficient energy for chilling resistance.

SDH plays a fundamental role in both the TCA cycle and the aerobic respiratory chain, catalyzing the oxidation of succinate to fumarate and the reduction of ubiquinone to ubiquinol (Acevedo et al., 2013). CCO is the last enzyme and the terminal electron acceptor in the mitochondrial respiratory electron transport chain, provides energy in the form of ATP for diverse physiological activities in plants (Siedow and Umbach, 1995). Alterations in CCO and SDH activities may lead to disturbance of electron flow in the mitochondrial respiratory chain, thus resulting in chilling injury symptoms (i.e., pitting) in cold-stored blueberries (Zhou et al., 2014). As depicted in Fig. 4, the activities of SDH and CCO in NO-treated banana fruit were significantly higher than that in control fruit. The results suggested that as important enzymes involved in energy metabolism, the enhanced activities of SDH and CCO may contribute to the alleviation in chilling injury by NO treatment. Similar results were obtained in oxalic-treated peach fruit under cold storage (Jin et al., 2014).

Glycolysis, as a catabolic pathway involved in energy metabolism, oxidizes hexoses to generate ATP, reductant, and pyruvate, and produces building blocks for anabolism (Plaxton, 1996). Hexokinases (HXK) is commonly known as the first essential enzyme that catalyzes the ATP-dependent conversion of hexoses to hexose 6-phosphates in glycolysis process of plants (Dai et al., 2002). HXK phosphorylates a large series of hexoses, including fructose, glucose and mannose, while fructokinase and glucokinase specifically phosphorylate fructose and glucose, respectively (Jang et al., 1997). NO-treated banana fruit displayed a higher level of glucokinase and fructokinase activities than control fruit (Fig. 5). These results suggest that the elevated activities of hexokinases may contribute to the enhanced chilling tolerance of NO-treated banana fruit. In agreement with the present study, Cao et al. (2013) found that compared with the chilling sensitive cultivar 'Dahongpao', the chilling resistant 'Ninghaibai' fruit showed higher activities of fructokinase and glucokinase, as well as higher content of hexoses during cold storage of loquat fruit.

The OPP pathway is a major source of NADPH, and maintains the redox potential necessary to protect against oxidative stress (Kruger and von Schaewen, 2003). The main enzymes of OPP pathway that generate reducing power in the form of NADPH are G6PDH and 6PGDH (Sgherri et al., 2002). Airaki et al. (2012) found that the NADPH-generating dehydrogenases such as G6PDH and 6PGDH may play an important role in the process of cold acclimation through their effect on the redox state of the cell. In the present study, changes of G6PDH and 6PGDH activities in banana fruit showed similar patterns during cold storage. The higher activities of G6PDH and 6PGDH observed in the NO-treated banana fruit (Fig. 6), coinciding with the lower CI index, indicated that NO treatment could enhance the chilling resistance of banana fruit through the activation of G6PDH and 6PGDH in the OPP pathway. In red kidney bean roots, G6PDH plays a pivotal role in the NO-involved antioxidant defense system against salt stress (Liu et al., 2007). The dehydrogenase-mediated recycling of NADPH is a key antioxidant system against oxidative stress (Valderrama et al., 2006). It is presumed that NO treatment might maintain the content of NADPH through promoting G6PDH and 6PGDH activities in the OPP pathway. The flux of NADPH may protect banana fruit against the oxidative stress induced by cold temperature, thus leading to a relatively high chilling tolerance.

In conclusion, NO treatment reduced the chilling injury symptoms in banana fruit during 20 d of cold storage. The results indicated that NO-treated banana fruit had an elevated chilling tolerance to low temperature stress. Higher resistance of banana fruit to chilling stress was related to higher levels of ATP and energy charge, which can be attributed to the more active energy metabolism. It is suggested that energy metabolism associated with metabolic pathways such as glycolysis, OPP pathway and TCA cycle, may be involved in the chilling resistance of banana fruit. However, further studies are required to clarify the complex molecular networks regulated by NO as a signal in response to chilling stress.

Acknowledgements

The research was financially supported by the National Basic Research Program of China(2013CB127101), the National Natural Science Foundation of China (31371856) and Zhejiang Provincial Natural Science Foundation of China(LR13C200001).

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