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Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell)

S.P. Singh[a], Zora Singh[a,*], E.E. Swinny[b]

[a] Curtin Horticulture Research Laboratory, School of Agriculture and Environment, Curtin University of Technology, GPO Box U1987, Perth 6845, WA, Australia
[b] Food and Biological Chemistry Laboratory, Chemistry Centre WA, 125 Hay Street, East Perth 6004, WA, Australia

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ABSTRACT

We investigated the effects of nitric oxide (NO) fumigation on fruit ripening, chilling injury, and quality of Japanese plums cv. 'Amber Jewel'. Commercially mature fruit were fumigated with 0, 5, 10, and 20 $\mu L\,L^{-1}$ NO gas at 20 °C for 2 h. Post-fumigation, fruit were either allowed to ripen at 21 ± 1 °C or were stored at 0 °C for 5, 6, and 7 weeks followed by ripening for 5 d at 21 ± 1 °C. NO-fumigation, irrespective of concentration applied, significantly ($P \leq 0.5$) suppressed respiration and ethylene production rates during ripening at 21 ± 1 °C. At 21 ± 1 °C, the delay in ripening caused by NO-fumigation was evident from the restricted skin colour changes and retarded softening in fumigated fruit. NO treatments (10 and 20 $\mu L\,L^{-1}$) delayed the decrease in titratable acidity (TA) without a significant ($P \leq 0.5$) effect on soluble solids concentration (SSC) during ripening. During 5, 6, and 7 weeks of storage at 0 °C, NO-fumigation was effective towards restricting changes in the ripening related parameters, skin colour, firmness, and TA. The individual sugar (fructose, glucose, sucrose, and sorbitol) profiles of NO-fumigated fruit were significantly different from those of non-fumigated fruit after cold storage and ripening at 21 ± 1 °C. CI symptoms, manifest in the form of flesh browning and translucency, were significantly lower in NO-fumigated fruit than in non-fumigated fruit after 5, 6, and 7 weeks storage followed by ripening for 5 d at 21 ± 1 °C. NO-fumigation was effective in reducing decay incidence in plums during ripening without storage and after cold storage at 0 °C for 5, 6, and 7 weeks. In conclusion, the postharvest exposure of 'Amber Jewel' plums to NO gas (10 $\mu L\,L^{-1}$) delayed ripening by 3–4 d at 21 ± 1 °C, and also alleviated chilling injury symptoms during cold storage at 0 °C for 6 weeks.

1. Introduction

Nitric oxide (NO), a highly reactive free radical gas, acts as a multifunctional signalling molecule in various physiological processes in animal and plants (Wendehenne et al., 2001). NO modulates hormonal, wounding, and defence responses in plant tissues (Wendehenne et al., 2004) and its endogenous levels are higher in immature than in mature and ripe tissues of climacteric and non-climacteric fruit (Leshem and Pinchasov, 2000). The endogenous levels of ethylene and NO during fruit development and maturation have inverse and stoichiometric relationships. NO levels decrease with maturation and senescence in horticultural crops (Leshem et al., 1998; Leshem and Pinchasov, 2000), thereby offering an opportunity for modulation of their levels with exogenous application to exert the opposite effect.

Short-term exposure of intact and fresh-cut horticultural commodities to very low concentrations of NO is known to retard their postharvest senescence (Wills et al., 2000; Pristijono et al., 2006; Zhu and Zhou, 2007; Zhu et al., 2008). Postharvest NO application to intact and fresh-cut produce delayed ripening (Wills et al., 2000; Harris et al., 2003; Zhu and Zhou, 2007), inhibited ethylene biosynthesis (Leshem et al., 1998; Zhu and Zhou, 2007; Zhu et al., 2008; Eum et al., 2009), inhibited cut-surface browning (Pristijono et al., 2006; Wills et al., 2008), and enhanced resistance to postharvest diseases (Zhu and Zhou, 2007; Fan et al., 2008). The mechanism of action of NO in delaying senescence of postharvest horticultural produce, though not completely understood, is via the inhibition of ethylene biosynthesis (Leshem et al., 1998; Zhu et al., 2006; Zhu and Zhou, 2007; Eum et al., 2009). However, adequate evidence does not exist to ascertain the mode of action of NO.

The postharvest life of plums is limited due to a rapid rate of ripening, which is regulated by endogenous and exogenous levels of ethylene (Abdi et al., 1998; Khan and Singh, 2007a). The regulation of ethylene biosynthesis and/or its action through postharvest application of 1-methylcyclopropene (1-MCP) delays ripening and alleviates CI during cold storage of Japanese plums (Khan and Singh, 2007a; Candan et al., 2008). The overall effects of the NO on fruit ripening and quality are presumed to be similar to those of 1-MCP.

* Corresponding author. Tel.: +61 8 9266 3138; fax: +61 8 9266 3063.
E-mail address: Z.Singh@curtin.edu.au (Z. Singh).

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We hypothesized that NO fumigation may delay fruit ripening and alleviate CI symptoms during cold storage of Japanese plums. Therefore, we investigated the effects of NO fumigation on fruit ripening under ambient conditions and on alleviation of CI symptoms during cold storage of 'Amber Jewel' plums. To our knowledge, this is the first report on the NO-induced postharvest life improvement of plums and alleviation of CI in any fruit.

2. Material and methods

2.1. Fruit material

Japanese plums (*P. salicina* Lindell cv. 'Amber Jewel') were harvested at commercial maturity (SSC: 13.0 ± 0.21%; TA: 1.19 ± 0.05%; firmness: 24.8 ± 0.45 N) from the Red Valley Orchard, Karagullen, Perth Hills (latitude 31°57′S; longitude 115°50′E), Western Australia. Fruit trees were 20 years old and were grafted on myrobalan (*Prunus cerasifera* Ehrh.) rootstock, planted in a north-south direction (4.25 m between rows and 1.8 m within rows) and trained on a palmate system. Fruit were transported to the laboratory immediately after harvest, and were subjected to various treatments. Fruit of uniform size and maturity, free from visual blemishes and disease were used for the experiments.

2.2. NO fumigation

Fruit were fumigated with different concentrations of NO (0, 5, 10, and 20 μL L^{-1}) in a sealed plastic container (90 L). The desired concentrations of NO were obtained from a cylinder containing 4810 ± 100 μL L^{-1} NO in nitrogen (BOC Gases Ltd, Sydney, NSW, Australia) and injected into the container through an injection port in the lid of the container. Fruit were held in an atmosphere containing NO for 2 h at 20 °C. NO has been reported to be sufficiently stable at the low concentrations and short treatment times required for produce to be treated in normal air (Soegiarto et al., 2003). Therefore, fruit were fumigated with NO in containers having normal air without depletion of O_2. Control fruit were sealed in a plastic container for the same duration except without addition of NO. After 1.5 h of fumigation, the average concentrations of CO_2 in the headspace of treatment containers injected with 0, 5, 10, and 20 μL L^{-1} NO were 0.29 ± 0.13%, 0.72 ± 0.08%, 0.65 ± 0.27%, and 0.69 ± 0.07%, respectively.

2.3. Experiments

2.3.1. Effects of NO fumigation on ripening under ambient conditions (21 ± 1 °C)

The fruit fumigated with different concentrations of NO (0, 5, 10, and 20 μL L^{-1}) were kept at 21 ± 1 °C, RH 60.4 ± 7.3% for ripening. The experimental design was completely randomized including two factors, NO-fumigation and ripening period. All treatments were replicated three times, and ten fruit were treated as an experimental unit. Respiration and ethylene production rates of fumigated and non-fumigated fruit were determined daily for up to 10 d. The fruit were assessed for various quality parameters (flesh firmness, skin colour, SSC, individual sugars, and TA) at 3 d intervals during fruit ripening, commencing from 0 d.

2.3.2. Effects of NO fumigation on development of CI symptoms and fruit quality during cold storage (0 °C)

The fruit fumigated with different concentrations of NO (0, 5, 10, and 20 μL L^{-1}) were kept in plastic crates lined with 30 μm thick low density polyethylene film (AMCOR Packaging, Pvt. Ltd., Melbourne, Australia) at 0 ± 0.3 °C, RH 86.5 ± 5.5%, for 7 weeks. During cold storage, 30 fruit per replication of each treatment (15 fruit per replication for immediate analysis, fruit allowed to warm to ambient before assessment, and 15 fruit per replication following ripening at 21 ± 1 °C for 5 d) were transferred from the cold store after 5, 6, and 7 weeks to ripen at 21 ± 1 °C for 5 d. The experimental design was completely randomized including two-factors, NO-fumigation and storage/ripening period. Fifteen fruit were treated as an experimental unit.

2.4. Determination of ethylene production and respiration rates

Ethylene production rates of the fruit (two fruit per experimental unit) were determined as previously described by Khan and Singh (2007a) using a gas chromatograph (6890N Network GC system; Agilent Technologies, Palo Alto, CA, USA) fitted with a 2 m long stainless steel column (Porapak-Q, 3.18 mm, mesh size 80/100; Supelco, Bellefonte, PA, USA) and a flame ionization detector (FID). Ethylene production rate was expressed as mmol kg^{-1} s^{-1}.

Respiration rates measured on the basis of amount of CO_2 evolved, were determined as described earlier by Khan and Singh (2007b) using an infra-red gas analyser (Servomex, Gas Analyser, Analyser Series 1450; Servomex Ltd., East Sussex, UK). The respiration rate of fruit was expressed as mol CO_2 kg^{-1} s^{-1}.

2.5. Fruit quality evaluation

Flesh firmness was measured using a texture analyser (TA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) interfaced to a personal computer with Nexygen® software. A 5/16 in. Magness-Taylor probe, with a 500 N load cell on, punctured the peeled fruit at a crosshead speed of 100 mm min^{-1} to 7.5 mm depth. Five fruit per replication were subjected to firmness testing with each fruit punctured on both the sides at equatorial region. The firmness was expressed as newtons (N).

The changes in fruit colour parameters including, L^*, a^*, b^* were measured with a Hunterlab ColorFlex 45°/0° Spectrophotometer (Hunter Associates Inc., Reston, VA, USA) using the 15 mm aperture. The chroma value (c^*) and hue angle ($h°$) were calculated from chromaticity values a^* and b^* as reported earlier by Khan and Singh (2007b). To measure skin colour, four readings were taken from opposite positions of each fruit. For flesh colour measurements, fruit were cut around the equatorial axis, and four readings were taken from the mesocarp tissue of each half of fruit. Ten fruit constituted one replication unit for skin and flesh colour measurements.

To determine the SSC of fruit juice, a digital refractometer (Atago-Palette PR 101; Atago Co., Tokyo, Japan) was used and SSC was expressed as % soluble solids. To determine the TA, juice was titrated against 0.1 N NaOH solution using phenolphthalein as an indicator to pH 8.2, and was expressed as % malic acid.

For extraction and determination of soluble sugars, flesh tissue (~15 g) was homogenized with 15 mL of extraction buffer containing 3% metaphosphoric acid, 2 mM ethylenediaminetetracetic acid (EDTA), and 1% polyvinylpolypyrrolidine (PVPP) followed by centrifugation at 15,000 × *g* for 20 min at 4 °C. After centrifugation, 10 mL of each supernatant was flushed through a pre-conditioned Sep-Pak C-18 cartridge (Waters, Milford, MA, USA). Finally, the sample extract was filtered through the 0.2 μm nylon syringe filter [Alltech Associates (Australia) Ltd., NSW, Australia] and loaded into the 1 mL glass vial.

Reverse phase-liquid chromatography was performed for the determination of individual sugars using a high performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA). An aliquot (20 μL) of the extract was injected using an autosampler. Separation of sugars was performed isocratically with 0.005 N H_2SO_4 + 16% acetonitrile as a mobile phase flowing at 0.3 mL min^{-1} using Aminex® 87 X-H column (300 mm × 7.8 mm; Bio Rad Laboratories, Hercules, CA, USA) which was preceded by a micro-guard cartridge (Carbo-C 30 mm × 4.6 mm; Bio Rad Laboratories, Hercules,