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

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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 µL 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 the respiration and ethylene production rates during fruit ripening at 21 ± 1 °C. At 21 ± 1 °C, the delay in fruit ripening caused by NO-fumigation was evident from the restricted skin colour changes and retarded fruit softening in fumigated fruit. NO treatments (10 and 20 µL L^{-1}) delayed the decrease in titratable acidity (TA) without a significant ($P \leq 0.5$) effect on soluble solids concentration (SSC) during fruit ripening. During 5, 6, and 7 weeks of storage at 0 °C, NO-fumigation was effective towards restricting changes in the fruit ripening related parameters, skin colour, firmness, and TA. The individual sugars (fructose, glucose, sucrose, and sorbitol) profiles of NO-fumigated fruit were significantly different from non-fumigated fruit after cold storage and ripening at 21 ± 1 °C. CI symptoms, manifested in the form of flesh browning and translucency, were significantly lower in NO-fumigated fruit as compared to non-fumigated fruit after 5, 6, and 7 weeks storage and followed by ripening for 5 d at 21 ± 1 °C. NO-fumigation was effective in reducing the decay incidence in plum fruit 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 µL L^{-1}) delayed fruit ripening by 3-4 d at 21 ± 1 °C, and also alleviated chilling injury symptoms during cold storage at 0 °C for 6 weeks.

Keywords: *Chilling injury; Ethylene; Nitric oxide; Plum; Respiration; Ripening; Storage*

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 fruits (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 in 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 the faster rate of ripening, which is regulated by the 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 fruit 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 the 1-MCP. 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 at ambient and 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: 24.80 ± 0.45 N) from the Red Valley Orchard, Karagullen, Perth Hills (lat. 31 ° 57 'S; long. 115 º 50 'E), Western Australia. Fruit trees were 20 years old and were grafted on myrobalan (*Prunus cerasifera* Ehrh.) rootstock. Fruit trees were planted in a north-south direction (4.25 m between rows and 1.8 m within rows) and were trained on a palmate system. Fruit were transported to the laboratory immediately after harvesting, 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 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 fruit ripening of 'Amber Jewel' plums at 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 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 quality of 'Amber Jewel' plums 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 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 rate

Ethylene production rate of the fruit (two fruit per experimental unit) was 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}.

2.5 Determination of respiration rate

Respiration rate of plum fruit, measured on the basis of amount of CO_2 evolved, was 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}.

2.6 Fruit quality evaluation

2.6.1 Flesh firmness

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" 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 test with each fruit punctured on both the sides at equatorial region. The firmness was expressed as newtons (N).

2.6.2 Fruit skin and flesh colour

The changes in fruit colour parameters including, *L**, *a**, *b** were measured with a Hunterlab ColorFlex 45 ° / 0 º Spectrophotometer (Hunter Associates Inc., Reston, VA, USA) using the 15 mm aperture. The chroma value (c*) and hue angle (h^o) were calculated from chromaticity values *a** and *b** as reported earlier by Khan and Singh (2007b). To measure skin colour, four readings were taken at the opposite directions 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.

2.6.3 SSC and TA

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.

2.6.4 Extraction and determination of soluble sugars

Flesh tissue (~15 g) was homogenized with 15 mL of extraction buffer containing 3 % metaphosporic 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 µ nylon syringe filter [Alltech Associates (Australia) Ltd., NSW, Australia] and loaded into the 1 mL glass vial.

The 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 x 7.8 mm; Bio Rad Laboratories, Hercules, CA, USA) which was preceded by a micro-guard cartridge (Carbo-C 30 mm x 4.6 mm; Bio Rad Laboratories, Hercules, CA, USA) maintained at 25 °C. The detection of sugars was carried out using a refractive index detector (Waters 2414, Milford, MA, USA). The concentrations of different sugars- fructose, glucose, sorbitol, and sucrose, were expressed as g $100g^{-1}$ on fresh weight basis.

2.7 Chilling injury (CI) index

The incidence of CI was assessed immediately after 5, 6, and 7 weeks of storage at 0 °C, and after 5 d of ripening at 21 ± 1 °C. Fifteen plums per replication (three replicates) for each treatment were cut around the equatorial axis, the two halves of each fruit twisted in opposite directions, and the mesocarp was examined for CI symptoms particularly, flesh browning and translucency. CI index was determined using an arbitrary scale (0-5) based on the surface area of fruit flesh affected. The

scale used was: 0, 0 % area affected; 1, 1-20 % area affected; 2, 21-40 % area affected; 3, 41-60 % area affected; 4, 61-80 % area affected; 5, 81-100 % area affected. CI index was calculated by multiplying the number of fruit scored with the same value of the hedonic scale with the corresponding scale number. Finally, the resultant number was divided by the total number of fruit.

2.8 Decay incidence

Fruit showing symptoms of rot, irrespective of the severity, were considered a loss. The decay incidence was recorded in fruit kept at 21 ± 1 °C after 9 and 12 d. Similarly, decay incidence was recorded in fruit stored for 5, 6, and 7 weeks at 0 °C, and also subsequent to ripening at 21 ± 1 °C for 5 d. The percent decay incidence was determined by following the formula: (X/Y) x 100, in which X is the number of fruit decayed and Y is the total number of fruit kept for observations at the beginning of storage.

2.9 Statistical analyses

The experimental data were subjected to Genstat (release 9.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK) using two-way analysis of variance (ANOVA) including treatments and storage period. The effects of various treatments and storage period were assessed using two-way ANOVA and Fisher's least significant differences were calculated following a significant ($P \leq 0.05$) F test. All the assumptions of ANOVA were checked to ensure validity of statistical analysis.

3. Results

3.1 Respiration and ethylene production rate

Exogenous application of NO, irrespective of concentration, significantly ($P \leq 0.5$) reduced the rate of respiration during fruit ripening in 'Amber Jewel' plums at 21 ± 1 °C (Fig. 1A). Non-fumigated fruit exhibited a respiratory climacteric on the 7th and 8th day of fruit ripening. Respiration rate of NO-fumigated fruit was about 2-fold lower than non-fumigated fruit on the 7th day of fruit ripening. The differences in the respiration rates of fruit fumigated with 10 and 20 µL L^{-1} NO were non-significant, but were significantly ($P \leq 0.5$) lower than those fumigated with 5 µL L^{-1} NO.

NO concentrations of 10 and 20 µL L^{-1} significantly ($P \leq 0.5$) inhibited the ethylene production rate in 'Amber Jewel' plums during fruit ripening at 21 ± 1 °C as compared to 0 and 5 µL L^{-1} NO (Fig. 1B). Non-fumigated fruit exhibited a rise in ethylene production during fruit ripening and achieved a climacteric peak on the 6th day. Fumigation of fruit with 10 and 20 µL L^{-1} NO completely inhibited the ethylene production for the first 8 d of ripening. However, ethylene production was resumed on the 5th day in fruit subjected to 5 µL L^{-1} NO-fumigation and a peak in ethylene production rate, about 4-fold lower than the non-fumigated fruit, was observed on the following day.

3.2 Flesh firmness

Throughout the ripening period at 21 ± 1 °C, NO-fumigated fruit had a significantly ($P \leq 0.5$) higher flesh firmness than non-fumigated fruit (Fig. 2A). During the first 9 d, the decrease in flesh firmness in fruit exposed to 0, 5, 10, and 20 µL L^{-1} NO was 2.9-, 1.6-, 1.5- and 1.6-fold, respectively. Fruit fumigated with 0, 5, 10, and 20 µL L^{-1} NO concentrations, respectively, retained 8.4, 26.5, 39.4, and 41.5 % of their initial firmness values on the 12th day. The differences in the flesh firmness of fruit exposed to different concentrations of NO were practically significant and broad on the 12th day in contrast to the differences on the 9th day.

During storage at 0 °C and subsequent ripening, all NO-fumigated fruit exhibited significantly ($P \leq 0.5$) higher flesh firmness than non-fumigated fruit (Fig. 2B). A significant decrease in flesh firmness was observed during storage at 0 °C for 5, 6, and 7 weeks, irrespective of the treatment. However, exposure of fruit to 20 µL L^{-1} NO concentration was more effective as compared to other treatments in reducing the firmness loss during storage up to 6 weeks. At the end of 7 weeks storage period, the flesh firmness values of fruit fumigated with 5, 10, and 20 µL L^{-1} NO concentrations were 1.4-, 1.6-, 1.5-fold higher than non-fumigated fruit. After each storage interval, 5, 6, and 7 weeks, flesh firmness further decreased during fruit ripening at 21 ± 1 °C for 5 d. All NO-treatments significantly reduced the fruit softening during ripening subsequent to storage for 5, 6, and 7 weeks. The positive effects of NO-fumigation on the retention of fruit firmness during ripening declined significantly on weeks 6 and 7, but fruit fumigated with 10 and 20 µL L^{-1} concentrations were, respectively, 1.6- and 2.4-fold firmer than non-fumigated ones.

3.3 Skin colour

All NO-fumigation treatments significantly ($P \leq 0.5$) restricted the changes in fruit skin chromaticity L* and chroma (c*) values during ripening at 21 ± 1 °C (data not shown). The decline in hue angle (h°) observed during 12 d of fruit ripening was slower in NO-fumigated fruit than non-fumigated fruit (Fig. 2C). The hue angle values of fruit fumigated with 0, 5, 10 and 20 µL L^{-1} NO were about 9.5-, 3.3-, 2.6-, and 2.3-fold lower after 12 d than at harvest, respectively.

During storage at 0 °C for 5, 6 and 7 weeks, chromaticity values L* decreased significantly ($P \leq 0.5$) in non-fumigated fruit than in fumigated ones (data not shown). The chroma values of fruit after 5 days at 21 ± 1°C subsequent to cold storage did not differ significantly ($P \leq 0.5$), irrespective of the NO-concentration applied (data not

shown). Hue angle values decreased during storage as well as ripening, and were lower in fruit fumigated with 20 µL L^{-1} NO compared to those fumigated with 10 µL L^{-1} NO after 5, 6, and 7 weeks storage as well as plus 5 d of ripening at 21 ± 1°C (Fig. 2D).

3.4 SSC, individual sugars, and TA

NO-fumigation did not significantly ($P \leq 0.5$) affect the changes in SSC during fruit ripening at 21 ± 1 °C (Fig. 3A). However, an increase in the SSC was restricted to some extent in the fumigated fruit. On the 12^{th} day, fruit fumigated with 20 µL L^{-1} NO concentration had the highest SSC (13.97 %) followed by those fumigated with 5, 10, and 0 µL L^{-1} NO concentrations (13.5, 13.4, and 12.97 %, respectively). NO-fumigation significantly ($P \leq 0.5$) influenced the changes in concentrations of glucose, sorbitol and sucrose, but not of fructose and total sugars, which is the sum of individual sugars concentrations. The concentrations of fructose and glucose increased during ripening in fruit from all treatments including control. No significant differences in fructose concentration were observed in fruit from different treatments after 12 d at 21 ± 1 °C (Fig. 4A), but glucose concentration was significantly higher in fruit exposed to 10 or 20 µL L^{-1} NO compared to non-fumigated fruit (Fig. 4B). The levels of sucrose and sorbitol decreased during the early phase of ripening (3 and 6 d) with a subsequent increase in their concentrations on the 12^{th} day of fruit ripening, and their concentrations were significantly higher in fruit exposed to 0 and 5 µL L^{-1} NO compared to those fumigated with 10 or 20 µL L^{-1} NO (Figs. 4C & 4D). Similar to SSC, total sugars concentration was not significantly affected by the NO-fumigation (data not shown).

NO-fumigation significantly ($P \leq 0.5$) reduced the decrease in TA during fruit ripening (Fig. 3C). The differences in TA of fruit fumigated with 10 and 20 µL L^{-1}

NO concentrations were not significant ($P \leq 0.5$) on the 9th and 12th day at 21 ± 1 °C. Fruit fumigated with NO gas had significantly ($P \leq 0.5$) lower SSC: TA ratio than non-fumigated ones, suggesting retarded fruit ripening. A significant improvement in the SSC: TA ratio was observed during fruit ripening for 12 d (data not shown).

A minor decrease in SSC of fruit for all treatments was observed after 5 weeks of storage period, followed by a slight increase for fumigated fruit and a further decrease for non-fumigated fruit after 6 and 7 weeks (Fig. 3B). Fruit fumigated with 20 µL L^{-1} NO concentration had lower SSC than those fumigated with either 5 or 10 µL L^{-1} NO concentrations after 5 d of ripening on each storage interval, 5, 6 and 7 weeks. Statistically, treatment effect on the totals sugars concentration in stored fruit was non-significant (data not shown). Nevertheless, NO-fumigation had a significant effect on the individual sugars profiles of fruit. Fructose and glucose concentrations increased significantly during cold storage in all treatments, but the increase in fructose was much higher in non-fumigated fruit compared to fumigated ones (Fig. 4E). Glucose concentration was found significantly higher in fruit fumigated with 10 and 20 µL L^{-1} NO compared to those fumigated with 0 and 5 µL L^{-1} NO after 5 and 6 weeks of storage, but the differences in glucose levels were smaller after 7 weeks (Fig. 4F). Regardless of treatment, a significant reduction in sucrose concentration was observed in fruit during cold storage (Fig. 4G). NO-fumigated fruit retained slightly higher sucrose concentration after 5 weeks of storage than non-fumigated fruit, but the differences were statistically significant. Sorbitol concentration also decreased during storage, but its concentration was significantly higher in non-fumigated fruit than fumigated ones (Fig. 4H).

NO-fumigated fruit retained TA better than non-fumigated fruit during storage for 5, 6, and 7 weeks (Fig. 3D). TA content decreased during 5 d ripening after cold storage; however, the fumigated fruit still had comparatively higher TA than non-

fumigated fruit. As a consequence of reduction in TA, an increase in SSC: TA ratio was observed both during storage and ripening (data not shown).

3.5 Chilling injury (CI)

CI symptoms in the form of flesh browning and translucency were manifested immediately after cold storage and also after 5 d of fruit ripening at 21 ± 1 °C. The severity of CI increased during fruit ripening after cold storage (Fig. 5A). All NO-treatments significantly ($P \leq 0.5$) reduced the CI symptoms during storage and ripening (Fig. 5A). Fruit exposed to 10 and 20 µL L^{-1} NO concentrations exhibited significantly lower CI index during storage and ripening after 5 and 6 weeks than non-fumigated and those fumigated with 5 µL L^{-1} NO. However, the NO-fumigation was more effective in alleviating CI symptoms after 5 and 6 weeks of storage than after 7 weeks of storage at 0 °C. The subjective evaluation of CI index was further confirmed by measuring the flesh chromaticity L* values of the stored fruit. Fruit suffered from CI exhibited a lower flesh chromaticity L* value, either due to flesh browning or translucency, than the healthy or fruit with minor injury (Fig. 5B).

3.6 Decay incidence

Fruit were not given any postharvest fungicide treatment in order to observe any beneficial effect of NO-fumigation. NO-fumigations (10 and 20 µL L^{-1}) were very effective in reducing the decay incidence in 'Amber Jewel' plums (Fig. 6). During the first 9 d at 21 ± 1 °C, the decay incidence in fruit exposed to 0, 5, 10, and 20 µL L^{-1} NO was ~ 26, 20, 2.2, and 2.2 %, respectively, which further increased to ~ 75, 41, 20, and 25 % on the 12^{th} day. NO-fumigated and non-fumigated fruit stored for 7 weeks and then held for 5 d at 21 ± 1 °C showed more decay incidence than those stored for 5 and 6 weeks.

4. Discussion

Postharvest exposure of 'Amber Jewel' plums to NO gas (5, 10, and 20 μL L^{-1}) significantly reduced the respiration rate and ethylene production during fruit ripening at 21 ± 1 °C (Fig. 1). The suppression of respiration during fruit ripening in NO-fumigated peaches and strawberries has been reported earlier (Zhu et al., 2006; Zhu and Zhou, 2007; Flores et al., 2008). We observed a strong inhibition of ethylene production in NO-fumigated fruit leading to a delay in the onset of ripening and senescence (Fig. 1B). The inhibition of ethylene biosynthesis has been reported in NO-fumigated kiwifruit, peach, strawberry, and tomato (Zhu et al., 2006; Zhu and Zhou, 2007; Flores et al., 2008; Eum et al., 2009). Anti-senescent action of NO on plant tissues has been proposed to take place *via* the inhibition of ethylene biosynthesis (Leshem et al., 1998; Zhu et al., 2006).

The reduction in ethylene production during fruit ripening in NO-fumigated fruit may be due to binding of NO with ACC oxidase and ACC to form a stable ternary complex, thus limiting the ethylene production (Tierney et al., 2005). Zhu et al. (2006) supported the proposed mechanism of action of NO in peach fruit and showed that ethylene biosynthesis was mainly due to decreased activity of ACC oxidase and accumulation of ACC and 1-malonylaminocyclopropane-1-carboxylic acid (MACC) without any significant effect on ACC synthase activity. Recently, it was confirmed that the inhibition of ethylene biosynthesis in NO-fumigated tomatoes was due to decreased and delayed expression of ACC oxidase genes (Eum et al., 2008). However, another study on strawberry revealed that NO decreased the activity of ACC synthase, but not ACC oxidase (Zhu and Zhou, 2007). Therefore, we speculate that either one of these proposed or any other mechanism of action of NO *via* ethylene inhibition may exist in Japanese plums. Exploring the possible mode of

action of NO in ethylene inhibition in climacteric and suppressed-climacteric type plums is worthy of further study.

Fruit softening was significantly reduced by NO-fumigation (Figs. 2A & 2B) which might be due to decreased activities of fruit softening enzymes caused by inhibition of ethylene production. Ethylene is directly involved in increasing the activities of fruit softening enzymes (pectin esterase, endoglucanase, exo- and endo-polygalacturonase) in the Japanese plums (Khan and Singh, 2007a). It is, therefore, possible that inhibition of ethylene production through NO-fumigation as observed in our study and blocking of its action though 1-MCP (Abdi et al., 1998; Khan and Singh, 2007a) helps to retard the fruit softening process. Fumigation of peaches with 5 or 10 μL L^{-1} NO (Zhu et al., 2006; Flores et al., 2008) and kiwifruit with 1 μmol L^{-1} NO (Zhu et al., 2008) has been reported to retard the fruit softening during storage and ripening, but a higher concentration of 15 μL L^{-1} NO in peaches and 2 μmol L^{-1} NO in kiwifruit enhanced the fruit softening. The increase in NO concentrations from 5 to 20 μL L^{-1} did not significantly increase fruit softening in plums during fruit ripening for 12 d. Higher concentration (20 μL L^{-1}) instead helped to retain higher firmness during storage at 0°C for 5 and 6 weeks. More research is required to examine if the enhanced fruit softening effect in plums may be obtained by applying higher concentrations of NO.

The magnitude of the effects of NO-fumigation on changes in the hue angle of skin during fruit ripening at 21 ± 1 °C was dose-dependent (Figs. 2C). The decrease in L* value and hue angle marked the increase in the skin colour intensity and accumulation of anthocyanin during fruit ripening in plums (Khan and Singh, 2007b). Abdi et al. (1998) also reported that 1-MCP treatment, whose effects are presumed to be similar to NO, delayed the decrease in hue angle of 'Beauty' plums during 14 d at 20 °C either in the presence of air or propylene. Skin colour changes were noticed in

NO-fumigated fruit even in the absence of ethylene production (Figs. 1B & 2C) which indicates that colour development in 'Amber Jewel' plums during fruit ripening may not be completely dependent on ethylene production. Abdi et al (1998) also proposed that ethylene acts as a catalyst in hastening and co-ordinating the pigment biosynthesis and chlorophyll loss in the climacteric and non-climacteric plums. The decrease in hue angle values of fruit fumigated with 20 µL L^{-1} NO was significantly higher than those fumigated with 10 µL L^{-1} NO after 5, 6, and 7 weeks storage plus 5 d at 21 ± 1 °C. It is still not elucidated why a higher concentration of NO is also counterproductive in peaches and kiwifruit (Zhu et al., 2006; Flores et al., 2008; Zhu et al., 2008).

The changes in other quality parameters such as, SSC, TA and SSC: TA ratios were restricted in NO-fumigated fruit during storage and ripening. Our results are in agreement with Zhu et al. (2006, 2008) who reported a delay in the increase of SSC in peaches and kiwifruit fumigated with 5 or 10 µL L^{-1} NO and 0.5 or 1 µmol L^{-1} NO, respectively, during fruit ripening and storage. The increase in SSC: TA ratio, also called ripening index, was significantly reduced in NO-fumigated fruit during fruit ripening and storage. Similar to Zhu et al. (2006, 2008), we also observed that SSC and SSC: TA ratio were higher in fruit exposed to 20 µL L^{-1} NO concentration than those exposed to 10 µL L^{-1} NO. Our data shows that NO-fumigation significantly influenced the changes in individual sugars during fruit ripening and storage without a significant effect on the SSC and totals sugars. It appears that the effects of NO-fumigation are not only limited to SSC, but are extended to postharvest sugar metabolism of fruit. The role of NO in influencing the metabolism of sugars in fruit has not been defined yet and would be worthy of studying. Previously, 1-MCP treatment has been reported to delay the increase in the SSC: TA ratio during cold storage and ripening of 'Santa Rosa' (Martínez-Romero et al., 2003). Plums fruit

ripening involves a slight increase in sugars concentration and a significant loss of malic acid, the major organic acid (Singh and Singh, 2008), which result into an increase in the SSC: TA ratio. Irrespective of treatment, a marked decrease in the sucrose concentration during cold storage may be attributed to its hydrolysis leading to increase in concentrations of fructose and glucose, depending upon the treatment. The patterns of the changes in different types of sugars during storage and ripening are very complex and inconclusive.

The occurrence of CI and increase in its severity during the fruit ripening at 21 ± 1 °C after cold storage were observed (Fig 5A). These observations are consistent with the findings of Candan et al. (2008) in 'Larry Ann' plums. Crisosto et al. (1999) also described similar CI symptoms in the various cultivars of plums stored for 5 weeks at 0 °C. The decrease in flesh chromaticity L* value supports the subjective evaluation of CI in affected fruit (Fig. 5B). The flesh browning may be related to the disintegration of tissue membrane resulting into the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to *o*-quinones, which are brown coloured polymers. Our data suggest that the beneficial effect of NO-fumigation on alleviation of CI decreased in fruit stored beyond 6 weeks. The alleviation of CI symptoms in plums could be attributed to the inhibition of ethylene production during cold storage of NO-fumigated fruit as reported in case of kiwifruit and peaches (Zhu et al, 2006, 2008). A reduction in CI symptoms during cold storage of 1-MCP- treated 'Larry Ann' plums was observed by Candan et al. (2008), which suggests a significant role of ethylene in the series of events associated with the initiation and development of CI symptoms in plums during cold storage.

The decay incidence was significantly lower in NO-fumigated fruit as compared to non-fumigated fruit during ripening at 21 ± 1 °C and cold storage at 0 °C for 7 weeks (Fig. 6). Exogenous application of NO has been reported to enhance the

disease resistance in postharvest strawberries and tomatoes (Zhu and Zhou, 2007; Fan et al., 2008). These authors proposed that NO application initially triggered the endogenous H_2O_2 level and delayed its accumulation in higher concentrations at later stages. More research is required to reveal the mechanism of NO-induced disease resistance in postharvest fruits and vegetables.

This study has shown that postharvest application of NO (10 μL L^{-1}) has potential to delay the fruit ripening in Japanese plums. NO fumigation may be useful to improve the storage potential of plums at 0°C through alleviation of CI symptoms to some extent. Further work is required to understand the mechanisms of action of NO gas in the climacteric and suppressed-climacteric type plums that may allow its commercial use in future.

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References

Abdi, N., McGlasson, W.B., Holford, P., Williams, M., Mizrahi, Y., 1998. Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene. Postharvest Biol. Technol. 14, 29–39.

Candan, A.P., Graell, J., Larrigaudière, C., 2008. Roles of climacteric ethylene in the development of chilling injury in plums. Postharvest Biol. Technol. 47, 107-112.

Crisosto, C.H., Mitchell, F.G., Ju, Z., 1999. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. HortScience 34, 1116-1118.

Eum, H.L., Kim, H.B., Choi, S.B., Lee, S.K., 2009. Regulation of ethylene biosynthesis by nitric oxide in tomato (*Solanum lycopersicum* L.) fruit harvested at different ripening stages. Euro. Food Res. Technol. 228, 331-338.

Fan, B., Shen, L., Liu, K., Zhao, D., Yu, M., 2008. Interaction between nitric oxide and hydrogen peroxide in postharvest tomato resistance response to *Rhizopus nigricans*. J. Sci. Food Agric. 88, 1238-1244.

Flores, F.B., Sánchez-Bel, P., Valdenegro, M., Romojaro, F., Martínez-Madrid, M.C., Egea, M.I., 2008. Effects of a pretreatment with nitric oxide on peach (*Prunus persica* L.) storage at room temperature. Euro. Food Res. Technol. 227, 1599-1611.

Harris, D.R., Wills, R.B.H., Seberry, J.A., Ward, K.R., 2003. Use of ISONOP200 for measurement of NO in the gas phase under controlled humidity conditions. Nitric Oxide: Biol. Chem. 9, 135-140.

Khan, A.S., Singh, Z., 2007a. 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of 'Tegan Blue' plum. Postharvest Biol. Technol. 43, 298-306.

Khan, A.S., Singh, Z., 2007b. Methyl jasmonate promotes fruit ripening and improves fruit quality in Japanese plum. J. Hortic. Sci. Biotechnol. 82, 695-706.

Leshem, Y.Y., Pinchasov, Y., 2000. Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries *Fragaria anannasa* (Duch.) and avocado *Persea americana* (Mill.). J. Exp. Bot. 51, 1471-1473.

Leshem, Y.Y., Wills, R.B.H., Ku, V.V.V., 1998. Evidence for the function of the free radical gas - nitric oxide ($NO^{\cdot}$) - as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol. Biochem. 36, 825-833.

Martínez-Romero, D., Dupille, E., Guillèn, F., Valverde, J.M., Serrano, M., Valero, D., 2003. 1-Methylcyclopropene increases storability and shelf life in climacteric and nonclimacteric plums. J. Agric. Food Chem. 51, 4680-4686.

Pristijono, P., Wills, R.B.H., Golding, J.B., 2006. Inhibition of browning on the surface of apple slices by short term exposure to nitric oxide (NO) gas. Postharvest Biol. Technol. 42, 256-259.

Singh, S.P., Singh, Z., 2008. Major flavor components in some commercial cultivars of Japanese plum. J. Am. Pomol. Soc. 62, 185-190.

Soegiarto, L., Wills, R.B.H., Seberry, J.A., Leshem, Y.Y., 2003. Nitric oxide degradation in oxygen atmospheres and rate of uptake by horticultural produce. Postharvest Biol. Technol. 28, 327-331.

Tierney, D.L., Rocklin, A.M., Lipscomb, J.D., Que, L., Jr., Hoffman, B.M., 2005. ENDOR studies of the ligation and structure of the non-heme iron site in ACC oxidase. J. Am. Chem. Soc. 127, 7005-7013.

Wendehenne, D., Pugin, A., Klessig, D.F., Durner, J., 2001. Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends Plant Sci. 6, 177-183.

Wendehenne, D., Durner, J., Klessig, D.F., 2004. Nitric oxide: a new player in plant signalling and defence responses. Current Opinions Plant Biol. 7, 449-455.

Wills, R.B.H., Ku, V.V.V., Leshem, Y.Y., 2000. Fumigation with nitric oxide to extend the postharvest life of strawberries. Postharvest Biol. Technol. 18, 75-79.

Wills, R.B.H., Pristijono, P., Golding, J.B., 2008. Browning on the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO). Food Chem. 107, 1387-1392.

Zhu, S., Liu, M., Zhou, J., 2006. Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. Postharvest Biol. Technol. 42, 41-48.

Zhu, S., Sun, L., Liu, M., Zhou, J., 2008. Effect of nitric oxide on reactive oxygen species and antioxidant enzymes in kiwifruit during storage. J. Sci. Food Agric. 88, 2324-2331.

Zhu, S., Zhou, J., 2007. Effect of nitric oxide on ethylene production in strawberry fruit during storage. Food Chem. 100, 1517-1522.

Figures captions

Fig. 1. Effects of nitric oxide (NO) fumigation on the respiration (A) and ethylene production rates (B) of 'Amber Jewel' plums during the fruit ripening period at 21 ± 1 °C. Vertical bars represent S.E. of means. Some error bars are invisible due to the lower value of S.E against the y-axis scale.

Fig. 2. Effects of nitric oxide (NO) fumigation on flesh firmness and hue angle of 'Amber Jewel' plums during the fruit ripening period at 21 ± 1 °C (A and C) and after 5, 6, and 7 weeks storage at 0 °C and subsequently ripened at 21 ± 1 °C for 5 d (B and D). Vertical bars represent S.E. of means. Some error bars are invisible due to the lower value of S.E against the y-axis scale. Black bars indicate the flesh firmness and hue angle values at harvest.

Fig. 3. Effects of nitric oxide (NO) fumigation on soluble solids concentration (SSC) and titratable acidity (TA) of 'Amber Jewel' plums during the fruit ripening period at

21 ± 1 °C (A and C) and after 5, 6, and 7 weeks storage at 0 °C and subsequently ripened at 21 ± 1 °C for 5 d (B and D). *n* = 3 replicates. Vertical bars represent S.E. of means. Some error bars are invisible due to the lower value of S.E against the y-axis scale. Black bars indicate the SSC and TA at harvest.

Fig. 4. Effects of nitric oxide (NO) fumigation on the concentrations of fructose, glucose, sucrose, and sorbitol of 'Amber Jewel' plums during the fruit ripening period at 21 ± 1 °C (A, B, C, and D, respectively) and after 5, 6, and 7 weeks storage at 0 °C and subsequently ripened at 21 ± 1 °C for 5 d (E, F, G, and H, respectively). *n* = 3 replicates. Vertical bars represent S.E. of means. Some error bars are invisible due to the lower value of S.E against the y-axis scale. Black bars indicate the concentrations of fructose, glucose, sucrose, and sorbitol at harvest.

Fig. 5. Effects of nitric oxide (NO) fumigation on chilling injury (CI) index (A) and flesh chromaticity L* (B) of 'Amber Jewel' plums stored for 5, 6, and 7 weeks at 0 °C and subsequently ripened at 21 ± 1°C for 5 d. Vertical bars represent S.E. of means. Black bar indicates the flesh chromaticity value at harvest.

Fig. 6. Effects of nitric oxide (NO) fumigation on decay incidence in 'Amber Jewel' plums either stored for 12 d at 21 ± 1 °C or stored for 5, 6, and 7 weeks at 0 °C and subsequently ripened at 21 ± 1 °C for 5 d. Vertical bars represent S.E. of means.