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The Responses of *Yukbo* Strawberry (*Fragaria ananassa* Duch.) Fruit to Nitric Oxide

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Abstract The quality of *Yukbo* strawberry (*Fragaria ananassa* Duch.) fruit declines rapidly after harvest. Therefore, we examined the effects of nitric oxide (NO) on its respiration rate, quality, and shelf life. Strawberries were fumigated for 5 hr at 0, 50, 100, 200, or 500 μL/L NO atmospheres, followed by a hold at 18°C in air. Treatment with NO delayed the onset of ethylene production and reduced respiration, which at 18°C resulted in a maintained quality and prolonged shelf life. The NO-treated strawberries were also firmer and had a lower incidence of disease than the untreated fruit. The effect of NO on fruit quality was dose-dependent. Strawberries that were treated with low and high concentrations of 50 and 500 μL/L NO, respectively, had severe disease incidence and were of poor quality. Treating with NO at a concentration of 200 μL/L appeared to slow down the ripening and senescence of fruit stored at 18°C. Calyx browning, respiration, and rot development progressed more quickly in strawberries treated with 500 μL/L NO compared to those treated with 200 μL/L NO.

Keywords: strawberry, nitric oxide, quality, calyx, browning

Introduction

Strawberry fruit has tender tissue and is very susceptible to microorganisms and mechanical damage (1). It has a high metabolic rate and degrades rapidly after harvest (2). Even under ideal conditions at 0°C its storage life may be less than one week. With a combination of water loss, softening, and bruising during handling and transport, greater than 40% of the fruit may be lost before it reaches the consumer.

Nitric oxide (NO) is a potentially toxic and relatively unstable free radical gas. Due to its highly lipophilic nature NO can diffuse through cell membranes without the aid of specific membrane transporters, and therefore mediates cellular activities (3). Leshem and Haramaty (4) have reported on the existence and direct characterization of endogenous NO in higher plants, finding that on a molar basis pea foliage emitted more NO than ethylene and that the ethylene precursor aminocyclopropane-1-carboxylic acid (ACC) enhanced both NO and ethylene emissions. NO has been found to be ubiquitous in post-harvest climacteric and nonclimacteric fruits, vegetables, and flowers, with higher levels present in unripe than in ripe tissues (5). NO production has been shown to decrease as plant tissue senesces; fresh flowers emitted approximately twice as much NO as senesced flowers (3).

NO may also play a messenger role in plant pathogen resistance (6-8) by causing increases in cGMP and salicylic acid levels, which work as key factors in the hypersensitive reaction (HR) and cell death. In addition to NO may have a protective role against pathogens. In potato leaves that were infected with the pathogenic fungus *Phytopthora infestans*, the NO donor sodium nitroprussede (SNP) was reported to affect several mechanisms by either preserving the chloroplast membrane of the infected leaf against toxic reactive oxygen species, or by the direct involvement in the chlorophyll breakdown pathway (9).

The objective of this study was to investigate if NO treatment could maintain the quality of strawberry fruit. This was done by evaluating the effects of NO on fungal decay, respiration, ethylene production, and the quality parameters of strawberry fruit. Since the effect of NO on the fruit quality was expected to occur in a dose-dependent manner, we tested various NO concentrations.

Materials and Methods

Plant material *Yukbo* strawberry fruit was supplied by commercial growers from the Chungnam, Korea. Strawberry fruit that was clean, bright, and free from physical damage and deterioration was selected for experimental use.

Treatments Each strawberry fruit chosen for testing was placed in a sealed 15 L plastic container. Due to the rapid oxidation of NO to nitrogen dioxide, it was necessary to maintain a near zero oxygen level when applying NO (10). Therefore, the container was flushed with humidified nitrogen gas at 3 L/min for about 20 min to displace all the oxygen; NO (Daehan special gas Ltd., Seoul, Korea) was then added. After treatments of 50, 100, 200, or 500 μL/L NO the containers remained sealed for 5 hr at 15°C, after which the fruit was stored at 18°C in air. A previously established laboratory test (unpublished) was used to assess the effects of the fumigation time on the extension of post-harvest life. The storage temperature (18°C) was determined based on wholesale market facilities.

Analysis of the gases The carbon dioxide and ethylene gas concentrations were measured by sampling the headspace in 1 L jars with a 1 mL gas-tight syringe. Thermal conductivity and flame ionization detectors with

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gas chromatography (model M600D; Young Lin, Korea) were used to analyze carbon dioxide and ethylene, respectively.

Quality assessment The quality evaluations included measurements of weight loss, flesh firmness, calyx browning, disease incidence, and overall acceptability throughout the storage period. The strawberries weighed every day and the fresh weight loss was calculated as a percentage of the original weight. Flesh firmness was measured using a texture analyzer with a needle tip (TA-XT2; Stable Micro Systems Ltd., Godalming, Surrey GU7 1YL, UK). Calyx browning was described by its visual appearance. Disease incidence was expressed as the percentage of infected fruit per punnet. The condition of the fruit inside each punnet was evaluated every day by the same individual, using rating scales from 1 to 10 for overall acceptability. In each case 10 represented the best quality and 1 the worst.

Data analysis All of the experiments were prepared using completely randomized designs. SAS statistical software (SAS Institute, Cary, NC, USA) was used to conduct variation analyses (ANOVA) on the treatment results.

Results and Discussion

The effect of NO on gas exchange Strawberry fruit has a high respiration rate and is more perishable than any other fruit. Thus, lowering its respiration rate may increase the post-harvest life of the fruit. All the experiment samples had increased rates of CO_2 production after 3 days of storage at 18°C. High CO_2 production rates continued in the strawberries that were left untreated, and after 5 days the production rate was more than 90 mL/kg/hr. Treatments with 50, 100, or 200 μL/L NO resulted in significantly lower levels of respiration. However, after 4 days there were significant differences among the treatments. The 500 μL/L NO treatment, the highest NO concentration that was administered, was found to be detrimental to the fruit (Fig. 1). Therefore, it appears NO can be useful for decreasing the respiration rate of strawberries when it is applied at proper concentrations. Borutaite and Brown (11) used isolated rat mitochondria to show that NO may inhibit respiration by disrupting mitochondrial electron transfer via damage to the iron-sulfur centers at the cytochrome oxidase and ubiquinone-cytochrome b regions of the respiratory chain.

The ethylene production of the untreated fruit that was stored at 18°C started to increase after 2 days and reached a maximum value of 0.64 μL/kg/hr after 3 days. Similarly, ethylene production in the 500 μL/L NO-treated fruit increased after 3 days and reached a maximum value after 4 days (Fig. 2). In the 200 mL/L NO treatment, however, the production of ethylene was very low.

The exogenous application of NO has been shown to delay the fruit ripening process in climacteric and non-climacteric fruit and, furthermore, can inhibit the onset of general senescence (12). In non-climacteric fruit ethylene can stimulate the respiration rate, although the effect is reversible and dependent on the levels of endogenous or

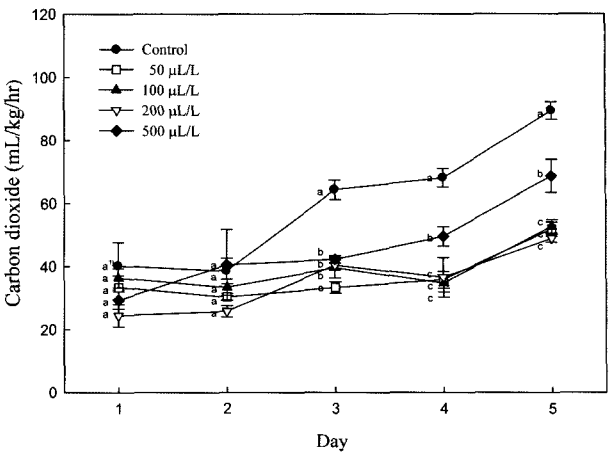

Fig. 1. Rates of CO_2 production by strawberries treated with 0 (control), 50, 100, 200, or 500 μL/L NO during storage at 18°C in air. Error bars indicate the standard error of each mean value (n=3 punnets each containing 10 fruits). Mean separation within column by Duncan's multiple range test at p=0.05.

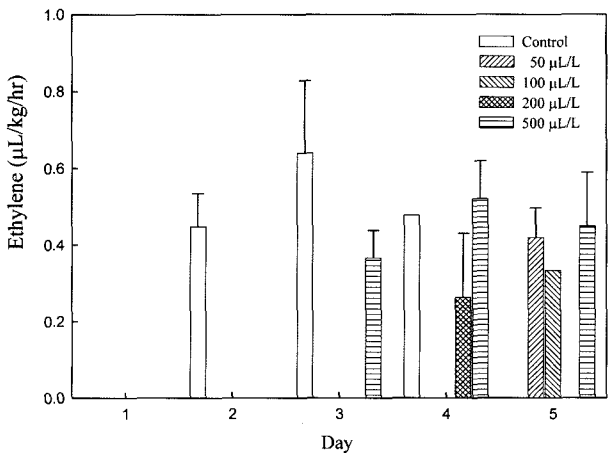

Fig. 2. Rates of ethylene production by strawberries treated with 0 (control), 50, 100, 200, or 500 μL/L NO during storage at 18°C in air. Error bars indicate the standard error of each mean value (n=3 punnets each containing 10 fruits).

exogenous ethylene (13). NO appears to have a similar effect to that obtained from low concentrations of the ethylene antagonist 1-methylcyclopropene (1-MCP). Ku *et al.* (14) found that the fumigation of strawberry fruit with 1-MCP at 5-15 nL/L for 2 hr extended the post-harvest life. It is possible that the mechanisms of action for NO and 1-MCP are similar.

In a wide variety of immature fruit, vegetables, and flowers endogenous levels of NO emissions significantly exceed those that are emitted in the mature or senescing conditions. This phenomenon applies to both climacteric and non-climacteric species (12). If the reduction of NO in mature tissues is a typical and overall trend in plants, then exogenously applied NO could delay ripening and/or senescence. Previous work has indicated that the emission of NO can proceed simultaneously with the emission of ethylene. It was also shown that a significant reduction in C_2H_4 production was induced by the addition of an NO

releasing compound (5). Since ethylene accumulation initiates the ripening of climacteric produce and enhances the senescence of non-climacteric produce, an application of NO may retard ripening and senescence in post-harvest tissues by reducing C_2H_4 production.

The effect of NO on fruit quality Weight loss was significantly reduced in the NO-treated strawberries compared to the untreated fruit. The smallest weight loss was seen when the fruit was exposed to 50 μL/L NO (Table 1). Ku *et al.* (15) proposed that the exogenous application of NO could stimulate horticultural crops to reduce transpiration.

The NO treatment at 200 μL/L had a beneficial effect on flesh firmness throughout the storage period, and the fruit treated with this dose remained significantly firmer than the control (Fig. 3). The untreated fruit had already begun to soften after 3 days, and firmness reduced from 0.41 to 0.34 N. The softening of the NO-treated fruit was delayed and fruit firmness was maintained above 0.40 N in the 200 μL/L NO treatment. Thus, NO has the potential to maintain optimum firmness during the average selling period.

Table 1. Weight loss percentages of strawberries treated with 0 (control), 50, 100, 200, or 500 μL/L NO during storage at 18°C in air

Treatment (μg/L)	Storage period (day)				
	1	2	3	4	5
Control	3.6±0.2[a1)]	6.1±0.3[a]	9.5±0.3[a]	12.8±0.5[a]	22.0±2.2[a]
50	1.7±0.1[c]	3.8±0.1[c]	5.9±0.2[c]	8.7±0.2[c]	11.4±0.2[c]
100	2.1±0.2[cb]	4.5±0.3[cb]	7.4±0.3[b]	10.8±0.4[b]	13.5±0.4[cb]
200	2.1±0.2[cb]	4.7±0.4[b]	7.4±0.6[b]	10.3±0.8[b]	13.6±0.9[cb]
500	2.4±0.1[b]	5.0±0.2[b]	8.6±0.3[a]	11.4±0.3[b]	15.2±0.4[b]

[1)]Mean separation within column by Duncan's multiple range test at p =0.05 (n=3 punnets each containing ten fruit).

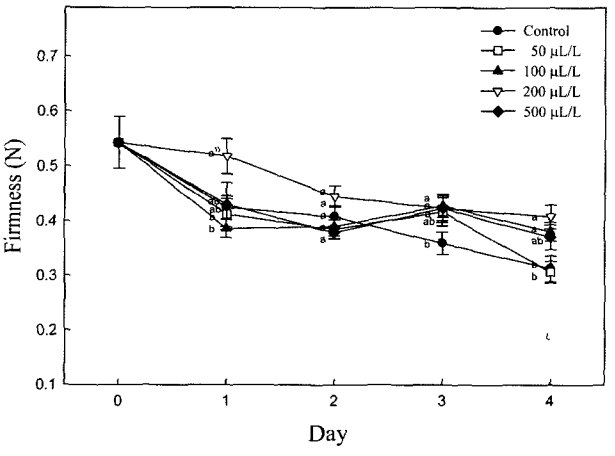

Fig. 3. Firmness of strawberries treated with 0 (control), 50, 100, 200, or 500 μL/L NO during storage at 18°C in air. Error bars indicate the standard error of each mean value (n=3 punnets each containing 10 fruits). Mean separation within column by Duncan's multiple range test at p=0.05.

The calyx color tends to change over time causing the overall acceptability of the strawberry fruit to decrease. NO was able to inhibit the browning of the calyx, especially at 200 μL/L (Fig. 4). However, in the 500 μL/L NO treatment and in the control, the browning was severe after 4 days of storage. NO treatments at high concentrations caused faster color degradation of the chlorophyll within the calyx. Similar results were observed with a previous study, in which 500 μL/L NO caused the development of browning around the calyx and 4,000 μL/L NO developed a dull color and severe blackening around the calyx soon after fumigation (16).

Leshem (17) reported that NO is toxic rather than protective at high concentrations. NO gas may diffuse from the apoplast, penetrate the periplastic membrane bilayer, and then react with the phospholipid components. Thus, increased NO concentrations may cause an attack of the unsaturated fatty acyl chains, whose carboxyl groups are especially prone to free radical attack (12). The inhibitory effect of higher NO concentrations could therefore be understood in terms of membrane impairment and leakiness. In previous research Laxalt *et al.* (9) observed that the chlorophyll content of infected potato leaf pieces markedly decreased in the absence of SNP, an NO-releasing compound. In contrast, SNP treatment effectively maintained the chlorophyll content in the infected leaves. In addition, early reports have demonstrated that chlorophyll degradation depends on respiration during senescence (18). We found that the NO-treated fruit had a lower respiration rate and a bright green colored calyx with 200 μL/L NO rather than with the 500 μL/L concentration.

A loss of quality in the fruit was due mostly to the onset of rotting. Disease development progressed more quickly in the untreated fruit than in the NO-treated fruit. NO did not completely prevent the incidence of rot, but in most cases it was reduced. Rot development progressed more quickly in the 50, 100, and 500 μL/L NO-treated fruit than in fruit treated with 200 μL/L NO (Fig. 5).

The NO-mediated stress coping response relates to the resistance of pathogenic infection. Active oxygen species (AOS) associated plant defenses are similar to the inflammatory and immune responses of animals, where

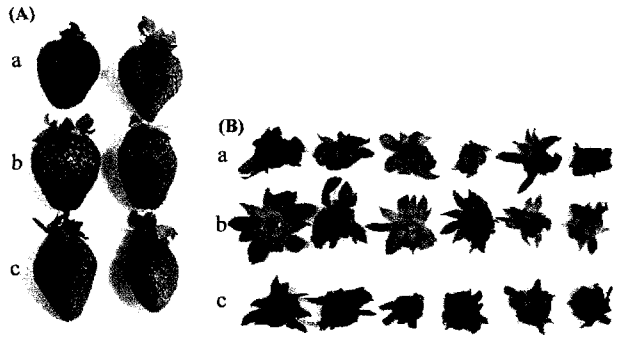

Fig. 4. Changes in strawberry fruit quality (A) and calyx browning (B) after 4 days storage at 18°C in air. a, Without treatment; b, treated with 200 μL/L NO; c, treated with 500 μL/L NO.

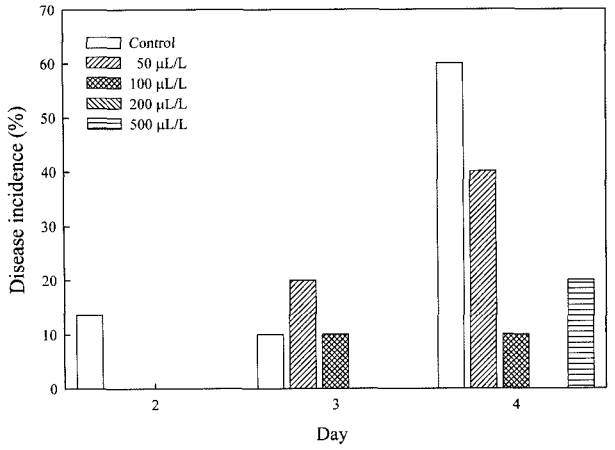

Fig. 5. Disease incidence of strawberries treated with 0 (control), 50, 100, 200, or 500 μL/L NO during storage at 18°C in air.

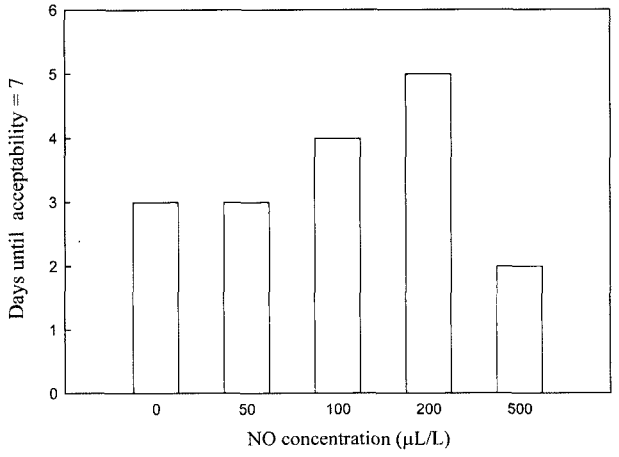

Fig. 6. Effect of exposure to different concentrations of NO on the number of days until the overall acceptability in strawberries stored at 18°C reached unacceptable levels.

NO is an important signaling molecule (19). NO as well as other AOS have been shown to stimulate the accumulation of salicylic acid (SA), and likewise SA induces the production of AOS, H_2O_2, and NO. This plays an important role in a plant's response to pathogen attack. One likely role for NO, SA, and AOS is to promote the HR and killing of pathogens. Both AOS and SA have been shown to work synergistically with NO to enhance host cell death in soybean suspension cells (6, 7, 20).

Figure 6 shows that the treatment with 200 μL/L NO had positive effects on fruit quality. An acceptable storage life was maintained for 5 days at 18°C with the 200 μL/L NO treatment. However, in the untreated fruit and the 50 and 500 μL/L NO-treated fruit, overall acceptability was lower than in the 200 μL/L NO treated fruit (Fig. 5).

In conclusion, NO treatment maintained the quality and prolonged the post-harvest life of strawberry fruit at 18°C. A concentration of 200 μL/L NO was more beneficial than lower or higher concentrations. This suggests that treating strawberries with an optimal concentration of NO is likely to be a useful technique for maintaining their shelf life. Further investigation is required to understand the physiological basis for this extended shelf life.

Acknowledgments

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