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Residual analysis of nitric oxide fumigation on fresh fruit and vegetables

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ABSTRACT

Nitric oxide (NO) is a newly discovered fumigant which is effective against a wide range of postharvest pests. To register NO with US EPA for commercial use as a pesticide and to ensure its safety to consumers, it is necessary to analyze residues of NO fumigated products. In this study, we analyzed nitrate (NO_3^-) and nitrite (NO_2^-) ion concentrations in liquid extracts as residues on 20 fresh products at 24 h after 16 h fumigation treatments and compared them from untreated controls to determine effects of nitric oxide fumigation. Each product was subjected to two identical NO fumigation treatments except one treatment was terminated by flushing with N_2 and the other terminated by flushing with air. For most products, there were no significant differences in NO_3^- or NO_2^- level between the treatment that was terminated with nitrogen flush and the control. Only when NO fumigation treatment was terminated by flushing with normal air, there were significantly higher NO_3^- and NO_2^- concentrations in all fumigated products than both control and N_2 flushed fumigated products. NO_2^- concentration was generally not detectable in both fumigated and control products. Therefore, our results indicated that there were no significant levels of residues from NO fumigated fresh products at 24 h after fumigation when fumigation was terminated properly with nitrogen flushing.

1. Introduction

There is a great demand for safe and effective postharvest treatments to control quarantine pests on internationally traded agricultural products. In USA, fresh products like apples and cherries are required to be fumigated to control insect pests before export to countries like Japan, Australia, Republic of Korea, and New Zealand (Terauds et al., 1978; Dentener et al., 1990; Hansen et al., 2000). The current methyl bromide fumigation treatments on exported commodities may not be sustainable due to global phase-out of methyl bromide production. Methyl bromide fumigation also causes unacceptable phytotoxic effects on some fresh products such as lettuce. Methyl bromide fumigation also leaves high levels of residue on fresh commodities like apples and cherries immediately after fumigation and the residues required long time to decline to levels below the maximum residue limits (MRL) (Hansen et al., 2000; Moffitt et al., 1992).

Due to the phase out of methyl bromide production, phosphine has emerged as the major alternative fumigant for postharvest pest control on fresh products. However, phosphine fumigation requires longer treatment time to achieve effective control of the pests. Also, some insects are tolerant or resistant to phosphine fumigation treatment and cannot be effectively controlled with phosphine fumigation (Liu, 2016). In comparison, a recently discovered new fumigant, nitric oxide (NO) has potential to become a safe and effective alternative fumigant for postharvest pest control on both fresh and stored products (Liu, 2013, 2015, 2016; Liu and Yang, 2016; Liu et al., 2016).

Nitric oxide (NO) is a free radical agent and a ubiquitous cell signal molecule which modulated various physiological and biochemical processes in almost all forms of organisms including microbes, plants, and animals (Lamattina et al., 2003). NO is also produced during natural electrical discharges from lightening in thunderstorm, and the combustion of fossil fuel by automobiles and power plants. NO has been found to enhance postharvest quality and extend the shelf-life of many fresh products including fruits like apple, kiwi, plum, and strawberry, and vegetables like broccoli, cucumber, carnations, green bean, lettuce, and mushroom (Leshem et al., 1998; Wills et al., 2000, 2007, 2008; Bowyer et al., 2003; Soegiarto and Wills, 2004; Zhang et al., 2007; Pristijono et al., 2008; Manjunatha et al., 2010; Saadatian et al., 2012). Recently, NO was discovered to be a potent fumigant under ultralow oxygen (ULO) conditions against insects and mites (Liu, 2013; 2015; Liu and Yang, 2016; Liu et al., 2016). NO fumigation is effective against all pests tested to date at different life stages, including both external and internal feeders (Liu, 2013; 2015; Liu and Yang, 2016). Since NO reacts to oxygen spontaneously to form nitrogen dioxide (NO_2), a reddish-brown gas, which can cause injuries to fresh vegetables and fruits, NO fumigation must be conducted under ULO conditions to minimize its

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oxidation. In addition, when terminating NO fumigation, the fumigation chamber needs to be flushed with inert gas like N_2 to dilute NO to avoid injuries to fresh products caused by NO_2 (Liu, 2013; Liu and Yang, 2016). When terminated properly with N_2 flushing, NO fumigation is safe to fresh products (Liu, 2016). Nitric oxide fumigation was also concluded to be technically feasible and cost-effective for commercial use (Liu, 2015).

Even though NO has good potential to be alternative fumigant to methyl bromide for postharvest pest control, it needs to be registered with US EPA as a chemical pesticide to be used commercially and studied for potential residues and their implications to human health on fumigated products. As a simple and old chemical, environmental fate of NO is well understood. Due to the oxidation nature of NO, it is speculated that the residue of NO fumigation on fresh products may include NO_2 from NO oxidation and products of endogenous oxidation of NO_2 on plant tissue. NO_2 is known to be converted to nitrate (NO_3^-) ion and then can be further reduced to nitrite (NO_2^-) ion (Hord et al., 2009). Fumigation procedures may have significant impact on residue levels. If fumigation chamber is not flushed with N_2 to dilute NO at the end of fumigation, NO will react with O_2 to produce NO_2 and will likely increase NO_2 as well as NO_3^- and NO_2^- as residues. In the present study, we analyzed NO_3^- and NO_2^- ion levels as residues on a variety of fresh produce and fruits from two NO fumigation treatments: one was terminated with N_2 flush and the other flushed with air to allow oxidation of NO to simulate the worst scenario. The importance of proper termination procedure of NO fumigation was also discussed.

2. Materials and methods

2.1. Chemicals

Nitric oxide (> 99.5% purity) and commercial grade N_2 in compressed cylinders were obtained from a commercial source (Praxair, Inc., Danbury, CT, USA) to be used for all experiments. NO was released and stored in a N_2-washed foil bag equipped with stopcock for easy sampling with an airtight syringe.

2.2. Fresh products

Twenty fresh vegetable and fruit products were used in this study, they were obtained from local supermarket and were stored at certain temperatures before the fumigation experiments (Table 1). Each product was visually screened to remove defective ones to maintain uniformity in color, texture, and size for fumigation experiments.

Table 1
Fresh vegetables and fruits and NO fumigation treatments for residue analysis.

Product	Species	NO treatment, %	temperature, °C
Apple	*Malus pumila,* cv. Fuji	5	2
Apricot	*Prunus armeniaca*	3	2
Asparagus	*Asparagus officinalis*	3	2
Avocado	*Persea americana,* cv. Fuerte, Hass	3	5
Blueberry	*Vaccinium corymbosum*	3	2
Broccoli	*Brassica oleracea* var. *Italica*	3	2
Cherry	*Prunus cerasus*	3	2
Garlic bulb	*Allium sativum*	3	2
Grape	*Vitis vinifera*	3	2
Kiwifruit	*Actinidia chinensis*	3	2
Lettuce	*Lactuca sativa L. var. longifolia*	2	2
Mango	*Mangifera indica*	3	13
Orange	*Citrus sinensis*, var. Navel	3	5
Pear	*Pyrus communis*	3	2
Peach	*Prunus persica*	3	2
Pepper	*Capsicum annuum*	3	5
Plum	*Prunus domestica*	3	2
Squash	*Cucurbita pepo*	3	8
Strawberry	*Fragaria ananassa*	2.5	2
Tangerine	*Citrus reticulata*	3	5

2.3. NO fumigation treatments

All products were fumigated in separated tests in 1.9 or 7.6 L airtight chambers depending on the size of the product. Chambers containing products were flushed with N_2 gas to purge the O_2 out to establish ULO conditions of $\leq$35 ppm O_2. An oxygen analyzer (Series 800, IL Instruments, Inc., Johnsburg, IL) was used to monitor O_2 levels in the fumigation chambers. NO from a preloaded foil bag was injected into fumigation chambers to start a fumigation treatment. NO concentrations were calculated based on volumes of NO and chambers. Because of the reactive nature of NO with O_2, the syringe and associated tubing were flushed with N_2 prior to NO injection (Liu, 2013). After NO injection, the chambers were kept in a temperature chamber at certain temperatures for 16 h to complete the fumigation treatment. The NO concentration applied to each of products was shown in Table 1.

In each fumigation test, a product was subject to two identical fumigation treatments, but one was terminated with N_2 flush (NO-N_2) and the other terminated with air flush (NO-Air). An untreated portion of the product was also stored at the same temperature as the treatments to serve as a control. The NO-N_2 treatments in the 1.9 L jar and 7.6 L chambers were terminated by flushing with N_2 at 2 L min^{-1} for 20 min and 3 L min^{-1} for 30 min respectively. The NO-Air treatments in the 1.9 L jar and 7.6 L chambers were terminated by flushing with air at the same flow rate and duration as NO-N_2 treatments. Treatment chambers were then opened to ambient air. For each product, all treatments were replicated at least 6 times. After fumigation treatments, all products were stored in a walk-in cooler at certain temperatures before being analyzed for residues.

2.4. Residue analysis

Nitrate (NO_3^-) and nitrite (NO_2^-) ion concentrations in liquid extracts of fumigated products at 24 h after fumigation were measured as residues using a nitric oxide analyzer (NOA 280i, GE Analytical Instruments, Boulder, CO, USA). A 15 g sample was randomly taken from each product in each treatment and homogenized in 100 mL deionized water in a blender (Blender 7010G, Waring Commercial, Torrington, Connecticut, USA) for 10 min. The homogenized sample was then vacuum-filtered and used for NO_3^- and NO_2^- analysis using the NO analyzer. For NO_3^- reduction analysis, the liquid sample was injected into the purge vessel with 5 mL solution of the vanadium chloride (VCl_3) in 1 M hydrochloride acid (HCl) as a reducing agent at 95 °C, and helium (He) was used as inert gas carrier. This method measures the total of NO_3^- and NO_2^- and NO_3^- content was calculated by subtracting the subsequent measurement of NO_2^- content from the total. NO_2^- was measured separately using NO_2^- reduction analysis. An aliquant of 5 mL solution of the sodium iodide (NaI) in 1 M HCl was used as the reducing agent, and the reaction was carried out at room temperature with He gas. The measurements of NO_3^- and NO_2^- for each sample was determined by calculating the peak area on the chromatogram using NOAnalysis software (v3.2, Sievers Instruments Inc., Boulder, CO, USA), and the area was converted to μM by using the regression curve of standard which were developed using the same NOA 280i parameters for NO_3^- or NO_2^- analyses. NO_3^- and NO_2^- contents were converted to mg kg^{-1}. Each treatment for each product was replicated 6 times.

2.5. Data analysis

Data on NO_3^- and NO_2^- ion concentration measurements for each product were subject to the one-way analysis of variance. Means of NO_3^- and NO_2^- concentrations among treatments for each product