Journal of Stored Products Research 84 (2019) 101521

Contents lists available at ScienceDirect

Journal of Stored Products Research

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

九大储粮以及坚果制品一氧化氮熏蒸残留分析*

Residue analysis of nitric oxide fumigation in nine stored grain and nut products☆

Xiangbing Yang [a], Yong-Biao Liu [b, *]

[a] University of California, 1636 East Alisal Street, Salinas, CA, USA
[b] Crop Improvement and Protection Unit, USDA-ARS, 1636 East Alisal Street, Salinas, CA, USA

ARTICLE INFO

Article history:
Received 17 June 2019
Received in revised form
6 September 2019
Accepted 30 September 2019
Available online 25 October 2019

Keywords:
Fumigation
Nitric oxide
Residue analysis
Nitrate
Nitrite
Nitrogen dioxide
Stored product

一氧化氮（NO）是最近发现的一种熏蒸剂，用于新鲜和储存的产品的收获后虫害控制。处理得当的情况下，氧化亚氮熏蒸并不会在新鲜水果和蔬菜上留下残留物。
在本研究中，我们分析了液体提取物中的硝酸盐（NO3）和亚硝酸盐（NO2）水平，以及二氧化氮（NO2）解吸率作为对九种储存类谷物和坚果产品进行不同时间NO熏蒸的残留物。
每个产品在两个处理中分别用3.0%NO熏蒸24h：一个处理（NO-N2）用氮气（N2）冲洗，另一个（NO-Air）是用正常的空气冲洗终止的。

ABSTRACT

Nitric oxide (NO) is a recently discovered fumigant for postharvest pest control on fresh and stored products. Nitric oxide fumigation also does not leave residues on fresh fruit and vegetables when conducted properly. In this study, we analyzed nitrate (NO_3^-) and nitrite (NO_2^-) levels in liquid extracts and nitrogen dioxide (NO_2) desorption rates as residues of NO fumigation at various times after fumigation on nine stored grain and nut products. Each product was fumigated separately with 3.0% NO for 24 h in two treatments: one treatment (NO–N_2) was terminated with nitrogen gas (N_2) flush and the other (NO-Air) was terminated with normal air flush. For NO–N_2, NO_3^- concentrations of all fumigated products were not significantly higher than those of untreated controls at 1, 7, and 14 d after fumigation. NO_2^- concentrations of all fumigated products from N_2 gas flush were not significantly higher than those of control products at 14 d after fumigation. NO_2 desorption rates for most products from NO–N_2 treatment showed no significant difference from those for the controls 1 d after NO fumigation, except for beans and wheat, which showed no significant difference at ≥7 d after fumigation. All products from NO-Air treatment, however, had significant higher NO_3^- and NO_2^- ion concentrations in liquid extracts at 14 d after fumigation than those from NO–N_2 treatment and the control. NO_2 desorption rates in all products from NO-Air treatment were also significantly higher than those from NO–N_2 treatment and the control at 21 d after fumigation. Therefore, when terminated properly with N_2 flush, NO fumigation did not result in significant increases of NO_3^-, NO_2^-, or NO_2 as residue in nut and grain products.

对于NO-N2，所有熏蒸产品的NO3浓度均不显著高于未经处理的对照组，分别为1、7和14d。
NO2浓度均不显著高于熏蒸后14d的对照产物。

NO-N2处理的大部分产品NO2解吸率与对照组NO熏蒸1d无显着性差异，但豆类和小麦则在熏蒸后7d无显著性差异。
然而，所有NO空气处理产品在熏蒸后14d液体提取物中的NO3和NO2离子浓度显著高于NO-N2的处理和控制。
所有NO-空气处理产物中NO2的解吸率也明显高于NO-N2处理产物和熏蒸后21d的对照。
因此，如果适当控制N2冲洗时间节点，NO熏蒸不会导致坚果和谷物产品中NO3、NO2或NO2作为残留物的显著增加。

1. Introduction

There is a great need for safe and effective alternative fumigants for postharvest pest control to replace methyl bromide which has been phased out of production globally as mandated by Montreal Protocol due to its depleting effects on atmospheric ozone (Montreal Protocol, 1987). Phosphine and sulfuryl fluoride have become mostly widely used alternative fumigants for postharvest pest control on stored products. However, both phosphine and sulfuryl fluoride have limitations for postharvest pest control. Phosphine fumigation acts slowly against pests and may take more than ten days to control stored product insects (Hole et al., 1976). Some stored product insects have developed resistance to phosphine (Nayak et al., 2003; Benhalima et al., 2004; Opit et al., 2012). Sulfuryl fluoride fumigation has low efficacy against insect eggs and, therefore, has limited potential for postharvest pest control on stored products (UNEP, 2011).

The recently discovered new fumigant nitric oxide (NO), however, is effective against all insects and mites tested to date and has potential for postharvest pest control (Liu, 2013, 2015; Liu and Yang, 2016; Yang and Liu, 2018). Nitric oxide fumigation has been demonstrated highly efficacious against a wide range of insect pests including external and internal feeders at all life stages on both fresh and stored products including rice weevil and confused flour beetle (Liu, 2013). Nitric oxide fumigation with desired levels of NO and NO_2 is also effective in controlling microbes such as *Aspergillus flavus* spores (Liu et al., 2019). Moreover, NO is an antagonist of ethylene biosynthesis of plants and NO fumigation has been demonstrated to improve postharvest quality and extend shelf life extension of fresh products (Soegiarto and Wills, 2004;

☆ This article reports the results of research only. Mention of proprietary products, trade names or commercial products in this publication is not for the purpose of providing specific information and does not constitute an endorsement or a recommendation by the U.S. Department of Agriculture for its use. The USDA is an equal opportunity provider and employer.

* Corresponding author.
E-mail address: yongbiao.liu@usda.gov (Y.-B. Liu).

https://doi.org/10.1016/j.jspr.2019.101521

Manjunatha et al., 2010).

Nitric oxide reacts with oxygen (O_2) spontaneously to form nitrogen dioxide (NO_2) and, therefore, NO fumigation must be conducted under ultralow oxygen (ULO) conditions in airtight fumigation chambers to minimize the oxidation of NO in fumigation chamber (Liu, 2013, 2015; Liu et al., 2017). NO fumigation also needs to be terminated by flushing the chamber with inert gas like nitrogen (N_2) to dilute NO to prevent NO_2 formation, which may cause injuries to fresh products (Liu, 2016). When terminated properly with N_2 gas flush, NO fumigation is safe to postharvest quality of fresh products (Liu, 2016; Liu et al., 2017; Yang and Liu, 2017, 2018).

NO fumigation may result in increases in nitrate (NO_3^-) and nitrite (NO_2^-) levels as residues in fumigated products. Both NO_3^- and NO_2^- already exist in various food products (Hord et al., 2009; Bahadoran et al., 2016). For fresh products, NO fumigation when terminated properly with N_2 gas flush does not increase levels of NO_3^- or NO_2^- residues (Yang and Liu, 2017). As NO is a recently discovered fumigant, it is essential to understand whether NO fumigation has an impact on quality and safety of fumigated products and these data are also necessary for approval of NO by regulatory agencies such as U.S. Environmental Protection Agency (EPA) for commercial use as a pesticide for postharvest pest control. In this study, we analyzed NO_3^- and NO_2^- ion concentrations and NO_2 desorption rate as residues on a variety of grain and nut products from two NO fumigation treatments, one terminated with N_2 gas flush to dilute NO concentration and the other with air flush to simulate oxidation of NO. The importance of proper termination of procedure of NO fumigation was also discussed.

2. Materials and methods

使用了来自当地超市的九种谷物和坚果产品，它们是杏仁、大麦、加班佐豆、山核桃、平托豆、开心果、核桃、小麦和大米。熏蒸前对产品进行视觉筛选，以去除受霉菌污染的谷物和坚果。

2.1. Fumigation gas and stored products

Nitric oxide with >99.5% purity (other ingredients: 120 ppm carbon dioxide, 400 ppm nitrous oxide, 800 ppm nitrogen, and <5 ppm moisture) (Advanced Specialty Gases, Sparks, NV, USA) and commercial grade N_2 gas in compressed cylinders (Praxair, Inc., Danbury, CT, USA) were used for all experiments. Nitric oxide was released and stored in a N_2-washed foil bag (40 × 20 cm) (Uline, Pleasant Prairie, WI, USA) equipped with stopcock for easy sampling with an airtight syringe.

Nine grain and nut products from local supermarket were used and they were almonds, barley, garbanzo bean, pecan, pinto bean, pistachio, walnut, wheat, and rice. They were stored in their original packages at ambient temperatures of 18–25 °C, 60–75% RH in the laboratory before the fumigation experiments. Products were visually screened to remove mold-contaminated grains and nuts before each fumigation experiment.

2.2. NO fumigation treatments

The nine products were fumigated separately with 3.0% NO under ULO conditions for 24 h at 25 °C using the procedures described previously (Liu, 2013; Liu et al., 2017; Yang and Liu, 2017). Eight of the products were fumigated in 1.9 L air-tight glass jar chambers and walnuts were fumigated in 7.6L air-tight chambers modified from pressure cookers. Two 3.0% NO fumigation treatments and one control were included in each test. About 400 g of each product was randomly sampled and sealed in each fumigation chamber (filling ratio of 30–35%). Fumigation chambers were then flushed with N_2 by releasing N_2 through a tube to the bottom of the chamber to establish an ULO atmosphere with ≤35 ppm O_2. Nitric oxide from a preloaded foil bag was then taken with an airtight syringe and injected into the fumigation chamber to establish 3.0% NO. To balance the air pressure inside fumigation chamber, 3.0% of air from ULO chamber was removed using an airtight syringe before NO injection. Nitric oxide concentrations were calculated based on volumes of NO injected and sizes of fumigation chambers. All chambers were kept at 25 ± 0.5 °C in an environment chamber for the 24 h fumigation treatment. An untreated portion of each product was stored in an air-tight chamber as a control under the same conditions as the two fumigation treatments.

The two NO fumigation treatments for each product were terminated differently: one was terminated with N_2 flush (NO–N_2) and the other was terminated with air flush (NO-Air). The NO–N_2 treatments for 1.9 and 7.6 L chambers were terminated by flushing chambers with N_2 gas for 30 min at flow rate of 2 and 3 L min^{-1} respectively. The NO-Air treatments in the 1.9 and 7.6 L chambers were terminated by flushing with air using an air pump (SP6000, Smart Products, Inc., Morgan Hill, CA, USA) at the same flow rate and duration as in NO–N_2 treatments. Chambers were then opened to ambient air. Treatments were replicated 4 times for each product. After treatment, all products were stored in open fumigation chambers at 25 ± 0.5 °C in a temperature chamber prior to residue analysis.

2.3. Residue analysis

NO_3^- and NO_2^- concentrations in liquid extracts of fumigated products, and NO_2 gas desorption rate from fumigated products were measured as residues at 1, 7, and 14 d after NO fumigation. For each treatment, a 10 g sample was randomly taken from each product and homogenized in 100 mL deionized water in a blender (Blender 7010G, Waring Commercial, Torrington, Connecticut, USA); the homogenized sample was then vacuum-filtered and analyzed using a NO analyzer (minimum detection limit: 1 pg) (NOA 280i, GE Analytical Instruments, Boulder, CO, USA) to determine NO_3^- and NO_2^- concentrations. The detailed procedures for measuring NO_3^- and NO_2^- in liquid extract using the NO analyzer was same as previously described (Liu et al., 2017; Yang and Liu, 2017). Total concentrations of NO_3^- and NO_2^- were measured by injecting 5 μL liquid sample into 5 mL vanadium chloride (VCl_3) (Acros Organics-Fisher Scientific, Geel, Belgium) as a reducing agent in 1 M hydrochloride acid (HCl) (Cole-Parmer, Vernon Hills, Illinois, USA) in the purge vessel of the analyzer at 95 °C. Concentrations of NO_2^- were measured separately using NO_2^- reduction analysis by injecting 5 μL liquid sample into 5 mL sodium iodide (NaI) (Fisher Chemicals, Hampton, NH, USA) solution as a reducing agent in 1 M HCl in the purge vessel at room temperature. Helium (He) gas (Praxair, Inc., Danbury, CT, USA) was used as carrier gas for both reduction reactions. The concentration of NO_3^- was calculated by subtracting NO_2^- concentration from the total concentration of NO_3^- and NO_2^-. NOAnalysis software (v3.2, Sievers Instruments Inc., Boulder, CO, USA) was used to determine concentrations of NO_3^- and NO_2^- and results were converted to μM by using a calibration curve established from standards using the same NOA 280i parameters (Yang and Liu, 2017). To create the standard curve, 69 mg sodium nitrite ($NaNO_2$) or 85 mg sodium nitrate ($NaNO_3$) (Fisher Chemicals, Hampton, NH, USA) were diluted in a 10 mL flask with deionized water to prepare the 100 mM standard stock solution. The stock solution was then used to prepare standard solutions containing 10, 50, 100 nM, 1, 5, 10, and 10 μM in microfuge tubes (1.5 mL) after a serial dilution. After preparing dilute standard solutions, the calibration curve was then constructed by injection of the standards into the purge vessel and analyzed by NOAanalysis software. The standard curves were then used to determine NO_3^- and NO_2^- concentrations in sample solutions. All NO_3^- and NO_2^- concentrations were converted to mg kg^{-1}. Each treatment for each product was replicated 4 times.

NO_2 desorption rates from each fumigated product and each fumigation treatment were measured using a Model 405 nm NO_2/NO/NO_x monitor (limit of detection: 0–10,000 ppb for NO_2, 0–2000 ppb for NO) (2B Technologies, Boulder, CO, USA) following procedures described earlier (Liu et al., 2017). To measure NO_2 desorption rate, fumigation chambers containing fumigated products from all three treatments for each product were sealed, and the two ports on the chamber lid were connected to the inlet and outlet of the NO_2 monitor to create a recirculation loop for air in the headspace of the chamber to pass through the NO_2 monitor continuously and NO_2 levels were shown on the display of the monitor. The chambers were then kept sealed with the two stopcocks closed and held at 25 °C for one hour. At the end of one hour, NO_2 levels were then measured again to determine the increases in NO_2 concentration during the one-hour period. After the second measurements, fumigation chambers were opened and held at 25 °C in the temperature chamber. The weight of product sample in each fumigation chamber for each treatment and each product was used to calculate NO_2 desorption rate in one hour based on chamber volume and product weight ($\mu g\ kg^{-1}h^{-1}$). To determine how fast NO_2 desorbs from a fumigated product after fumigation, NO_2 desorption rates were measured for all three treatments for each product at 3 h and 1, 7, 14, and 21 d after fumigation. The residue analysis for NO_2 desorption rate was replicated 4 times for each treatment and each product.

2.4. Data analysis

NO_3^-, NO_2^-, and NO_2 data for each product were subject to one-way analysis of variance. Means of NO_3^- and NO_2^- ion concentrations and NO_2 desorption rates among treatments for each product at each time after fumigation were compared using Tukey's HSD multiple range test in repeated measurement analysis of variance by using SAS program (PROC GLM). All statistical analyses were conducted using SAS program (SAS Institute, 2012).

3. Results

There were no significant differences between NO–N_2 which was terminated with N_2 flush and control treatments for NO_3^- or NO_2^- levels for any of the nine products 14 d after fumigation treatment (Table 1). Some products from NO–N_2 treatment had significantly higher levels of NO_3^- or NO_2^- 1 and 7 d after fumigation as compared with the control, showing gradual declines of both NO_3^- and NO_2^- levels over time (Table 1). The NO-Air treatment, however, which was terminated with air flush, resulted in significantly higher NO_3^- and NO_2^- concentrations as compared with the control 1, 7, and 14 d after fumigation in all products, with the exception that the NO_3^- level in almond from NO-Air treatment was not significantly higher than that in the control 14 d after fumigation (Table 1). Therefore, termination of NO fumigation with N_2 flush was critical to prevent increases of NO_3^- and NO_2^- as residues in fumigated stored products.

NO_3^- concentration of control samples ranged from 7.39 mg kg^{-1} (rice) to 28.37 mg kg^{-1} (pinto bean) during post-treatment storage. None of the NO–N_2 fumigated products differed significantly from the control for NO_3^- concentration one day after fumigation. In contrast, all products from NO-Air treatment had significantly higher NO_3^- concentrations than their respective controls at all times after fumigation, with the exceptions of almonds, pinto bean, and walnut, which were not significantly greater in NO_3^- concentrations compared to their respective NO–N_2 treatment or controls 14 d after fumigation (Table 1).

The NO_2^- concentrations in control treatments were 0 mg kg^{-1} at all times during post-treatment storage for all species tested. No significant difference in NO_2^- concentration was found between NO–N_2 treatment and control at any time after fumigation for any product. Concentrations of NO_2^- in all products subjected to NO-Air treatment were significantly above the 0 mg kg^{-1} value for controls 1, 7, and 14 d after fumigation (Table 1).

NO_2 desorption rates of fumigated products for all species showed no significant difference between NO–N_2 treatment and the control 7 d after fumigation, except for pinto bean, which showed no significant difference between NO–N_2 and the control 14 d after fumigation (Table 2). At 3 h after fumigation, NO_2 desorption rates of pecan, almonds, walnut, and barley from NO–N_2 treatment showed no significant difference from the control. All products from NO-Air treatments showed significantly higher NO_2 desorption rates than those from the control at all times after NO fumigation, with four exceptions: wheat (14 d), barley (14 d), rice (1 d), and pistachio (7 and 14 d) (Table 2). The mean NO_2 desorption rate measurements in controls ranged from 0.7 $\mu g\ kg^{-1}\ h^{-1}$ for pinto bean, to 54.9 $\mu g\ kg^{-1}\ h^{-1}$ for barley at 3 h after fumigation, and from 0.6 $\mu g\ kg^{-1}\ h^{-1}$ for pinto bean, to 3.6 $\mu g\ kg^{-1}\ h^{-1}$ for rice at 21 d after fumigation. NO_2 desorption measurements for most unfumigated control products showed gradual declines over time of the five measurements (Table 2).

4. Discussion

在NO-N2处理的任何熏蒸产品中，NO3或NO2离子浓度没有显著增加，表明用N2冲洗正确终止的NO熏蒸没有留下大量的NO3或NO2残留物。 NO-N2与对照组熏蒸后14d产物的NOeN2解吸率无显著差异。

No significant increases in NO_3^- or NO_2^- ion concentration in any fumigated product from NO–N_2 treatment indicate that NO fumigation does not leave significant amounts of NO_3^- or NO_2^- residue if it is terminated properly with N_2 flush. There was also no significant difference in NO_2 desorption rate between NO–N_2 and controls for any product 14 d after fumigation. The 3.0% NO concentration used in this study is higher than the concentrations used previously to successfully control all life stages of rice weevil and confused flour beetle (Liu, 2013). A conclusion of no significant increase in NO_3^-, NO_2^- or NO_2 residue for NO fumigation terminated with N_2 flush is likely, therefore, to be conservative and valid for NO fumigations to control insects on stored products. When NO fumigation was terminated with air flush, there were significant increases in NO_3^- and NO_2^- levels in fumigated products as compared with controls, and the magnitude of increases for NO_3^- ranged from 32.9% in almond to 268.2% in barley 14 d after fumigation (Table 1). Levels of NO_2^- in controls were zero in most products. NO_2^- levels in fumigated products from NO-Air treatment had significant levels of NO_2^- ranging from 1.1 mg kg^{-1} (pinto bean and wheat) to 8.03 mg kg^{-1} (pistachio) at 14 d after fumigation (Table 1). For the majority of products, NO-Air treatment also had significantly higher NO_2 desorption rates as compared with controls (Table 2). These results indicate that NO fumigation needs to be terminated with N_2 flush to prevent NO_3^-, NO_2^-, or NO_2 residues in fumigated stored products.

NO_3^- ions are essential nutrient in food and are the primary source of nitrogen for vegetable and fruits (Hord et al., 2009; Bahadoran et al., 2016; Yang and Liu, 2017). Plants normally take up NO_3^- from soil during growth and the level of nitrogen in plants is primarily controlled by fertilization practices. For example, vegetables like spinach and lettuce normally accumulate high concentrations of NO_3^- from soil and yield high levels of NO_3^- in these vegetables with amount up to 1000 mg kg^{-1} (Muramoto, 1999). Excessive NO_3^- may, however, be reduced to NO_2^-, which in turn under certain conditions such as high heat or strong acidic environment may form nitrosamine, which is carcinogenic to human (Cammack et al., 1999; Pannala et al., 2003; Santamaria, 2006).

Our results showed that NO_3^- levels in stored products are generally lower than those in fruits and vegetables, ranging from 7.76 mg kg^{-1} (rice) to 28.37 mg kg^{-1} (pinto beans). Although NO-

Table 1
Nitrate and nitrite levels on stored products at different times after 24 h fumigation treatments with 3.0% nitric oxide.

Product	Treatment	Nitrate (NO_3^-), mg kg^{-1}			Nitrite (NO_2^-), mg kg^{-1}		
		1 d	7 d	14 d	1 d	7 d	14 d
Almonds	NO-Air	16.86 ± 1.10a	14.95 ± 0.85a	15.85 ± 5.21a	4.22 ± 0.37a	3.15 ± 0.51a	2.61 ± 0.79a
	NO−N_2	12.21 ± 1.83 ab	9.92 ± 0.65b	11.92 ± 2.72a	1.91 ± 0.89b	0.46 ± 0.20b	0.10 ± 0.05b
	Control	11.34 ± 0.79b	9.53 ± 1.19b	11.92 ± 1.88a	0b	0b	0b
	$F_{2,9}$	5.10	10.70	0.41	14.48	29.14	10.44
	P	0.0330	0.0042	0.6782	0.0015	0.0001	0.0045
Barley	NO-Air	26.36 ± 0.50a	21.90 ± 1.20a	29.79 ± 4.58a	6.23 ± 0.35a	6.23 ± 0.34a	3.62 ± 0.15a
	NO−N_2	8.29 ± 1.10b	7.54 ± 1.23b	7.64 ± 1.58b	2.04 ± 0.36b	0.53 ± 0.17b	0.09 ± 0.05b
	Control	8.48 ± 0.56b	7.84 ± 0.86b	8.09 ± 1.17b	0c	0b	0b
	$F_{2,9}$	181.4	54.71	19.38	118.40	245.63	513.41
	P	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	<0.0001
Garbanzo bean	NO-Air	37.72 ± 5.26a	30.38 ± 6.17a	29.69 ± 2.57a	8.02 ± 0.55a	7.14 ± 1.26a	4.12 ± 1.25a
	NO−N_2	19.03 ± 2.53b	14.12 ± 0.45b	9.56 ± 0.38b	5.78 ± 0.93a	1.19 ± 0.37b	0.26 ± 0.09b
	Control	15.41 ± 1.04b	13.0 ± 2.29b	9.70 ± 3.14b	0b	0b	0b
	$F_{2,9}$	12.23	6.52	24.20	44.0	25.37	10.12
	P	0.0027	0.0178	0.0002	<0.0001	0.0002	0.0050
Pecan	NO-Air	23.01 ± 4.28a	22.51 ± 1.02a	19.68 ± 2.09a	3.74 ± 0.54a	3.63 ± 0.22a	2.30 ± 0.07a
	NO−N_2	17.15 ± 4.11b	10.02 ± 0.09b	10.96 ± 1.78b	1.83 ± 0.21b	1.44 ± 0.17b	0.06 ± 0.02b
	Control	16.49 ± 1.51b	11.96 ± 1.29b	10.51 ± 0.65b	0c	0c	0b
	$F_{2,9}$	10.3	49.66	10.05	31.38	128.49	873.49
	P	0.0395	<0.0001	0.0051	<0.0001	<0.0001	<0.0001
Pinto bean	NO-Air	39.58 ± 3.53a	36.96 ± 4.12a	23.60 ± 11.47a	9.54 ± 1.47a	3.07 ± 0.43a	1.11 ± 0.36a
	NO−N_2	33.62 ± 9.0b	18.82 ± 1.07b	13.61 ± 5.73a	1.12 ± 0.16b	0.88 ± 0.39b	0.22 ± 0.10b
	Control	28.37 ± 5.84b	17.07 ± 0.88b	16.11 ± 6.16a	0b	0b	0b
	$F_{2,9}$	7.4	19.27	0.40	37.57	22.21	7.42
	P	0.042	0.0006	0.6810	<0.0001	0.0003	0.0125
Pistachio	NO-Air	34.86 ± 1.23a	34.11 ± 0.90a	31.19 ± 3.68a	9.08 ± 0.49a	8.62 ± 0.74a	8.03 ± 2.06a
	NO−N_2	21.00 ± 2.25b	20.07 ± 1.77b	17.75 ± 3.88b	6.48 ± 0.67b	3.54 ± 0.26b	0.54 ± 0.20b
	Control	21.49 ± 2.67b	19.19 ± 1.32b	16.16 ± 0.42b	0c	0c	0b
	$F_{2,9}$	13.52	37.08	7.12	96.54	90.38	14.13
	P	0.0019	<0.0001	0.0140	<0.0001	<0.0001	0.0017
Rice	NO-Air	14.41 ± 2.02a	14.37 ± 0.60a	11.31 ± 0.69a	3.44 ± 0.28a	2.10 ± 0.11a	1.81 ± 0.33a
	NO−N_2	8.53 ± 1.60 ab	7.46 ± 1.13b	7.45 ± 0.59b	1.69 ± 0.13b	0.71 ± 0.26b	0.15 ± 0.10b
	Control	7.76 ± 0.71b	7.80 ± 0.41b	7.39 ± 0.91b	0c	0c	0b
	$F_{2,9}$	5.53	25.22	9.19	95.48	41.85	25.40
	P	0.0271	0.0002	0.0067	<0.0001	<0.0001	0.0002
Walnut	NO-Air	19.04 ± 3.61a	17.39 ± 1.11a	16.41 ± 3.47a	3.20 ± 0.07a	3.15 ± 0.42a	2.57 ± 0.16a
	NO−N_2	11.73 ± 2.12a	10.46 ± 0.68b	9.73 ± 1.19a	0.82 ± 0.47b	0.43 ± 0.19b	0b
	Control	13.84 ± 0.22a	10.18 ± 0.54b	11.18 ± 0.45a	0b	0b	0b
	$F_{2,9}$	2.42	25.17	2.72	36.36	41.24	244.39
	P	0.1444	0.0002	0.1194	<0.0001	<0.0001	<0.0001
Wheat	NO-Air	38.05 ± 5.08a	31.75 ± 5.93a	25.58 ± 1.66a	9.52 ± 1.48a	3.07 ± 0.44a	1.11 ± 0.36a
	NO−N_2	14.49 ± 0.77b	13.76 ± 0.55b	7.49 ± 0.57b	1.12 ± 0.16b	0.88 ± 0.39b	0.22 ± 0.10b
	Control	12.86 ± 1.14b	8.59 ± 2.75b	7.56 ± 0.25b	0b	0b	0b
	$F_{2,9}$	21.52	10.30	103.59	36.92	21.90	7.38
	P	0.0004	0.0047	<0.0001	<0.0001	0.0003	0.0127

Mean ± SE were presented. Means were separated by Tukey honest difference test (PROC GLM). Means in each column for each product at a specific time followed by same letter were not significantly different at $P = 0.05$.

Air treatments resulted in higher NO_3^- and NO_2^- ion concentrations than those in the control and NO−N_2 treatments in the current study, the level of NO_3^- and NO_2^- ion concentrations in products from NO-Air treatments were still well below the maximum limits (2500−4500 mg kg^{-1}) set by European Commission (EC) Regulation (Santamaria, 2006).

Our study showed that NO_2 desorption rates from NO−N_2 treated products were not significantly different from control products 21 d after fumigation. Some products in our tests showed no significant difference in NO_2 desorption rates immediately after fumigation, e.g., pecan, almonds, walnut, and barley. Formation of NO_2 after fumigation is mostly due to incomplete flush during termination which resulted in oxidation of some of NO. Under moist conditions, NO_2 can react with water to form nitric and nitrous acids, which can eventually be converted to nitrosamine under certain conditions such as high temperature or strong acidic environment. However, most stored products after harvest are dried to recommended low moisture levels before storage (Walker et al., 2018). NO_2 has a boiling point of about 21 °C and it is, therefore, expected that NO_2 residue on nuts and grain products, which are normally stored at ambient temperature instead of low temperature for most fresh products, will likely desorb faster after treatment than NO_2 residue on fresh products stored at low temperatures.

Due to the toxicity of NO and NO_2 gases to human, the Occupational Safety and Health Administration (OSHA) has set exposure limits for both gases in workplace air for worker safety purposes (Agency for Toxic Substances and Disease Registry, 2002). Proper flush of fumigation chamber with inert gas like N_2 at the end of NO fumigation can reduce the NO_2 level on fumigated products, especially for small grains, and thereby lower the risk of worker exposure to NO_2. This is another important factor for proper termination of NO fumigation with N_2 flush for stored products, particularly for small grains.

There are growing interest and developmental efforts in hermetic grain storage in recent years, especially in Asia and Africa (De Groote et al., 2013; Njoroge et al., 2014). Metal, hermetic silos are specifically promoted since they are suitable for hermetic bulk

Table 2
NO_2 desorption rates from stored products at different times after 24 h fumigation treatments with 3.0% nitric oxide.

Product	Treatment	NO_2, μg kg^{-1} h^{-1}				
		3h	1 d	7 d	14 d	21 d
Almonds	NO-Air	187.3 ± 8.0a	34.2 ± 7.9a	18.4 ± 6.9a	12.4 ± 8.0a	4.6 ± 1.0a
	NO–N_2	25.6 ± 8.6b	23.8 ± 8.4a	13.4 ± 4.6a	7.5 ± 0.9 ab	2.2 ± 0.9a
	Control	22.8 ± 8.6b	19.9 ± 8.0a	7.6 ± 0.9a	4.9 ± 0.8b	2.0 ± 0.5a
	$F_{2,9}$	125.25	0.82	1.28	4.59	3.24
	P	<0.0001	0.4707	0.3249	0.044	0.0873
Barley	NO-Air	461.1 ± 99.5a	36.6 ± 9.8a	25.3 ± 9.6a	7.4 ± 0.9a	5.1 ± 0.9a
	NO–N_2	65.1 ± 9.1b	31.3 ± 8.4b	19.1 ± 6.8a	5.5 ± 0.9a	3.2 ± 0.8a
	Control	54.9 ± 7.7b	18.2 ± 5.1b	9.6 ± 1.0b	5.7 ± 1.0a	3.5 ± 0.9a
	$F_{2,9}$	16.02	6.12	6.02	1.32	1.53
	P	0.0011	0.03	0.0309	0.3138	0.2686
Garbanzo bean	NO-Air	149.1 ± 49.0a	11.3 ± 0.7a	9.7 ± 1.1a	5.7 ± 0.8a	5.2 ± 0.9a
	NO–N_2	11.6 ± 1.5b	10.1 ± 0.9 ab	6.3 ± 0.9 ab	3.9 ± 0.8a	2.7 ± 0.8a
	Control	7.2 ± 0.8b	6.4 ± 0.9b	4.2 ± 1.0b	3.4 ± 0.7a	2.0 ± 0.8a
	$F_{2,9}$	8.14	9.1	7.86	2.31	4.12
	P	0.0096	0.0069	0.0106	0.1554	0.0537
Pecan	NO-Air	217.4 ± 37.2a	18.1 ± 8.0a	16.1 ± 7.0a	9.9 ± 1.0a	5.6 ± 0.8a
	NO–N_2	18.4 ± 6.5b	12.4 ± 2.7a	9.8 ± 1.0a	5.7 ± 0.8b	2.4 ± 0.8b
	Control	13.7 ± 4.2b	12.3 ± 6.7a	7.5 ± 0.9a	5.7 ± 0.9b	1.9 ± 0.7b
	$F_{2,9}$	28.17	0.29	1.15	6.82	6.64
	P	0.0001	0.7569	0.3589	0.0158	0.0169
Pinto bean	NO-Air	166.7 ± 20.7a	17.3 ± 5.1a	12.0 ± 4.0a	8.0 ± 1.5a	1.9 ± 0.1a
	NO–N_2	10.0 ± 1.8b	12.6 ± 1.0b	7.6 ± 0.8 ab	5.0 ± 1.2a	0.6 ± 0.2b
	Control	0.7 ± 0.1c	0.6 ± 0.1c	0.4 ± 0.2b	0.2 ± 0.1b	0.6 ± 0.1b
	$F_{2,9}$	60.38	8.04	6.1	12.5	27.22
	P	<0.0001	0.0099	0.0212	0.0025	0.0002
Pistachio	NO-Air	230.1 ± 41.8a	29.1 ± 3.9a	17.4 ± 6.2a	5.6 ± 1.0a	5.5 ± 0.7a
	NO–N_2	65.8 ± 9.5b	18.8 ± 1.3 ab	12.9 ± 1.5a	5.0 ± 1.1a	1.2 ± 0.2b
	Control	43.2 ± 2.2b	14.3 ± 4.3b	13.4 ± 2.9a	5.3 ± 1.7a	1.3 ± 0.1b
	$F_{2,9}$	6.69	4.92	0.38	0.05	31.28
	P	0.0166	0.036	0.6919	0.9518	<0.0001
Walnut	NO-Air	278.4 ± 64.7a	22.5 ± 8.2a	13.4 ± 2.1a	9.9 ± 2.7a	6.2 ± 0.9a
	NO–N_2	19.8 ± 5.5b	14.9 ± 6.5a	8.5 ± 0.8 ab	6.6 ± 0.9a	1.9 ± 0.1b
	Control	14.5 ± 1.8b	15.5 ± 7.2a	7.8 ± 0.9b	6.8 ± 1.0a	1.9 ± 0.5b
	$F_{2,9}$	16.2	0.33	4.78	1.1	21.31
	P	0.001	0.7249	0.0386	0.3732	0.0004
Wheat	NO-Air	360.7 ± 85.6a	25.9 ± 2.3a	15.6 ± 5.9a	8.7 ± 1.3a	6.3 ± 0.9a
	NO–N_2	28.7 ± 4.9b	25.6 ± 8.9a	13.5 ± 7.9 ab	6.3 ± 0.7a	3.5 ± 0.7 ab
	Control	8.5 ± 5.9c	9.2 ± 7.7b	6.7 ± 1.9b	6.2 ± 1.8a	2.9 ± 0.6b
	$F_{2,9}$	15.88	1.91	0.66	1.13	6.2
	P	0.0011	0.2032	0.5420	0.3661	0.0203
Rice	NO-Air	460.2 ± 93.4a	41.5 ± 8.6a	25.1 ± 8.2a	8.6 ± 0.5a	5.1 ± 0.5a
	NO–N_2	152.5 ± 19.6 ab	33.3 ± 8.0a	18.8 ± 7.0a	5.3 ± 0.8b	3.5 ± 0.6a
	Control	53.5 ± 8.7b	33.7 ± 8.8a	8.7 ± 0.9a	4.9 ± 1.3b	3.6 ± 1.0a
	$F_{2,9}$	6.49	0.3	1.76	4.65	1.65
	P	0.018	0.7470	0.2260	0.0411	0.2452

Mean ± SE (standard error) were presented. Means were separated by Tukey honest difference test (PROC GLM). Means in each column for each product at a specific time followed by same letter were not significantly different at $P = 0.05$.

storage and can be made in different sizes based on needs of growers or farmers (Walker et al., 2018). With some modifications, hermetic silos can be suitable for NO fumigation applications. If harvested grains are fumigated after being sun-dried and before storage to control insect pests and microorganism as pretreatment, the treatment will likely reduce or prevent issues of pest infestation and microbial development during postharvest storage. The cost of NO fumigation has been estimated and discussed earlier, and NO fumigation was concluded to be likely cost effective (Liu, 2015). Even though advantages of NO fumigation toward stored products treatment are promising, the lack of large gastight facilities and uncertainty of registration of nitric oxide as a pesticide in the future remain to be challenging for commercial application of NO fumigation.

Nitric oxide fumigation was previously demonstrated safe to fresh products with no residue. The results of current study provided additional residue data on stored products. These data are important in determining safety of NO fumigation and meeting data requirements for eventual registration of NO as a pesticide for postharvest pest control with regulatory agencies, e.g., EPA. Given that NO fumigation does not leave toxic residues in fresh and stored products, and controls both pests and microbes, more research and developmental efforts are warranted to gain regulatory approval and commercial application for NO fumigation. Specifically, large or commercial-scale studies are needed to develop treatment protocols and demonstrate efficacy in controlling pests and pathogens and safety of NO fumigation to postharvest quality of fresh and stored products.

5. Conclusions

Nitric oxide (NO) is a recently discovered as a potent fumigant for postharvest pest control. Nitric oxide fumigation did not leave significant amounts of residue in forms of NO_3^-, NO_2^- ions or NO_2 gas on nut and grain products tested at certain times after fumigation when the treatments were terminated properly with N_2 flushing. Previous and present results indicated no safety concerns for NO fumigation of stored product in terms of residues, and that

termination of NO fumigation with N_2 flush is critical for reducing residues.

Acknowledgements

We thank T. Masuda and R. Singh for technical assistance for this study. This study was supported in part by a TASC grant (C2017-04) from U.S. Department of Agriculture, Foreign Agricultural Service.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2019.101521.

References

Agency for Toxic Substances and Disease Registry, 2002. Nitrogen dioxide fact sheet. Available from: https://www.atsdr.cdc.gov/toxfaqs/tfacts175.pdf.

Bahadoran, Z., Mimiran, P., Jeddi, S., Azizi, F., Ghasemi, A., Hadaegh, F., 2016. Nitrate and nitrite content of vegetables, fruits, grains, legumes, dairy products, meats and processed meats. J. Food Compos. Anal. 51, 93–105.

Benhalima, H., Chaudhry, M.Q., Mills, K.A., Price, N.R., 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J. Stored Prod. Res. 40, 241–249.

Cammack, R., Jonanou, C.L., Cui, X.-Y., Martinez, C.T., Maraj, S.R., Hughes, M.N., 1999. Review: ntrite and nitrosyl compounds in food preservation. Biochim. Biophys. Acta 1411, 475–488.

De Groote, H., Kimenju, S.C., Likhayo, P., Kanampiu, F., Tefera, T., Hellin, J., 2013. Effectiveness of hermetic systems in controlling maize storage pests in Kenya. J. Stored Prod. Res. 53, 27e36.

Hole, B.D., Bell, C.H., Mills, K.A., Goodship, G., 1976. The toxicity of phosphine to all developmental stages of thirteen species of stored product beetles. J. Stored Prod. Res. 12, 235–244.

Hord, N.G., Tang, Y., Bryan, N.S., 2009. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am. J. Clin. Nutr. 90, 1–10.

Liu, Y.-B., 2013. Nitric oxide as a potent fumigant for postharvest pest control. J. Econ. Entomol. 106, 2267–2274.

Liu, Y.-B., 2015. Nitric oxide as a new fumigant for postharvest pest control on fresh commodities. Acta Hortic 1105, 317–321.

Liu, Y.-B., 2016. Nitric oxide fumigation for control of western flower thrips and its safety to postharvest quality of fresh fruit and vegetables. J. Asia Pac. Entomol. 19, 1191–1195.

Liu, Y.-B., Yang, X., 2016. Prospect of nitric oxide as a new fumigant for post-harvest pest control. In: Navarro, S., Jayas, D.S., Alagusundaram, K. (Eds.), Proceedings of 10th International Conference of Controlled Atmosphere and Fumigation in Stored Products (CAF2016). Nov. 7–11, 2016, New Delhi, India, pp. 161–166.

Liu, Y.-B., Yang, X., Masuda, T., 2017. Procedures of laboratory fumigation for Pest control with nitric oxide gas. J. Vis. Exp. 129, 56309.

Liu, Y.-B., Oh, S., Jurick II, W.M., 2019. Response of Aspergillus flavus spores to nitric oxide fumigations in atmospheres with different oxygen concentrations. J. Stored Prod. Res. 83, 78–83.

Manjunatha, G., Lokesh, V., Neelwarne, B., 2010. Nitric oxide in fruit ripening: trends and opportunities. Biotechnol. Adv. 28, 489–499.

Montreal Protocol, 1987. The montreal protocol on Substances that deplete the ozone layer. Available from. https://www.epa.gov/ozone-layer-protection/international-treaties-and-cooperation-about-protection-stratospheric-ozone.

Muramoto, J., 1999. Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Available from. https://pdfs.semanticscholar.org/6218/91ffac9e3568856a44552db93280dd47fb61.pdf.

Nayak, M.K., Collins, P.J., Pavic, H., 2003. Developments in phosphine resistance in China and possible implications for Australia. In: Wright, E.J., Webb, M.C., Highley, E. (Eds.), Proceedings of the Australian Postharvest Technical Conference Canberra, 25-27 June. CSIRO Stored Grain Research Laboratory, Canberra, pp. 156–159.

Njoroge, A., Affognon, H., Mutungi, C., Manono, J., Lamuka, P., Murdock, L., 2014. Triple bag hermetic storage delivers a lethal punch to *Prostephanus truncates* (Horn) (Coleoptera: bostrichidae) in stored maize. J. Stored Prod. Res. 58, 12e19.

Opit, G.P., Phillips, T.W., Aikins, M.J., Hasan, M.M., 2012. Phosphine resistance in Tribolium castaneum and Rhyzopertha Dominica from stored wheat in Oklahoma. J. Econ. Entomol. 105, 1107–1114.

Pannala, A.S., Mani, A.R., Spencer, J.P.E., Skinner, V., Bruckdorfer, K.R., Moore, K.P., Rice-Evans, C.A., 2003. The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. Free Radic. Biol. Med. 34, 576–584.

Santamaria, P., 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agric. 86, 10–17.

SAS Institute, 2012. SAS/STAT v.9.0. SAS Institute, Cary, North Carolina.

Soegiarto, L., Wills, R.B.H., 2004. Short term fumigation with nitric oxide gas in air to extend the postharvest life of broccoli green bean, and bok choy. HortTechnology 14, 538–540.

United Nations Environment Programme (UNEP), 2011. Special Review On Achieving Control Of Pest Eggs By Sulfuryl Fluoride, Report Of The Technology And Economic Assessment Panel, pp. 110–136. Available from: http://ozone.unep.org/Assessment_Panels/TEAP/Reports/TEAP_Reports/TEAP_Progress_Report_May_2011.pdf.

Walker, S., Jaime, R., Kagot, V., Probst, C., 2018. Comparative effects of hermetic and traditional storage devices on maize grain: mycotoxin development, insect infestation and grain quality. J. Stored Prod. Res. 77, 34–44.

Yang, X., Liu, Y.-B., 2017. Residual analysis of nitric oxide fumigation on fresh fruit and vegetables. Postharvest Biol. Technol. 132, 105–108.

Yang, X., Liu, Y.-B., 2018. Nitric oxide fumigation for control of spotted wing drosophila (Diptera: drosophilidae) in strawberries. J. Econ. Entomol. 111, 1180–1184.