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Browning on the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO)

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Abstract

Freshly cut lettuce slices (*Latuca sativa* L.) were fumigated with nitric oxide (NO) gas at concentrations between 5 and 1000 μl/l in air at 20 °C for 1–4 h or dipped in an aqueous solution of the NO-donor compound, 2,2′-(hydroxynitrosohydrazino)-bisethanamine (DETANO) at concentrations between 10 and 1000 mg/l for 15 s to 60 min at 20 °C. Development of browning on the cut surfaces was inhibited during subsequent storage at 0 °C. The most effective treatments for extending postharvest life of lettuce slices were fumigation with 500 μl/l NO for 1 h, and dipping in 500 mg/l DETANO for 5 min. Dipping in DETANO solution was, however, more effective as it generated a 100% increase in postharvest life compared with a 70% increase due to NO gas. Solutions of DETANO in water were found to be relatively stable as the same extension in postharvest life was obtained for five batches of lettuce sequentially dipped in the same solution.

Keywords: Nitric oxide; Cut lettuce; Postharvest life; Browning

1. Introduction

The convenience of minimally processed lettuces pieces has resulted in such products becoming an important segment of the fresh fruit and vegetables market at retail and food service level. However, various degradative changes are induced by the act of cutting and present additional challenges to the maintenance of quality during marketing. An important factor causing loss of quality is the development of browning on cut surfaces. While a number of metabolic pathways can lead to browning, an important route is through a range of endogenous phenolic compounds (containing an *o*-dihydroxy group) being oxidised to the corresponding *o*-quinones in the presence of oxygen by an oxidising enzyme, particularly polyphenoloxidase (PPO), with subsequent reactions leading to the formation of a coloured polymer (Robards, Prenzler, Tucker, Swatsitang, & Glove, 1999). Various chemical treatments give some inhibition of browning, the most widely used being ascorbic acid, often in combination with organic acids and calcium salts, while a common physical treatment is use of packages that allow development of a modified atmosphere of elevated carbon dioxide and reduced oxygen (Salcini & Massantini, 2005). Notwithstanding these treatments, browning on cut surfaces remains an impediment to the successful marketing of cut lettuces.

Nitric oxide (NO) is a highly reactive free radical gas that was initially notable as an industrial pollutant but is now known to be synthesized by mammals and plants Wendehenne, Pugin, Klessig, & Durne, 2001). Short term exposure to a low concentration of NO gas has been shown to extend the postharvest life of various intact fresh fruits and vegetables (Leshem, Wills, & Ku, 1998; Soegiarto & Wills, 2004; Sozzi, Trinchero, & Fraschina, 2003; Wills, Ku, & Leshem, 2000; Zhu, Liu, & Zhou, 2007; Zhu & Zhou, 2007).

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Pristijono, Wills, and Golding (2006) examined the effect of NO gas on the development of browning on the cut surface of apple slices and found that exposure to 10 μl/l NO for 1 h delayed the onset of browning by about 50% compared with untreated produce. While most of the study was with Granny Smith apples, NO was also found to similarly delay browning in Royal Gala, Golden Delicious, Sundowner, Fuji and Red Delicious apple slices.

Research into the role of NO in mammalian physiology has led to emergence of NO donor technology, that is solid compounds that store NO chemically but regenerate it under appropriate physical conditions (Keefer, 1998). Of the many NO-donor compounds that now exist, the diazeniumdiolates are of considerable interest, due to their high water solubility, chemical stability and ease of synthesis (Hrabie & Keefer, 2002). Of these, 2,2′-(hydroxynitrosohydrazino)-bisethanamine (DETANO) is of interest for use on horticultural crops as it contains 50% nitric oxide by mass and releases two equivalents of nitric oxide gas per molecule and is quite stable in solution at pH >7.0 (Hrabie, Klose, Wink, & Keefer, 1993). Studies on the action of DETANO with horticultural crops have been conducted by Noritake, Kawakita, and Doke (1996), who found that application of DETANO induced an accumulation of the endogenous phytoalexin, rishitin, in potato tubers and by Bowyer, Wills, Badiyan, and Ku (2003), who extended the vase life of carnation flowers by about 50% by standing flower stems in a solution containing DETANO.

The aim of this work was to examine the development of browning on the cut surface of freshly cut iceberg lettuce strips following exposure to NO gas or dipping in an aqueous solution containing DETANO.

2. Materials and methods

Mature heads of iceberg lettuce (*Lactuca sativa* L.) were obtained from a retail outlet and transported to the laboratory. Outer, damaged and wilted leaves were discarded and high quality individual leaves of uniform size and shape were selected. The mid-rib section of each lettuce leaf was cut horizontally into 1 × 3 cm slices (Saltveit, 2004) and slices from a single leaf were evenly distributed into the required number of 4 l plastic containers. Each plastic container became a treatment unit and held 32–34 slices from leaves obtained from 2 to 3 lettuce heads with a total weight of 40–45 g. There were two units per treatment in an experiment. Experiments were replicated 3–9 times with lettuces from different times of purchase. The numbers of replicates used in each trial are denoted with the results.

NO gas was applied after sealing a container by injecting the desired concentration of NO into the container through an injection port in the lid. The aliquot of NO was obtained from a cylinder of 4500 ± 170 μl/l NO in nitrogen (BOC Gases, Sydney). The container remained sealed for 1–4 h at 20 °C. Control containers of lettuce slices were similarly stored in a sealed container for 1–4 h at 20 °C but without the addition of NO gas. After treatment, the lids of all containers were opened to allow the entry of atmospheric air. The lid was then replaced on the container but with an open injection port to prevent the accumulation of carbon dioxide in the container. A beaker of water was placed inside containers to maintain a high humidity. Containers were stored at 0 °C.

DETANO (supplied as a powder by Dr. M.C. Bowyer, University of Newcastle) was applied by dipping a unit of sliced lettuce in a solution of DETANO at the desired concentration that was dissolved in water or a 0.01 M phosphate buffer solution at pH 7.0 and 6.5. The buffer solution at pH 7.0 was prepared at 20 °C by dissolving sodium dihydrogen phosphate (1.2 g) in distilled water (900 ml),then adding sodium chloride (7.733 g), with the resulting solution titrated with potassium hydroxide solution (0.1 M) to obtain pH 7.0 and the volume then brought to 1 l by adding distilled water. The buffer solution at pH 6.5 was similarly prepared, except that 8.058 g was added to the sodium dihydrogen phosphate solution and the titration with potassium hydroxide was to pH 6.5. The sliced lettuce was placed into a strainer which was placed into the DETANO solution (2 l) for periods ranging from 15 s to 60 min at 20 °C. After allowing to drain for 60 s, the slices were stored in a plastic container at 0 °C in air with a hole in the lid and a beaker of water placed in the container as was done for slices treated with NO gas. Control units were similarly dipped in water.

The development of browning was assessed daily by visual observation of the cut surface of each lettuce slice, using a scoring scale of 1–5 where 5 = fresh without any browning or discoloration, 4 = slight browning, 3 = moderate browning, 2 = severe browning, 1 = complete browning or discoloration of the cut surface. The mean score for all lettuce slices in a treatment unit was calculated for each observation and plotted against time. The time taken for the slice colour to decline to a score of 3 was determined from the graph and taken as the postharvest life of the lettuce in that treatment unit. Statistical analysis was performed on the quality data (SAS version 9, SAS Institute, Cary, NC) and least significant difference (LSD) at $P = 0.05$ was used to determine significant difference between means. Linear regression equations were calculated, where appropriate, to determine the relationship between postharvest life and the applied treatment (SPSS version 12, Chicago IL).

3. Results and discussion

3.1. Effect of fumigation with nitric oxide gas on browning

Initial experiments examined NO gas concentrations ranging from 5 to 1000 μl/l. The first experiment showed an increase in postharvest life due to the application of 100 μl/l NO gas compared with control lettuce (Table 1). The use of higher concentrations of NO gas in experiment 2 showed that application of 500 μl/l NO gas gave a higher

Table 1
Postharvest life of lettuce slices fumigated with NO gas in air at 20 °C for 2 h, followed by storage at 0 °C in humid air

NO concentration (μl/l)	Postharvest life (days)		
	Experiment 1 ($n = 5$)[a]	Experiment 2 ($n = 4$)	Experiment 3 ($n = 12$)
0	6.5	7.2	7.4
5		7.5	
10	8.5	7.1	
50		8.3	
100	9.7	9.0	9.4
500		16.7	12.5
1000			–[b]
l.s.d. ($P = 0.05$)	2.39	4.66	2.18

[a] Number of replicate units in the experiment with 2 units per treatment in each replicate.
[b] No browning had developed after 14 days but overall appearance was unacceptable after 1 day.

Table 2
Postharvest life of lettuce slices dipped in DETANO solution at 20 °C for 60 s followed by storage at 0 °C in air

DETANO concentration (mg/l)	Postharvest life (days)		
	Experiment 1 ($n = 7$)[a]	Experiment 2 ($n = 4$)	Experiment 3 ($n = 4$)
Untreated	6.74	7.5	8.4
0 (water only)	7.4	8.4	9.1
10	7.5		
50	8.1		
100	8.9		
250	10.5	10.2	
500		12.0	13.8
1000			13.8
l.s.d. ($P = 0.05$)	1.68	1.52	0.93

[a] Number of replicate units in the experiment with 2 units per treatment in each replicate.

postharvest life than all other concentrations, including untreated lettuce. The beneficial effect of 500 μl/l over 100 μl/l NO gas was confirmed in experiment 3. A concentration of 1000 μl/l NO was also applied but the lettuce slices lost all green colour and crispiness soon after the fumigation and, although no browning had developed after 14 days at 0 °C, the slices were considered of unacceptable quality for marketing. The high concentration of 1000 μl/l would seem to have caused irreversible damage to lettuce cells.

The effect of fumigation time was examined by holding lettuce slices in 500 μl/l of NO gas for 1, 2 and 4 h. While the use of 500 μl/l of NO significantly increased postharvest life (mean value 10.8 days) over control slices (7.4 days; LSD ± 1.24), there was no significant difference between times of fumigation. Thus, the shorter 1 h fumigation time was considered to be the preferred treatment period for operational efficiency. Fumigation times longer than 4 h were not considered, as the half-life of a low concentration of NO in air has been reported to be 3.5 h due to aerial oxidation (Soegiarto, Wills, Seberry, & Leshem, 2003).

3.2. Effect of dipping in aqueous DETANO solution on browning

The initial experiment examined the development of browning on lettuce slices dipped in water containing 10–250 mg/l DETANO for 60 s at 20 °C, followed by storage at 0 °C. The data for experiment 1 in Table 2 show that the postharvest life of lettuce dipped in 250 mg/l of DETANO was longer than that of the water control but regression analysis of the data showed a significant linear relationship between postharvest life (y) and DETANO concentration (x) of $y = 0.012x + 7.5$ ($R^2 = 0.99$, $P < 0.001$). The effect of higher concentrations of DETANO was therefore examined. Dipping lettuce slices in 500 mg/l of DETANO resulted in a longer postharvest life than dipping in 250 mg/l (experiment 2) but no further increase in postharvest life was achieved by dipping in 1000 mg/l DETANO (experiment 3). The optimum DETANO treatment for iceberg lettuce slices was therefore considered to be 500 mg/l.

The effect of dipping time was initially examined by holding lettuce slices in a solution containing 500 mg/l of DETANO for 15 s to 5 min at 20 °C, followed by storage at 0 °C. The longest dipping time of 5 min resulted in a longer postharvest life than all the shorter dipping times of 15–60 s (Table 3). A subsequent, comparison with longer dipping times of up to 1 h showed no additional extension in postharvest life over a 5 min dip (experiment 2). However, the general appearances of lettuce slices dipped for 30 and 60 min were paler than those of all other treatments and were considered unacceptable for marketing. There was no significant difference in postharvest life for slices dipped in water for only 15 s to 60 min, indicating that the beneficial effect of longer dipping times in a DETANO solution was due to DETANO. The optimum

Table 3
Postharvest life of lettuce slices dipped in 500 mg/l of DETANO solution or water only at 20 °C for different times, followed by storage at 0 °C in air

Dip treatment	Postharvest life (days)	
	Experiment 1 ($n = 4$)[a]	Experiment 2 ($n = 3$)
Untreated	6.3	6.3
Water, 15 s	6.8	
Water, 30 s	6.3	
Water, 1 min	6.8	
Water, 5 min	7.0	7.1
Water, 60 min		6.5
DETANO, 15 s	10.7	10.4
DETANO, 30 s	11.0	
DETANO, 1 min	11.6	
DETANO, 5 min	13.8	13.6
DETANO, 10 min		13.4
DETANO, 15 min		13.2
DETANO, 30 min		14.3
DETANO, 60 min		13.8
l.s.d. ($P = 0.05$)	1.80	2.71

[a] Number of replicate units in the experiment with 2 units per treatment in each replicate.

treatment regime for DETANO was thus considered to be dipping in 500 mg/l for 5 min.

Given the effect of pH on the stability of DETANO in solution (Hrabie et al., 1993) and the potential for leakage of acidic materials from the cut lettuce slices, an examination was made of the postharvest life of lettuce slices following dipping in water buffered to pH 6.5 and pH 7.0 with phosphate. A longer postharvest life was found for lettuce slices dipped in DETANO dissolved in water or buffer than for slices dipped in water or buffer only (Table 4). The postharvest lives were, however, not significantly different between DETANO dissolved in water and dissolved in either buffer solution. There was also no significant increase in postharvest life due to dipping in either buffer over that obtained with water. Thus there appeared to be no need to buffer the water used to dissolve DETANO.

3.3. Comparison of DETANO solution and NO gas on browning

Comparison was made of the relative effectiveness of the optimum treatments of DETANO solution and NO gas found in previous experiments. DETANO solution and NO gas were found to result in longer postharvest lives than the respective control treatments of water and untreated, respectively (Table 5). However, lettuce slices dipped in 500 mg/l DETANO for 60 s or 5 min had a longer postharvest life than had slices fumigated with 500 μl/l of NO gas for 2 h. There was a 70% increase in postharvest life of lettuces fumigated with NO gas compared with an 80% increase for lettuces dipped in DETANO for 60 s and 100% when dipped for 5 min compared with the respective control treatments of untreated and water.

Throughout the study, it was noticed that the postharvest life of lettuce slices dipped in water was always slightly longer than that of untreated slices with the difference being statistically significant in some experiments and not in others. Combining the data for these treatments from all experiments resulted in a significantly higher ($P < 0.001$) postharvest life, by about 10%, in water dipped slices (7.28 days) compared with untreated (6.66 days; mean of 54 replicate units with 2 units per replicate). This effect raised the possibility that the postharvest life of lettuce slices fumigated with NO gas might be further increased if the slices were dipped in water prior to fumigation. However, it was found that lettuces dipped in 500 mg/l DETANO for 5 min still had a longer postharvest life than slices dipped in water before fumigation with 500 μl/l of NO gas for 2 h (Table 6).

Table 4
Postharvest life of lettuce slices dipped in a buffered 500 mg/l of DETANO solution for 60 s at 20 °C, followed by storage at 0 °C in air

Dip treatment	Postharvest life (days)
Untreated	5.8
Water (pH 6.6)	5.9
DETANO in water	10.7
Buffer, pH 6.5 (phosphate)	7.0
DETANO in buffer, pH 6.5	9.1
Buffer, pH 7.0 (phosphate)	6.5
DETANO in buffer, pH 7.0	9.2
l.s.d. ($P = 0.05$)	2.29

Values are the mean of 5 replicates with 2 units per treatment in each replicate.

Table 5
Postharvest life of lettuce slices dipped in DETANO solution and fumigated with NO gas at 20 °C, followed by storage at 0 °C in air

Treatments	Postharvest life (days)	
	Experiment 1 ($n = 6$)[a]	Experiment 2 ($n = 4$)
Untreated	6.4	6.6
Water, 60 s	7.0	
Water, 5 min		7.4
500 μl/l NO gas, 2 h	11.0	11.0
500 mg/l DETANO, 60 s	12.6	
500 mg/l DETANO, 5 min		13.8
l.s.d. ($P = 0.05$)	0.81	2.73

[a] Number of replicate units in the experiment with 2 units per treatment in each replicate.

3.4. Repeated dipping of lettuce slices into a DETANO solution

An examination was made of the ability of DETANO solution to extend postharvest life when batches of lettuce slices were sequentially dipped in the same solution of 500 mg/l of DETA/NO for 60 s with 3 min between dips. The stability of DETANO in the solution was also assessed. The data in Table 7 confirm the previous result that lettuce slices dipped in a solution containing 500 mg/l of DETANO had a longer postharvest life than had those dipped in water. Table 7 also shows that sequential dipping of an additional four batches of lettuce slices into the same solution gave the same increase in postharvest life as did the initial dipped slices. The stability of DETANO in solution was measured with a UV–VIS spectrophotometer at 258 nm, with five readings taken on each solution and Table 7 shows that there was no significant change in DETANO concentration arising from the dipping of five batches of lettuce slices. Table 7 also shows that there was no significant change in the pH of the solution after

Table 6
Postharvest life of lettuce slices washed in water prior to fumigation with NO gas at 20 °C, followed by storage at 0 °C in air, compared with slices dipped in DETANO solution

Treatment	Postharvest life (days)
Water, 60 s	7.8
Water 60 s + 500 μl/l NO gas, 2 h	10.2
500 mg/l DETANO, 5 min	13.3
l.s.d. ($P = 0.05$)	1.17

Values are the means of 4 replicates with 2 units in each replicate.

Table 7
Postharvest life of lettuce slices sequentially dipped at 20 °C in the same solution of 500 mg/l DETANO at pH 6.5 followed by storage at 0 °C in air, and the concentration of DETANO and pH of the solution after dipping

Treatment	Postharvest life (days)	DETANO concentration (mg/l)	Solution pH
Untreated	6.4[a]		
Water	6.4		
DETANO, 1st dipping	12.5	497[a]	6.57[a]
DETANO, 2nd dipping	11.3	491	6.55
DETANO, 3rd dipping	12.5	494	6.53
DETANO, 4th dipping	12.7	488	6.49
DETANO, 5th dipping	11.6	495	6.47
l.s.d. ($P = 0.05$)	2.09	14.8	0.08

Values are the mean of 4 replicates with 2 units per replicate.
[a] Readings were taken on the dipping solution after lettuce slices were removed.

sequential dipping. Thus, lettuce slices did not have any marked effect on the stability of DETANO during the dipping process.

4. Conclusions

The data thus show that the application of NO, by either fumigation with gas or dipping in a solution of the NO-donor compound, DETANO, will extend the postharvest life of cut lettuce slices stored at 0 °C by inhibiting the development of browning on the cut surfaces. The optimum treatment for NO gas of an initial concentration of 500 μl/l for 1 h resulted in about a 70% increase in postharvest life over untreated slices. While the application of 1000 μl/l of NO gas strongly inhibited browning on the cut surface, it caused rapid severe tissue damage and loss of colour to the leaf surface However, dipping lettuce slices in a solution of DETANO was more effective than was fumigation for inhibiting cut surface browning with use of a solution of 500 mg/l for 5 min increasing postharvest life by about 100% over water-dipped slices. Use of a shorter dipping time of 60 s also resulted in a shorter but still useful extension in postharvest life and may be more desirable in a flow-through lettuce processing operation. The stability of the DETANO, when dissolved in water and subjected to sequential dipping of lettuce slices, suggests that any substrate leaking from the cut surface of the lettuce is not acidic enough to react with DETANO. This stability (and the lack of additional extension in postharvest life when DETANO was dissolved in buffered solution) indicates that a commercial processing operation could use water without addition of a buffering agent, which has an obvious cost advantage.

The inhibition of browning on the cut surface of lettuces by NO, found in this study, when coupled with the inhibition of browning on apples by NO gas reported by Pristijono et al. (2006), raises the possibility that NO may inhibit browning on a range of other minimally processed fruit and vegetables. If so, then NO must be acting on a system that is common to all such produce. While it is not known how NO is acting in its inhibition of cut surface browning, Leshem (2000) proposed that the oxidative properties of NO are important mediating factors and that NO or its reaction products can oxidatively inactivate enzyme co-factors, such as ascorbate and ferrous ion. A possible effect of NO in modulating polyphenolase activity and oxidative enzymes in lettuce and apples is worthy of further study. Perhaps the free radical-scavenging ability of NO may scavenge and thereby interrupt normal browning reactions on the cut surface. However, it seems that the most effective NO gas concentration for apples was 10 μl/l whereas, for lettuce, it was 500 μl/l. This could indicate some difference in the control over the mode of action of NO between products. The greater effectiveness of DETANO over NO gas would seem to be a commercial advantage as most minimally processed produce is invariably passed through some washing process. Thus, it may be possible to utilise the DETANO, or some other NO-donor compound, in an existing process operation. However, where a gas injection system is in use, for example, for modified atmosphere packaging, it may easily be possible to inject NO gas into the package.

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