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Nitric Oxide Inhibition of Alcohol Dehydrogenase in Fresh-Cut Apples (*Malus domestica* Borkh)

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ABSTRACT: The effects of nitric oxide (NO) and nitrite treatment on alcohol dehydrogenase activity and the shelf life of apple tissue were investigated. Fresh-cut apple slices were stored for 2 days at 6 °C in 0.25−1% NO (v/v, balance N_2) or 100% N_2 atmospheres. Slices were also treated with 1% NO or 2 mM sodium nitrite ($NaNO_2$) for 20 min, stored for 6 weeks in 100% N_2 at 6 °C, and analyzed for acetaldehyde, ethanol, and ethyl acetate accumulation, firmness, and color. Compared with N_2 or deionized water controls, treatment with 1% NO or 2 mM $NaNO_2$ inhibited ethanol accumulation, whereas that of acetaldehyde increased. Ethyl acetate accumulation was inhibited only by NO. Slice firmness was not affected by NO or $NaNO_2$ treatment, but slices were darker than the untreated controls. NO and nitrite may extend the shelf life of fresh-cut produce with low concentrations of phenolic compounds.

KEYWORDS: *nitric oxide, fresh-cut, alcohol dehydrogenase, fermentation products, browning, firmness, shelf life, Malus domestica Borkh*

INTRODUCTION

The endogenous synthesis of nitric oxide (NO), a lipophilic, free radical gas, was first discovered in animals.[1−3] NO is involved in many mammalian physiological processes including the relaxation of blood vessels,[2] blood pressure regulation,[4] and neurotransmission.[5] NO modification of proteins may reversibly activate or inhibit enzymes such as the activation of soluble guanylate cyclase[6] and the inhibition of mitochondrial respiration by NO binding to cytochrome *c* oxidase.[7,8] NO stimulates seed germination[9−11] and plays an important signaling role in plant disease resistance[12,13] The postharvest application of NO to produce can extend the shelf life of broccoli, green bean, and bok choy[14] and inhibit surface browning of fresh-cut apples and lettuce.[15−18]

Modified atmosphere packaging (MAP) utilizing low O_2 and elevated CO_2 levels has been used to extend produce shelf life.[19] However, the low O_2 atmospheres associated with MAP result in undesirable anaerobic fermentation and the accumulation of acetaldehyde, ethanol, and ethyl acetate, which renders the cut produce organoleptically unacceptable. The inhibition of alcohol dehydrogenase (ADH) in produce would reduce the accumulation of ethanol and ethyl acetate and address this limitation of MAP. NO has been shown to inhibit rat and equine liver ADH catalyzed conversion of ethanol to acetaldehyde.[20] NO inhibition of the reverse reaction (acetaldehyde to ethanol) catalyzed by ADH2 has been demonstrated in *Entamoeba histolytica*[21] but not in plant tissue. The objective of this study was to determine whether NO and nitrite could be used to inhibit ADH activity and ethanol formation and extend the shelf life of fresh-cut apples.

MATERIALS AND METHODS

Apple Preparation. Delicious apples (*Malus domestica* Borkh cv. Red Chief), average weight 230 g, were harvested from the Cornell Orchards and stored in controlled atmospheres (CA) of 2−2.2% O_2 and 1.4−1.5% CO_2 (balance N_2) at 1 °C for 1−5 months. Apples were removed from CA, stored in air at 2 °C, and processed within 2 weeks. Prior to processing, apples were removed from cold storage and kept overnight at room temperature (25 °C). The apples were washed in deionized water (25 °C), peeled, and placed in deionized water (25 °C) until they were cored (1 cm diameter) and sliced into 8 or 16 wedges each, using a hand-operated corer/slicer and a sharp knife, or sliced horizontally 1.5 or 3 mm thick using a mandoline (Zyliss USA, Foothill Ranch, CA, USA). Slices were pooled and placed in deionized water (25 °C), removed, and blotted dry with cheesecloth. The slices were weighed and randomly distributed among the treatments.

Color Measurement. Average color values (CIE *L** values) of six slices per anaerobic vessel or pouch (three readings per slice) were measured with a color meter (model CR-100, Minolta Camera Co. Ltd., Ramsey, NJ, USA) using the method described by Amissah et al.[22]

Firmness Measurement. Firmness was measured using a back extrusion cell in the procedure described by Bourne and Moyer.[23] An Instron Universal Testing Machine (model 1122, Instron Corp., Canton, MA, USA) fitted with a 5.8 cm i.d. × 9.7 cm high stainless steel cup and a 4.9 cm wide plunger was used. Each slice was cut into two pieces and 70 g placed in the cup and then extruded at 50 mm/min with the plunger. Firmness was the maximum force expressed as newtons (N).

Acetaldehyde, Ethyl Acetate, and Ethanol Analysis. Apple slices from each pouch or anaerobic vessel were treated with liquid N_2 and pulverized in a Hamilton Beach blender (Scovill Inc., Washington, NC, USA). Pulverized apple (5 g) was placed in a 20 mL septum-stoppered glass vial (Fisher Scientific, Pittsburgh, PA, USA), and 0.25 mL of aqueous 4.16 mM 1-propanol was added as an internal standard. Vials were stored at −20 °C until analyzed using the method described

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by Amissah et al.[22] with modifications. Vials were heated in a heating block (Isotemp 125D, Fisher Scientific) at 64 °C for 24 min. A headspace sample (0.5 mL) was withdrawn from the vial with a gastight glass syringe and injected into a gas chromatograph equipped with a flame ionization detector (Varian 3800, Walnut Creek, CA, USA). A DB-Wax fused silica capillary column 15 m × 0.53 mm with a 1.0 μm film (J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds. The column temperature was set at 65 °C. An integrator (Varian 4290) was used to quantify the acetaldehyde, ethanol, and ethyl acetate peaks.

NO Assay. NO in the prepared gas mixtures was assayed with Greiss reagent [1:1 v/v solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED 0.1% in distilled water) and sulfanilamide (1% sulfanilamide in 5% concentrated H_3PO_4)].[24] Greiss reagent (1.8 mL) was pipetted into a 2 mL glass vial. The vial was sealed with a screw-cap top containing a rubber septum. NO gas mixtures of 0.1 mL were injected into the vial, and absorbance (546 nm) was measured after the vial had been incubated at room temperature (25 °C) for 15 min. A standard curve was prepared from the absorbance of known concentrations of $NaNO_2$ added to Greiss reagent.

ADH Activity of Fresh-Cut Apple Treated with NO or $NaNO_2$. Apples were prepared as above and sliced into wedges (8 or 16 per apple) or horizontally 1.5 or 3 mm thick. The horizontal slices (200 g) were placed on stainless steel rods with 0.5 mm stainless steel separators between slices. Apple wedges and horizontal slices (200 g) were each placed in three 3.3 L anaerobic vessels (Torbal model AJ-2, Torsion Balance Co., Clifton, NJ, USA) fitted with a vacuum/pressure gauge and Swagelok regulating stem valves at the inlet and outlet ports. The vessels were flushed with 100% N_2 for 10 min at a flow rate >700 mL/min to obtain an atmosphere with <0.1% O_2. The composition of the atmosphere in the anaerobic vessel was determined by gas chromatography (Varian Aerograph moduline 2700; Varian) with a molecular sieve N_2/O_2 5A packed column. Vacuum (3.3 kPa) was pulled on all vessels and released with 1% NO (balance N_2) or 100% N_2 (control) for 20 min. The experiment was repeated by dipping apple wedges and horizontal slices (200 g) in triplicate in solutions of 2 mM $NaNO_2$ or deionized water (control) for 20 min. ADH activity of sliced apple was determined using the method of Ke et al.[25] with modifications. For each 200 g portion of wedges or horizontal slices, 3 g was homogenized in 10 mL of 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0) with added 2 mM dithiothrietol (DTT) and 1% (w/v) polyvinylpyrolidone (PVP), filtered through four layers of cheesecloth, and then centrifuged at 27000*g* for 10 min at 4 °C. The supernatant was decanted and placed on ice as enzyme extract. ADH activity of the enzyme extract was then analyzed at 30 °C by adding 0.05 mL of 80 mM acetaldehyde to a mixture of 0.8 mL of 100 mM MES buffer (pH 6.0), 0.05 mL of 1.6 mM NADH, and 0.1 mL of enzyme extract. The decrease in absorbance (340 nm) due to the oxidation of NADH was measured using a spectrophotometer (model 6300, Jenway Ltd., Felsted, UK).

Minimum NO Concentration Required To Inhibit ADH in 400 g of Apple Tissue. 'Delicious' apples were prepared as above and sliced into 16 wedges. Apple slices (400 g) were packed into 3.3 L stainless steel anaerobic vessels. The vessels were flushed with 100% N_2 as above. Vacuum (3.3 kPa) was pulled on all vessels and released with 0.25, 0.5, 0.75, and 1% (v/v) NO (balance N_2) or 100% N_2 (control). NO headspace concentrations were determined as described above. Table 1 shows the total number of moles of NO in the headspace. The anaerobic vessels were stored at 6 °C for 2 days. Apple slices in three anaerobic vessels for each storage atmosphere were analyzed (one reading per vessel) for firmness and the accumulation of acetaldehyde, ethanol, and ethyl acetate. Average color values (CIE *L** values) of six wedges from each vessel; three readings per wedge were also measured.

Shelf Life of Fresh-Cut Apple Slices Treated with NO or $NaNO_2$. Eight wedges per apple were prepared as above. Apple wedges (650 g) were placed in 3.3 L anaerobic vessels and flushed with 100% N_2 for 10 min as described above. Vacuum (3.3 kPa) was pulled on all vessels and released with 100% N_2 (control) or 1% (v/v) NO (balance N_2). Anaerobic vessels were stored at 25 °C for 20 min and then flushed with 100% N_2 for 10 min at a flow rate >700 mL/min. Wedges (200 g) were packed in 20 × 22 cm pouches made from a high O_2 barrier plastic film (CVP Systems, Cold Stream, IL, USA). The film was a laminate of nylon (0.8 mil), ethyl vinyl acetate (1.2 mil), and Surlyn (2 mil) with gas transmission rates (cm^3/m^2/24 h) of 28−38 for O_2, 4−7 for N_2, and 108−128 for CO_2. The pouches were flushed with 100% N_2 for 2 min and heat sealed as above. The O_2 content of the pouches was <0.1% as determined by gas chromatography. The pouches were stored in the dark at 6 °C.

Table 1. NO Headspace Concentrations in Anaerobic Vessels (3.3 L) Containing 400 g of Apple Slices[a]

NO headspace concentration		vessel headspace
% (v/v)	mol/L of NO[b]	total NO (mol)
1	4.1×10^{-4}	11.4×10^{-4}
0.75	3.1×10^{-4}	8.6×10^{-4}
0.5	2.0×10^{-4}	5.5×10^{-4}
0.25	1.0×10^{-4}	2.8×10^{-4}
0.125	5.1×10^{-5}	1.4×10^{-4}

[a]Weight of apple was 400 g, volume of apple was 0.53 L, and the headspace of the vessel was 2.77 L. [b]Concentration of NO (mol/L) was calculated using a temperature of 25 °C.

For $NaNO_2$ treatment, apple wedges were dipped in deionized water (control) or 2 mM $NaNO_2$ for 20 min and then blotted dry with cheesecloth. Apple wedges (200 g) were packed in high O_2 barrier (20 × 22 cm) pouches, flushed with 100% N_2 for 2 min, and heat sealed. The pouches were stored at 6 °C. Each week, three pouches were analyzed (one reading per pouch) for firmness and the accumulation of acetaldehyde, ethanol, and ethyl acetate. Average color values (CIE *L** values) of six wedges from each pouch, three readings per wedge, were also measured.

Statistical Analysis. The data were analyzed using Minitab release 15 (Minitab Inc., State College, PA, USA). The General Linear Model was used to determine the treatment and interaction effects. Tukey's test was used to compare the different factor levels, and the data for ethanol accumulation were analyzed by regression.

RESULTS AND DISCUSSION

ADH Activity of Fresh-Cut Apple Treated with NO or $NaNO_2$. NO treatment inhibited ADH activity in 'Delicious' apples compared with the N_2 controls (Figure 1). Increasing the surface area to volume ratio of the apple slices by decreasing slice thickness (from 8 wedges/apple to 16 wedges/apple or from 3 mm to 1.5 mm) resulted in a decrease in ADH activity with the lowest ADH activity observed in the 1.5 mm thick slices, even though (for each slice thickness) the same weight of apple was treated (Figure 1). Similar results were obtained in the slices treated with deionized water or 2 mM $NaNO_2$. Compared with the deionized water controls, ADH activity in all slices was inhibited by treatment with 2 mM $NaNO_2$ (Figure 1). Greater inhibition of ADH activity was observed as slice surface area to volume ratio was increased (Figure 1). The lowest ADH activity was observed in the 1.5 mm thick slices.

Soegiarto et al.[26] reported the importance of surface area in the absorption of NO by produce. For the same weight of produce (100 g), the rate of NO absorption in green leafy vegetables was over 100 times greater than in lime, but when measured over the same surface area (100 cm^2) only 15 times greater.[26] The marked difference in the rate of NO absorption for a unit weight of leafy vegetables compared with lime reported by Soegiarto et al.[26] and the increased inhibition of ADH activity with an increase in apple surface area to volume ratio suggest that compared with thick slices or whole produce,

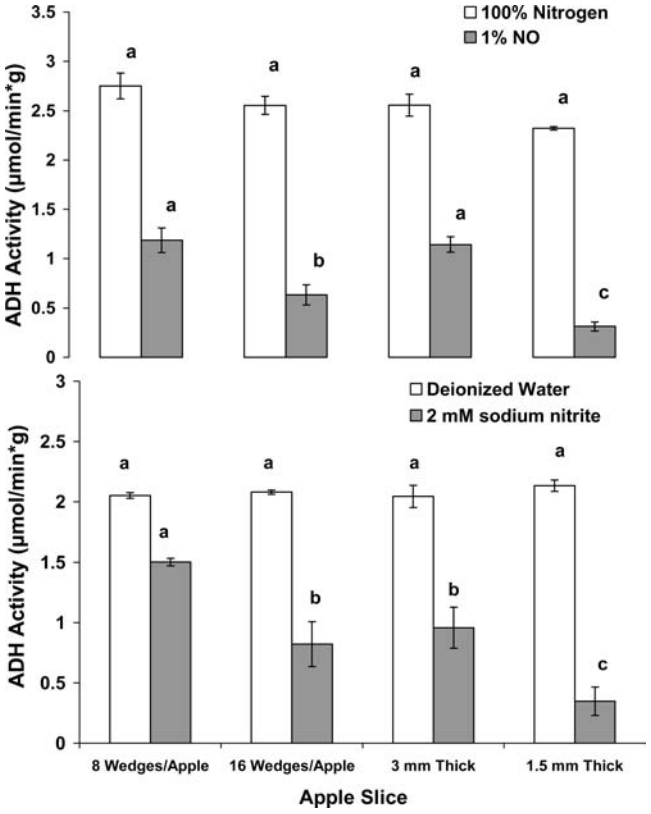

Figure 1. ADH activity (μmol/min·g) of 200 g of 'Delicious' apple slices treated with 100% N_2 or 1% NO (balance N_2) (■) atmosphere for 20 min in 3.3 L anaerobic vessel or with deionized water (□) only or 2 mM $NaNO_2$ (■) for 20 min. Bars represent the standard error (±SE). Means for the same treatment with different letters are significantly different (p = 0.05, n = 3).

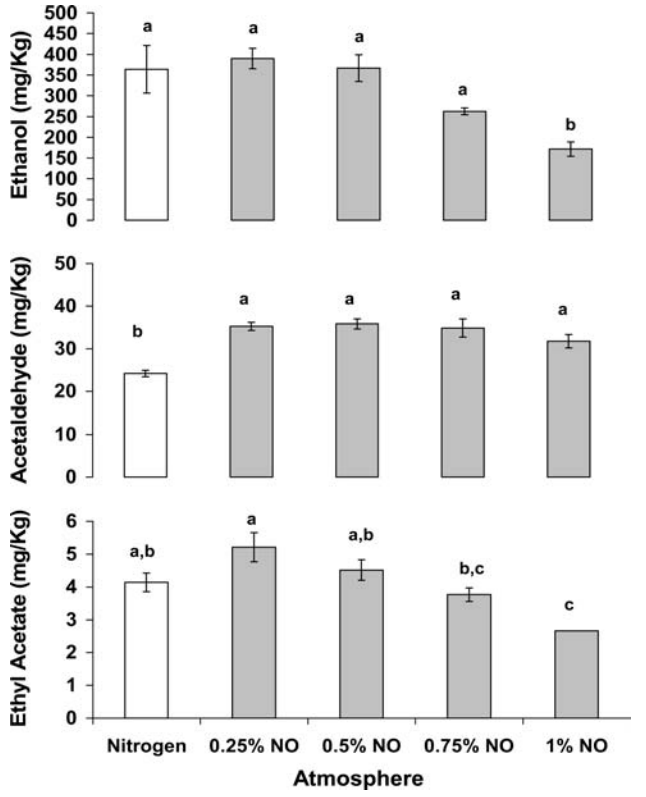

Figure 2. Ethanol, acetaldehyde, and ethyl acetate concentration (mg/kg) of sliced 'Delicious' apples (400 g) stored in 3.3 L anaerobic vessels in 100% N_2 (□) or 0.25−1% (v/v) NO (■), (balance N_2) atmospheres at 6 °C for 2 days. Bars represent the standard error (±SE). Means for the same compound with different letters are significantly different (p = 0.05, n = 3).

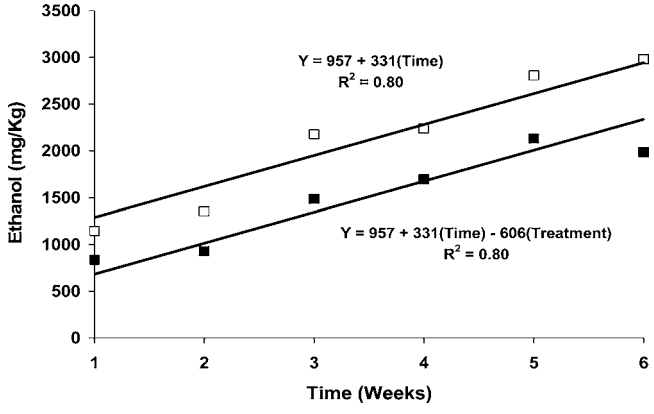

Figure 3. Ethanol concentration (mg/kg) of sliced 'Delicious' apples treated with N_2 (□) only or 1% NO (■) (balance, N_2) atmosphere for 20 min and stored for 1−6 weeks in 100% N_2 atmosphere (n = 3).

lower fumigation times and NO concentrations may be used to inhibit ADH activity in thin slices of produce.

Minimum NO Concentrations Required To Inhibit ADH Activity in 400 g of Apple Tissue. Ethanol accumulation of apple slices treated with 1% NO (v/v, headspace) was inhibited 52% compared with slices treated with N_2 only, but the effects of NO concentrations <1% were not significant (Figure 2). Slices treated with NO concentrations of 0.25−1% (v/v headspace) accumulated 42% more acetaldehyde compared with slices treated with N_2 only, and ethyl acetate accumulation was inhibited 35% only in slices treated with 1% NO (Figure 2).

Shelf Life of Fresh-Cut Apple Slices Treated with NO or Nitrite. Concentrations of 1% NO and 2 mM $NaNO_2$ were used for the shelf life experiment. NO-treated slices accumulated less ethanol than slices treated with N_2 only when packaged in 20 × 22 cm (high O_2 barrier) pouches and stored at 6 °C (Figure 3). In slices treated with N_2 only or NO (balance N_2) ethanol accumulation increased linearly (Figure 3) with storage time. The accumulation of ethanol is described by the linear regression model: $Y = 957 + 331(a) - 606(b)$ with $R^2 = 0.80$, where Y = ethanol accumulation, a = time, and b = treatment. On average, NO treatment decreased ethanol accumulation by 606 mg/kg over the 6 week storage period. Slices treated with 2 mM $NaNO_2$ accumulated less ethanol than slices treated with deionized water (Figure 4). There was a linear increase in ethanol accumulation with storage time in slices treated with either deionized water or $NaNO_2$. The accumulation of ethanol is described by the linear regression model $Y = 718 + 598(a) - 441(b)$ with $R^2 = 0.91$, where Y = ethanol accumulation, a = time, and b = treatment. On average, treatment with 2 mM $NaNO_2$ decreased ethanol accumulation by 441 mg/kg over the 6 week storage period. ADH activity was inhibited in the apple slices before the slices were packaged in pouches and stored for 6 weeks in N_2 (Figure 1), perhaps accounting for the identical ethanol accumulation slopes for slices treated with NO or $NaNO_2$ when compared with the N_2 and deionized water controls, respectively (Figures 3 and 4).

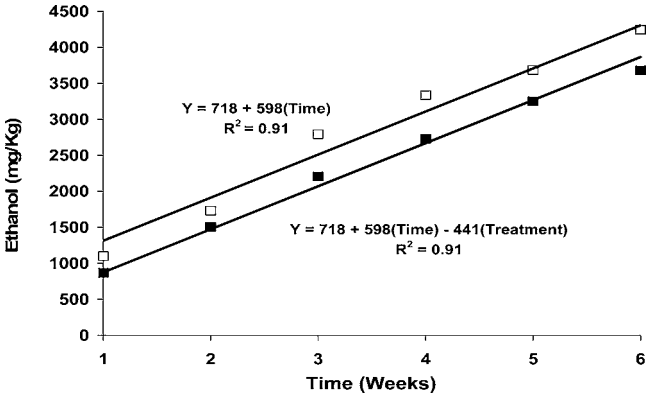

Figure 4. Ethanol concentration (mg/kg) of sliced 'Delicious' apples treated with deionized water (□) or 2 mM $NaNO_2$ (■) for 20 min and stored for 1−6 weeks in 100% N_2 atmosphere (n = 3).

Acetaldehyde accumulation in NO-treated slices was higher than in N_2-treated slices but was not affected by storage time (Table 2). Slices treated with $NaNO_2$ accumulated higher

Table 2. Acetaldehyde Concentration (Milligrams per Kilogram) of Sliced 'Delicious' Apples Stored for 1−6 Weeks in 100% N_2 Atmosphere[a]

time (weeks)	N_2	1% NO	deionized H_2O	2 mM $NaNO_2$
1	41	54	20 a	26 a
2	46	100	20 a	28 a
3	69	96	24 a	34 a
4	46	90	24 a	36 a
5	52	91	16 ab	34 a
6	35	95	7 b	23 a
		*		*

[a]Slices were treated with N_2 only or 1% NO (balance N_2) atmosphere for 20 min or treated with deionized water or 2 mM $NaNO_2$ for 20 min, prior to storage in 100% N_2 atmosphere for 1−6 weeks. Means for the same column with different letters are significantly different (p = 0.05). Responses to 1% NO or 2 mM $NaNO_2$ treatment are noted as significant (*) compared with N_2 or deionized water controls at p = 0.05 (n = 3).

concentrations of acetaldehyde than slices treated with deionized water (Table 2). Acetaldehyde accumulation was unaffected by storage time in slices treated with $NaNO_2$, but decreased at week 6 in slices treated with deionized water (Table 2). The higher acetaldehyde and lower ethanol concentrations observed over 6 weeks in both NO- and $NaNO_2$-treated apple slices than in the N_2- and deionized water-treated slices, respectively, are consistent with the inhibition of ADH, which catalyzes the conversion of acetaldehyde to ethanol. NO inhibition of the reverse reaction (ethanol to acetaldehyde) also catalyzed by ADH has been demonstrated in vitro by Gergel and Cederbaum[20] using rat and equine ADH.

Slices treated with 1% NO accumulated less ethyl acetate than slices treated with N_2 only, but compared with the deionized water treatment, 2 mM $NaNO_2$ had no effect on ethyl acetate accumulation (Table 3). Nitrite is converted to NO and nitrate in the presence of acid.[27] $NaNO_2$ in contact with the acidic apple surface is likely converted to NO, which, in turn, inhibits ADH. $NaNO_2$ concentrations >2 mM may be required to provide sufficient NO to reduce ethyl acetate accumulation. Storage time had no effect on ethyl acetate

Table 3. Ethyl Acetate Concentration (Milligrams per Kilogram) of Sliced 'Delicious' Apples Stored for 1−6 Weeks in 100% N_2 Atmosphere[a]

time (weeks)	N_2	1% NO	deionized H_2O	2 mM $NaNO_2$
1	8.0 ab	4.2 a	11.5 c	9.5 c
2	5.9 b	4.6 a	16.6 b	16.5 b
3	14.0 a	7.9 a	22.4 a	21.1 a
4	12.8 a	7.5 a	17.6 b	16.2 b
5	7.0 b	7.0 a	14.6 bc	16.8 bc
6	5.3 b	3.3 a	14.0 c	9.7 c
		*		ns

[a]Slices were treated with N_2 only or 1% NO (balance N_2) atmosphere for 20 min or treated with deionized water or 2 mM $NaNO_2$ for 20 min, prior to storage in 100% N_2 atmosphere for 1−6 weeks. Means for the same column with different letters are significantly different (p = 0.05). Responses to 1% NO or 2 mM $NaNO_2$ treatment are noted as significant (*) or not significant (ns) compared with N_2 or deionized water controls at p = 0.05 (n = 3).

accumulation in slices treated with 1% NO (balance N_2) or N_2 only, but increased with storage time in slices treated with deionized water or $NaNO_2$, reaching a maximum by week 3 and decreasing thereafter (Table 3).

Compared with the N_2 and deionized water controls, slice firmness was not affected by the NO or $NaNO_2$ treatments (Table 4). However, firmness of all slices decreased with

Table 4. Firmness (Newtons) of Sliced 'Delicious' Apples Stored for 1−6 Weeks in 100% N_2 Atmosphere[a]

time (weeks)	N_2	1% NO	deionized H_2O	2 mM $NaNO_2$
initial	1009 a	965 a	1113 a	1084 a
1	934 ab	712 ab	1007 a	984 a
2	689 bc	720 bc	977 ab	881 ab
3	451 c	526 c	983 ab	802 ab
4	516 c	485 c	716 bc	730 bc
5	440 c	598 c	571 c	612 c
6	549 c	620 c	624 c	555 c
		ns		ns

[a]Slices were treated with N_2 only or 1% NO (balance N_2) atmosphere for 20 min or treated with deionized water or 2 mM $NaNO_2$ for 20 min, prior to storage in 100% N_2 atmosphere for 1−6 weeks. Initial firmness was determined at the start of the experiment. Means for the same column with different letters are significantly different (p = 0.05). Responses to 1% NO or 2 mM $NaNO_2$ treatment are noted as not significant (ns) compared with N_2 or deionized water controls at p = 0.05 (n = 3).

storage time (Table 4). Zhu et al.[28] also observed a decrease in firmness with storage time in whole peaches fumigated with N_2 or NO (5−15 μL/L) for 3 h and then stored in air, but loss of peach firmness was inhibited by NO (5−10 μL/L).

L Value of Fresh-Cut Apple Slices Treated with NO or Nitrite.* The highest L^* value (lightest flesh color) was observed in the initial apple slices immediately after preparation and before storage in anaerobic vessels or high-barrier polymer pouches, in 100% N_2 or 0.25−1% NO (balance N_2) atmosphere (Figure 5; Table 5). Browning occurred in all slices treated with 0.25−1% NO or N_2 only and stored in anaerobic vessels (Figure 5). Slices treated with 0.25−1% NO were darker than slices treated with N_2 only (Figure 5), with lower L^* values (darker flesh color) as the NO concentration increased. Browning occurred in slices treated with N_2 or NO

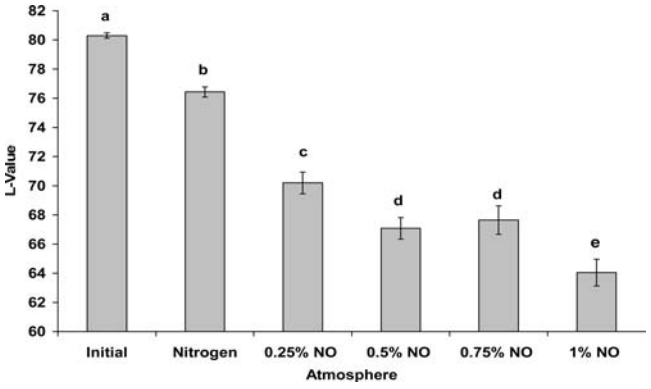

Figure 5. L^* value of 400 g of sliced 'Delicious' apples ($^1/_{16}$ wedges) stored in modified atmosphere for 2 days at 6 °C. Bars represent the standard error (±SE). Means with different letters are significantly different (p = 0.05, n = 54).

Table 5. Lightness (L^* Value) of Sliced 'Delicious' Apples Stored for 1− 6 Weeks in 100% N_2 Atmosphere[a]

time (weeks)	N_2	1% NO	deionized H_2O	2 mM $NaNO_2$
initial	78.9 a	77.3 a	79.5 a	81.9 a
1	75.4 b	70.4 b	73.8 b	71.4 b
2	74.3 b	70.5 b	73.2 b	71.1 b
3	70.9 cd	68.8 b	71.8 bc	69.8 bc
4	72.1 c	70.3 b	70.3 c	68.1 c
5	69.8 cd	68.2 b	67.7 d	65.0 d
6	69.4 d	66.3 c	67.0 d	65.2 d
		*		*

[a]Slices were treated with N_2 only or 1% NO (balance N_2) atmosphere for 20 min or treated with deionized water or 2 mM $NaNO_2$ for 20 min, prior to storage in 100% N_2 atmosphere for 1−6 weeks. Initial L^* value was determined at the start of the experiment. Means for the same column with different letters are significantly different (p = 0.05). Responses to 1% NO or 2 mM $NaNO_2$ treatment are noted as significant (*) compared with N_2 or deionized water controls at p = 0.05 (n = 54).

for 20 min and stored in N_2 (in pouches) (Table 5). Slices treated with NO were darker than slices treated with N_2 only (Table 5). Slices treated with $NaNO_2$ were lighter than slices treated with deionized water immediately after preparation; however, they were darker (Table 5) than slices treated with deionized water throughout the 6 week storage period. L^* values decreased with storage time in all slices. Pristijono et al.[15] reported that fumigation with 10−100 μL/L NO for 1−6 h inhibited browning, whereas browning was observed when apple slices were fumigated with 500 μL/L NO. It is possible that the low concentrations of NO (10−100 μL/L) used by Pristijono et al.[15] inhibit polyphenol oxidase on the cut apple surface, whereas higher NO concentrations cause browning due to the reaction of NO with phenolic compounds to form quinones as reported by Urios et al.[29] Increasing the surface area to volume ratio of NO-treated produce may also reduce browning because there will be a reduction in the number of moles of NO per unit surface area of produce.

NO and $NaNO_2$ inhibit ADH activity in apple tissue. Inhibition of ADH activity results in an increase in the accumulation of acetaldehyde and a decrease in ethanol and ethyl acetate accumulation. However, the NO concentrations required for the inhibition of ADH activity accelerated undesirable browning. NO and nitrite may be used to inhibit ADH activity of fresh-cut produce. Over the 6 week storage period, L^* value and firmness of all slices decreased, whereas ethanol accumulation increased linearly. Slices treated with NO or $NaNO_2$ accumulated less ethanol compared with N_2 or deionized water controls. The shelf life of fresh-cut produce with low concentrations of phenolic compounds in which browning is not a major factor may be extended by treatment with NO or nitrite followed by storage in nitrogen gas.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

$NaNO_2$, sodium nitrite; NO, nitric oxide; MAP, modified atmosphere packaging; ADH, alcohol dehydrogenase

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