Storage of ‘Oso Grande’ Strawberries in Controlled Atmosphere Containing Nitrous Oxide (N₂O)

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Abstract. The objective of this study was to evaluate the quality of ‘Oso Grande’ strawberries during controlled atmosphere (CA) storage with different concentrations of nitrous oxide (N₂O). The strawberries were stored at 10 °C for 10 days in hermetic mini-chambers with a continuous flow of 2.5 10⁻⁶ m³·s⁻¹ of gaseous mixtures of N₂O at 10 kPa, 30 kPa, 60 kPa, and 80 kPa combined with 21 kPa O₂ balanced with nitrogen (N₂) or in 21 kPa O₂, 0.03 kPa CO₂, and 0 kPa N₂O (control). Strawberries stored in the atmospheres that contained N₂O exhibited a reduced incidence of decay and presented better quality than the control group. After 10 days of storage, 37.2% decay was observed in the control group fruit, and 13.6% and 16.4% decay was observed in the fruit maintained at 60 kPa and 80 kPa N₂O, respectively. There was a significant reduction ($P < 0.05$) of 36% in the respiratory rate of the fruit maintained in the CA containing N₂O, and the levels of acetaldehyde (0.004 to 0.008 g·kg⁻¹) and ethanol (0.006 to 0.016 g·kg⁻¹) were unaffected by the treatments. The N₂O concentrations of 60 kPa and 80 kPa proved to be more adequate for storing the ‘Oso Grande’ strawberries because they reduce the incidence of decay, lowered the respiratory rate, and maintained the quality of the fruit.

The strawberry (*Fragaria ananassa* Duch.) is appreciated by consumers in various regions of the world as a result of its attractive characteristics such as red color and its characteristic aroma and flavor. However, this fruit has a reduced postharvest life and rapidly deteriorates after being harvested (Browne et al., 1984; Malgarim et al., 2006; Rosen and Kader, 1989). This deterioration is related to a high respiratory rate (Mitcham et al., 2004; Wills and Kim, 1995) although strawberry is a non-climacteric fruit. Furthermore, strawberry is highly susceptible to mechanical damage (Flores-Cantillano, 1998), the development of postharvest decay (Qadir and

Hashinaga, 2001), and a high rate of water loss (Nunes et al., 1995).

One of the techniques used to extend the postharvest shelf life of strawberries is the storage in a CA in combination with refrigeration (Kader, 2003a; Mitchell, 1992; Nunes et al., 2002; Seymour et al., 1993). Because strawberries are highly susceptible to the development of postharvest decay, in particular *Rhizopus* sp. and *Botrytis cinerea*, they have been stored in CAs with high concentrations of carbon dioxide (CO₂) (10 kPa to 30 kPa) to reduce the development of pathogens and to maintain the quality of the fruit (Ertan et al., 1990; Flores Cantillano, 1998; Mitchell, 1992; Van der Steen et al., 2002). However, the use of atmospheres with high concentrations of CO₂, particularly above the tolerance limits can cause cellular disruption and lead to increased levels of acetaldehyde, ethanol, ethyl acetate, and ethyl lactate, which confer undesirable aromas to the fruit (Kader, 2003b).

In an attempt to reduce the risks caused by CAs with high levels of CO₂, other gases

have been tested, and the following gases have shown particular promise: nitric oxide (Navarre et al., 2000), nitrous oxide (N₂O) (Palomer et al., 2005; Qadir and Hashinaga, 2001), and superatmospheric oxygen (Zheng et al., 2008). N₂O is a gas that is present in the atmosphere and is the product of denitrifying bacteria that are present in the soil. N₂O inhibits the action of ethylene and its synthesis in higher plants (Leshem and Wills, 1998). Another interesting characteristic of N₂O is its stable linear chemical structure, which confers similar physical properties to those of CO₂ such as relative stability and high solubility in water (Benkeblia and Varoquaux, 2003; Leshem and Wills, 1998). The biophysical similarity between N₂O and CO₂ suggests that N₂O has a beneficial effect on the conservation of fruit and vegetables (Leshem and Wills, 1998) with the added advantage that N₂O is not toxic to human beings (Benkeblia and Varoquaux, 2003).

Studies conducted with N₂O and climacteric products such as apples, persimmons (Qadir and Hashinaga, 2001), and bananas (Palomer et al., 2005) and non-climacteric fruit such as strawberries and tangerines (Qadir and Hashinaga, 2001) have shown promising results in the maintenance of quality and the reduction of postharvest decay. However, further studies on the use of N₂O with strawberries are needed because the physiological aspects of this treatment have not yet been evaluated. Thus, the objective of the present study was to evaluate the quality of ‘Oso Grande’ strawberries stored in CAs with different concentrations of N₂O.

Materials and Methods

Plant material. Strawberries of the Oso Grande cultivar were collected from a commercial fruit farm in the municipality of Valinhos in the state of São Paulo, Brazil. The strawberries were collected during the first hours of the day with 50% to 70% of the surface of the fruit presenting a bright red color according to the guidelines proposed by Flores Cantillano (2005). After being collected, the fruit were selected and stored in a cold chamber at 10 ± 1 °C and 95% ± 2% relative humidity for 12 h. The fruit were then stored in hermetic mini-chambers with different CAs. The mini-chambers were 8.6 L translucent boxes (Sanremo® 960) and had the capacity to hold 1.2 kg of strawberries (110 fruit in a single layer ≈ 1.1 kg) at 10 ± 1 °C for 10 d. Four chambers were used (replicate) per gas mixture per day of analyses.

Treatment application: controlled atmosphere and application of nitrous oxide. Storage under CA was conducted using the following five gaseous mixtures: 10 kPa, 30 kPa, 60 kPa, and 80 kPa of N₂O with 21 kPa oxygen (O₂) balanced with nitrogen (N₂). The control group was stored under normal atmosphere (21 kPa O₂ + 0.03 kPa CO₂, and 0 kPa N₂O).

“Flowboard” equipment was used to obtain the CA and was assembled according to Calbo (1989) with modification to the pressure regulator. The column water barostat was replaced

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by a differential valve (used in LPG domestic cylinders), which enabled the pressure to be regulated without wasting gas (Cerqueira et al., 2009). O₂, N₂O, and N₂ were supplied by individual cylinders with 99.99% purity. The gases, which were connected to individual feed lines, were humidified by passing them through a 9-L glass container containing distilled water. The desired flow rates were obtained using flexible copper capillaries, and the diameters were adjusted by compressing the capillaries in a mechanical vise. The gas flow of each capillary was calibrated using a standard 0.050-L bubble flow meter.

The gaseous mixtures were applied to the mini-chambers used for storage at a continuous flow of $2.5 \times 10^{-6} \text{ m}^3 \cdot \text{s}^{-1}$, and the atmospheric gas composition was measured daily using a gas analyzer (Checkmate 9001 model; Dansensor, Denmark).

Postharvest decay. The decay incidence was determined using 440 fruit (110 fruit per replicate) per treatment group (gas mixtures). The fruit were considered affected if they had characteristic lesions of at least 25 mm² in area. The results were expressed in percentages (%).

Texture. Pulp firmness was determined using a manual digital penetrometer (Samar 53200; Tr-Turoni, Italy) fixed in a stand drill with a 6-mm diameter tip. The penetration depth was of ≈ 9 mm in the pulp, and the results are expressed in Newtons (N). The readings were taken from the sides of the fruit with one reading per fruit using four replicates of 10 fruit randomly taken from each treatment group (gas mixtures).

Coloration. External coloration was determined using a colorimeter (Minolta CR-300), and the results are expressed in luminosity (*L**), hue angle (*h*), and chromaticity according to McGuire (1992). These analyses were conducted with four replicates of 10 fruit (the same used in to texture determination) per treatment group with two readings per fruit on opposite sides.

Physicochemical analyses. The titratable acidity (TA) content was determined according to the guidelines described by the Association of Official Analytical Chemistry (AOAC), and the levels are expressed as the equivalent in grams of citric acid per kilogram of sample (AOAC, 1997). The soluble solids content (SSC) was also determined according to the AOAC, and the results are expressed in °Brix (AOAC, 1997). The ascorbic acid content was determined according to the technique described by Strohecker and Henning (1967) and is expressed as the equivalent in grams of ascorbic acid per kilogram of sample. These analyses were conducted with four replicates of 30 strawberries randomly taken from each treatment group (gas mixtures).

Respiratory activity. Four replicates were used to determine the respiratory activity. Each replicate was composed of a mini-chamber containing ≈ 1.2 kg of strawberries. The amount of CO₂ produced was determined every 2 d by measuring the difference between the concentration of CO₂ in the exit and the entrance of the gas into the chambers.

Samples of 1.0 mL of gas were collected in the gas input and output of the mini-chambers. The samples were then analyzed using a gas chromatograph (GC Trade 2000 model; Thermofinnigan) with a separating column of “Porapak N” of the 400 mm and a flame ionization detector (FID). The carrier gas used was N₂ at a flow rate of $39.1 \text{ mL} \cdot \text{min}^{-1}$. The temperatures maintained in the machine were 120 °C in the column, 120 °C in the injector, 250 °C in the detector, and 350 °C in the catalyst for methane conversion. The production of CO₂ was calculated using the equation described by Kays (1991) and is expressed in $10^{-6} \text{ g} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$.

Levels of acetaldehyde and ethanol. To quantify the acetaldehyde and ethanol levels, 1 g of crushed pulp was taken immediately after homogenization during physicochemical analyses (four replicates per treatment group – gas mixtures) and the samples were maintained in 40-mL hermetic flasks at –18 °C. The standard curve for acetaldehyde was prepared with 0.085 g of acetaldehyde diluted in 400 mL of deionized water. Aliquots of 1.0, 10.0, and 20.0 mL of the sample were extracted in volumetric flasks and brought up to a volume of 100 mL with deionized water. The samples were then transferred to hermetic flasks. The ethanol curve was prepared by weighing and placing 0.01, 0.14, and 0.81 g of ethanol directly into the volumetric flasks and bringing the volume up to 200 mL with cold deionized water. Subsequently, the compounds were determined, and 1.0 mL of each solution was transferred to 40-mL flasks, which were hermetically closed and maintained in a warm bath at 50 °C for 30 min. One milliliter of air was collected from the free space in the flask and then injected into a gas chromatograph (GC Trace 2000 model; Thermofinnigan) with an FID using the “Porapak N” column. The chromatogram peak area was then recorded. All of the samples were processed in the same manner. The results are expressed in grams of acetaldehyde or ethanol per kilogram of plant material.

Statistical analysis. The statistical design was completely randomized with a five $\times$ six factorial arrangement with four replicates (four mini-chambers). The factors were constituted by the concentration of N₂O in the storage atmospheres (0 kPa, 10 kPa, 30 kPa, 60 kPa, and 80 kPa N₂O) and the control, and the storage period (0, 2, 4, 6, 8, and 10 d). The data were analyzed with an analysis of

variance and a test of the means (Tukey’s test at a 5% level of probability). The levels of acetaldehyde and ethanol and respiratory activity were evaluated by SE and were considered significant when the differences between the means of two treatments were greater than the sum of the two SEs (Shamaila et al., 1992). The results of the percentage of postharvest decay were analyzed by transformation using $\arcsin \sqrt{\frac{x}{100}}$ (Nishijima et al., 1992).

Results

Postharvest decay. The enrichment of the storage atmosphere with N₂O delayed the occurrence of postharvest decay. In addition, as the concentration of N₂O increased, a decrease in postharvest decay was observed (Table 1). The lowest concentration of N₂O (10 kPa) did not affect the postharvest decay incidence, indicating that this level did not differ from the control treatment, which showed similar percentages of decayed fruit at the end of the storage period. Conversely, decay only appeared on the eighth day of storage on the fruit maintained in atmospheres with 10 kPa, 30 kPa, 60 kPa, and 80 kPa N₂O (Table 1). At the end of the storage period, the 60-kPa and 80-kPa concentrations of N₂O produced the lowest percentages of fruit with decay ($P < 0.05$) compared with the other treatments (Table 1).

Respiratory activity. The presence of postharvest decay, particularly in the control group fruit and on the fruit maintained in the atmosphere containing 10 kPa N₂O, influenced the respiratory activity of the fruit (Fig. 1). The respiratory rate of the control group fruit increased during storage from $11.15 \times 10^{-6} \text{ g} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ (Day 0) to $17.19 \times 10^{-6} \text{ g} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ (Day 9). Conversely, the amount of CO₂ produced by the fruit maintained in N₂O-enriched atmosphere decreased by 36.30%. In addition, Figure 1 shows that the highest concentrations of N₂O (60 kPa and 80 kPa) produced lower respiratory rates.

Levels of acetaldehyde and ethanol. The enrichment of the storage atmosphere with N₂O did not affect ethanol production ($P > 0.05$), and levels of 0.006 to $0.016 \text{ g} \cdot \text{kg}^{-1}$ were observed throughout the storage period (Fig. 2). However, at the end of the storage period, the levels of acetaldehyde produced by the strawberries from the control group and those maintained in atmospheres with 10

Table 1. Decay incidence (%) on ‘Oso Grande’ strawberries stored in a controlled atmosphere containing different concentrations of N₂O at 10 °C and 95% relative humidity.

Storage (d)	N ₂ O concentrations									
	0 kPa		10 kPa		30 kPa		60 kPa		80 kPa	
0	0.0 ^a	1.57 Aa ^y	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa
2	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa
4	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa
6	15.5	1.17 Bb	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa	0.0	1.57 Aa
8	28.2	1.02 Cc	19.1	1.12 Bb	8.2	1.29 Ba	5.5	1.33 Ba	5.5	1.33 Ba
10	37.2	0.91 Dc	33.6	0.95 Cc	26.4	1.03 Cb	13.6	1.19 Ca	16.4	1.15 Ca

^aAverage of 110 fruit expressed in percentages.

^yTransformed means ($\arcsin \sqrt{\frac{x}{100}}$) followed by at least one common uppercase letter in the column and a lowercase letter in the line do not differ according to Tukey’s test ($P < 0.05$).

Fig. 1. Respiratory activity in 'Oso Grande' strawberries stored in controlled atmospheres (CAs) with different concentrations of N₂O with 20 kPa O₂ at 10 °C and 95% relative humidity. The vertical bars represent the SEM (N = 4).

Fig. 2. Ethanol production in 'Oso Grande' strawberries stored in controlled atmospheres (CAs) with different concentrations of N₂O with 20 kPa O₂ at 10 °C and 95% relative humidity. The vertical bars represent the SEM (N = 4).

kPa and 30 kPa N₂O were greater (0.0059 g·kg⁻¹) than the levels of acetaldehyde produced by the strawberries maintained with 60 kPa and 80 kPa N₂O (0.0034 g·kg⁻¹) (Fig. 3).

Quality evaluation (texture, color, and physicochemical analysis). Regarding the other quality parameters, no difference was observed among the treatments (Table 2). Firmness was not influenced by the levels of N₂O, and a decrease in firmness was only observed throughout the storage period.

The fruit color was unaffected by the levels of N₂O (Table 2). Lightness (*L*^{*}) decreased during the storage period and did not differ among treatments up to the tenth day of storage. A similar behavior was observed in

the hue angle (*h*^{*}), which decreased from 33.2° to 26.1° over the storage period. However, no significant differences among the treatment groups were observed (Table 2). The chromaticity increased during the first 4 to 6 d of storage and then decreased, but no treatment effect was observed.

Some differences were observed in the physicochemical parameters among the treatments (Table 2). The SSC decreased (0.52° Brix) during the storage period (Table 2). The levels of ascorbic acid also decreased during the storage period, and the fruit stored in the atmosphere with 80 kPa N₂O presented the lowest contents. The level of TA did not change throughout the storage period, and the

differences observed between the treatments with N₂O and the control were minimal and did not affect the quality of the fruit (Table 2).

Discussion

The decay control related to the presence of N₂O in the storage atmosphere observed in the present study was also observed by other authors. For example, Qadir and Hashinaga (2001) observed a reduced incidence of *Botrytis cinerea* in strawberries treated with different concentrations of N₂O (50 kPa and 80 kPa). The control of postharvest decay by N₂O is related to its inhibitory effect on the development of pathogens and/or by activating the defense mechanisms of the fruit (Benkeblia and Varoquaux, 2003) because the action of N₂O occurs through the interaction with the plant's defense mechanism (Navarre et al., 2000). This interaction is likely the result of the action of molecules that are produced or released in the place where the pathogen attacks occur and the fact that these molecules can be transported by diffusion through the vascular system (Navarre et al., 2000). This mechanism is believed to be activated in response to atmospheric N₂O. Considering the lowest percentage of decay present as the desired outcome, the storage life of the fruit exposed at 60 kPa and 80 kPa was longer (≈8 d) compared with the storage life of fruit exposed to other treatments (Table 1).

Although the postharvest decay affected the respiratory activity of the fruits from the control group and that of the fruit maintained in the atmosphere containing 10 kPa N₂O (Fig. 1), the presence of N₂O in the storage atmosphere may inhibit the respiratory activity because N₂O does inhibit ethylene action and synthesis in higher plants (Palomer et al., 2005). The effect of N₂O on the respiratory activity is partial and reversible because this gas regulates the activity of cytochrome c from the mitochondria, which are isolated from the seeds, leaves, and cellular suspensions (Sowa and Towill, 1991). Sowa et al. (1993) also reported that CAs with 80% N₂O reduced respiration in seedlings of *Phaseolus vulgaris* L.

Unlike atmospheres with high concentrations of CO₂, which can cause cellular disruption and lead to increased levels of acetaldehyde and ethanol (Kader, 2003b), the enrichment of the storage atmosphere with N₂O did not contribute to the increase of these compounds (Figs. 2 and 3). Despite the observed differences, the levels of acetaldehyde and ethanol did not compromise the quality of the strawberries. Thus, the effect of N₂O on the production of ethanol and acetaldehyde differs from that of CO₂ when applied in a CA. High concentrations of CO₂ (higher than 20 kPa) increase the production of acetaldehyde and ethanol throughout the storage period, which affects the aroma and flavor of the fruit (Ke et al., 1993). In the present study, this effect was not observed when a high concentration of N₂O (60 kPa and 80 kPa) was used. Benkeblia and Varoquaux (2003) also observed that the action of N₂O

differs from that of CO₂, as suggested by Qadir and Hashinaga (2001).

The enrichment of the storage atmosphere with N₂O did not affect the other quality parameters (Table 2). ‘Oso Grande’ strawberries are firmer than other strawberry cultivars even during storage (Nunes et al., 2006) and the absence of N₂O effect on firmness was also reported by other studies. N₂O (60 kPa) applied to cv. Dwarf Cavendish bananas stored under CA storage did not affect the firmness of the pulp (Palomer et al., 2005). Thus, the slight loss of firmness may have occurred as a natural consequence of senescence (Seymour

et al., 1993) mainly as a result of loss of cell wall material (Koh and Melton, 2004).

The fruit color was unaffected by the levels of N₂O (Table 2). Pelayo et al. (2003) studied ‘Diamante’ and ‘Selva’ strawberries stored in CAs enriched with 20 kPa CO₂ and did not observe differences in the lightness and hue angle during the cold storage (5 °C). Brackmann et al. (2001) stored ‘Oso Grande’ strawberries in CA and found significant differences in color (red coating); however, the differences were slight and visually not noticeable. Additionally, Palomer et al. (2005) did not observe significant differences in the

parameters that comprise color (*L**, *a**, and chromaticity) of bananas stored in a CA with 60 kPa N₂O compared with those from the control group (atmospheric air).

The physicochemical parameters were unaffected by the levels of N₂O (Table 2). On the other hand, strawberries stored under 100 kPa N₂O at 10 to 20 °C showed a decrease in TA (Al-Jamali and Hani, 2007), but this trend might be related to the temperature abuse rather than N₂O effect. Additionally, Palomer et al. (2005) did not observe significant differences in the levels of SSC and TA in bananas treated with 60 kPa N₂O compared with those maintained in atmospheric air. According to Lavee and Nir (1986), changes in the SSC and TA as well as sugars and organic acids are minimal during the storage of non-climacteric fruit such as strawberries. A decrease in the levels of sugar is only observed during the final stage of senescence (Garcia et al., 1996).

In conclusion, ‘Oso Grande’ strawberries stored at 10 °C in CAs containing 60 kPa or 80 kPa N₂O had a storage life of 8 d based on the incidence of postharvest decay, which was lower than that of fruit exposed to other N₂O concentrations. N₂O levels also reduce the respiratory rate and did not promote increases in ethanol and acetaldehyde production commonly observed in atmospheres enriched with high levels of CO₂. Higher N₂O concentrations (60 kPa and 80 kPa) showed better results and proved to be more adequate for storing ‘Oso Grande’ strawberries.

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Fig. 3. Acetaldehyde production (C) in ‘Oso Grande’ strawberries stored in controlled atmospheres (CAs) with different concentrations of N₂O with 20 kPa O₂ at 10 °C and 95% relative humidity. The vertical bars represent the SEM (N = 4).

Table 2. Contents of soluble solids (SSC), titratable acidity (TA), ascorbic acid (AA), and texture (Tx), lightness (*L**), hue angle (*a**), and chromaticity (chroma) in ‘Oso Grande’ strawberries in controlled atmospheres with different concentrations of N₂O with 21 kPa O₂ that were stored at 10 °C and 95% relative humidity.

Parameters	SSC (°Brix)	TA (g·kg ⁻¹)	AA (g·kg ⁻¹)	Tx (N)	<i>L</i> *	<i>a</i> *	Chroma
N ₂ O levels							
0 kPa N ₂ O	7.2 A	8.3 A	0.54 A	7.3 A	36.1 AB	27.5 A	28.9 A
10 kPa N ₂ O	7.2 A	8.0 B	0.53 AB	6.9 A	35.5 B	28.5 A	29.4 A
30 kPa N ₂ O	7.3 A	8.1 AB	0.54 A	7.0 A	36.3 A	28.2 A	28.5 A
60 kPa N ₂ O	7.3 A	8.2 AB	0.54 A	7.1 A	36.4 A	28.3 A	29.2 A
80 kPa N ₂ O	7.1 A	8.1 AB	0.51 B	6.9 A	36.4 A	28.4 A	29.0 A
MSD	0.2 NS	0.2*	0.022*	0.6 NS	0.67*	1.26 NS	1.0 NS
Storage (d)							
0	7.4 AB	8.2 A	0.58 A	8.1 A	42.7 A	33.2 A	26.0 C
2	7.4 A	8.2 A	0.56 AB	7.1 B	34.2 DE	29.3 B	30.7 A
4	7.2 AB	8.1 A	0.52 C	7.5 AB	35.3 C	26.9 C	27.2 B
6	7.2 AB	8.3 A	0.55 B	7.0 B	33.5 E	26.8 C	30.8 A
8	6.9 C	8.2 A	0.50 CD	7.5 AB	36.3 B	26.9 C	31.4 A
10	7.1 BC	8.3 A	0.48 D	6.1 B	34.8 CD	26.1 C	27.8 B
MSD	0.2*	0.3 NS	0.026*	0.6*	0.77*	1.44 NS	1.1*
Interaction							
N ₂ O × d	0.1 NS	0.02 NS	20.6*	4.8 NS	15.4*	55.7*	43.2*
CV (%)	3.6	2.56	5.3	30.6	7.4	17.8	13.8

Averages followed by at least one common letter in the column for each factor do not differ according to Tukey’s test (*P* < 0.05).

NS = nonsignificant; *significant at *P* < 0.05.

MSD = minimum significant difference.

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