Effect of nitrous oxide (N\textsubscript{2}O) on respiration rate, soluble sugars and quality attributes of onion bulbs \textit{Allium cepa} cv. Rouge Amposta during storage

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Abstract

Effects of nitrous oxide (N\textsubscript{2}O) on respiration rate (RR), soluble sugars, and organic acids during treatment, and sprouting and rotting during storage of onion bulbs \textit{(Allium cepa} cv. Rouge Amposta) were investigated. Concentrations of 50, 80 and 100 kPa of N\textsubscript{2}O were applied for 5, 10 and 15 days at 18 °C and compared to control (atmospheric air) samples and samples kept under 100 kPa N\textsubscript{2}. RR\textsubscript{s} reduced by 50% after 5 days were restored progressively and the difference between control and N\textsubscript{2} and N\textsubscript{2}O treated bulbs was approximately 17 and 25% less after 10 and 15 days, respectively. Soluble sugars were slightly higher in treated onions and averaged 6.97% under 100 kPa N\textsubscript{2}, and 7.17, 6.18 and 6.58% under 50, 80 and 100 kPa N\textsubscript{2}O. However in control bulbs, soluble sugars averaged 5.33%. During treatments of bulbs with N\textsubscript{2} and N\textsubscript{2}O, organic acid contents increased and accumulation was observed throughout the time of exposure. After 5, 10 and 15 days of treatment, five acids—citric acid, succinic acid, fumaric acid, malic acid and oxalic acid—increased in both N\textsubscript{2} and N\textsubscript{2}O treated bulbs. Large variability and randomized levels of sprouting of treated bulbs were observed, but no significant difference was noted between control and N\textsubscript{2}O or N\textsubscript{2} treated samples. N\textsubscript{2}O effectively reduced rotting of bulbs; those kept under N\textsubscript{2} showed higher rotting than control and N\textsubscript{2}O treated bulbs.

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1. Introduction

Onions are usually held for long periods between harvesting and marketing so as to fulfill the market demand. In this respect, several investigations have been carried out to develop new techniques to extend control of sprouting and rotting, which are the main problems in storage of onion bulbs, thus improving shelf-life and
quality attributes of the bulbs. Recently there has been a great interest in the potential benefits of using some rare gases for storage of vegetables (Spencer, 1995). These gases are thought to be chemically neutral, non-toxic, naturally occurring atmospheric gases that are biochemically active probably due to their enhanced solubility in water (Spencer, 1995). Nitrous oxide \((\text{N}_2\text{O})\) is similar to \(\text{CO}_2\) in relative stability and high solubility in water (Anonymous, 1991). It is classified as a miscellaneous additive and is permitted for food use. \(\text{N}_2\text{O}\) has been shown to cause a reversible partial inhibition of oxygen consumption by mitochondria and respiration (Sowa et al., 1987; Sowa and Towill, 1991). It also has extended shelf-life of lychee and longan seeds (Sowa and Roos, 1991), and Gouble et al. (1995) reported anti-ethylene effects of \(\text{N}_2\text{O}\) in ripening and senescence in tomato and avocado fruit. Thom and Marquis (1984) reported that at low pressure \(\text{N}_2\text{O}\) was a potent growth inhibitor of \textit{Escherichia coli}, \textit{Saccharomyces cerevisiae} and \textit{Tetrahymena thermophila}, as well as multiple postharvest fungi in vitro (Qadir and Hashinaga, 2001b). However, numerous biochemical and physiological aspects of the effects of \(\text{N}_2\text{O}\) remain unclear.

Application of \(\text{N}_2\text{O}\) to onion bulbs gave encouraging results for control of rotting (Benkeblia et al., 2001). Although production of, and sensitivity to ethylene (Kubo et al., 1990) are very low \((< 5 \text{ nmol kg}^{-1} \text{ h}^{-1})\) in onion, ethylene could play a secondary role in sprouting of bulbs (Benkeblia and Selselet-Attou, 1999). It is possible that any potential effect of \(\text{N}_2\text{O}\) on rotting could be due to: (1) its biophysical properties being similar to \(\text{CO}_2\), which inhibits microbial growth, (2) its competitiveness with \(\text{C}_2\text{H}_4\) production and action, and (3) its ability to interfere with methionine synthesis. However, there is no published information on the independent effect of \(\text{N}_2\text{O}\) on respiration rate (RR) and biochemical changes occurring during treatment or storage of onion bulbs. Improved storage of onion bulbs requires knowledge of physiological parameters i.e. respiration, anoxia and effects of gas composition, to extend the postharvest life of the commodity.

We investigated the effect of \(\text{N}_2\text{O}\) applied at different concentrations during different periods on sprouting and rotting of onion bulbs, as well as its influence on respiratory stress and soluble sugars.

2. Materials and methods

2.1. Onions

Onion bulbs \textit{Allium cepa} cv. Rouge Amposta (organic product, free of any preharvest chemical treatments), which had been freshly harvested (September 2, 1997 and September 5, 1998) and dried in the field for 2 weeks, were obtained from the local market (supplied by Pronatura, M.I.N, Avignon, France). They were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays each of 12 kg and placed at 18°C prior to treatments.

2.2. \(\text{N}_2\text{O}\) treatments

Onion bulbs (10±0.05 kg) were placed in 120 l vessels, the lid of which was tightly secured. These onions had been kept at 18°C for 12 h before use. The vessels were flushed with pure nitrogen \((\text{N}_2)\) (except the vessels of control bulbs and of the 100 kPa \(\text{N}_2\text{O}\) treatment) and the final gas composition within the flushed vessels averaged from 99.1 to 99.4 kPa \(\text{N}_2\), and from 0.87 to 0.6 kPa \(\text{O}_2\). Treatment chambers were then flushed with pure \(\text{N}_2\text{O}\) for different times to obtain the desired final concentrations of 50, 80 and 100 kPa \(\text{N}_2\text{O}\). Gas composition within the vessels was checked with a gas chromatograph (MTI, Fremont, USA) as described below. The residual \(\text{O}_2\) within the vessels was less than 0.4 kPa. Onions were treated with 100 kPa \(\text{N}_2\) for 15 days and with 50, 80 and 100 kPa \(\text{N}_2\text{O}\) for 5, 10 and 15 days. To avoid \(\text{CO}_2\) accumulation and fermentation of bulb tissues, the vessels were opened for 1 h after 5 and 10 days for 10 and 15 days treatment periods respectively and the same flushing operation with \(\text{N}_2\) and \(\text{N}_2\text{O}\) was repeated. RRs were determined prior to each opening of the vessels. Control bulbs were maintained in open vessels without any gas application. Final experimental treatments were: control bulbs (no gas application), 100 k\(\text{Pa \text{N}_2}\) (0 kPa \(\text{N}_2\text{O}\), 50
2.3. Storage conditions

Immediately after N₂ and 5, 10 or 15 days of N₂O treatment, onions were stored in a ventilated room set at 18±0.1 °C and 65±1% relative humidity. Every 15 days, all onions were evaluated and sprouted or rotted bulbs were counted and discarded.

2.4. N₂O measurement

N₂O within the vessels was measured according to the method of Benkeblia et al. (2001), using a MTI gas chromatograph analyser (model M200, Microsensor Technology Inc., Fremont, USA). The analyser involved two manifolds: one fitted with an MS-5A, 4 m capillary column set at 80 °C with argon as carrier gas at a pressure of 110 kPa, and the other fitted with a capillary Poraplot 4.6 m column set at 30 °C with helium as carrier gas at a flow rate of 30 ml min⁻¹. Both manifolds were coupled with a thermal conductivity detector. In such conditions argon does not interfere with O₂ peaks, and CO₂ and N₂O peaks are separated provided that the diluted N₂O partial pressure does not exceed 10 kPa. The gas standard used was 10:10:80% of CO₂:O₂:N₂, respectively, (L’Air Li- quide supplier, France).

2.5. Respiration rate measurement

RR was determined in the sealed vessels by the same method of Benkeblia et al. (2000). At specific time intervals (after 1, 2, 3, 4 and 5 days), gas samples (50 μl) were taken from the vessels through a silicone septum and analysed by a gas chromatograph (MTI). The silicone septa on the vessels were changed after each experiment to prevent any air leakage. RR was calculated by linear regression from O₂ or CO₂ depletion curves and expressed as mmol kg⁻¹ h⁻¹. The mean value of the RR was determined from triplicate measurements.

2.6. Soluble sugars analysis

Glucose, fructose and sucrose contents were determined by HPLC (Doyon et al. 1991). Five bulbs were removed from the vessels for analysis and 100 g of each bulb were freeze-dried. Samples (5 g) of freeze-dried tissues were homogenised in 50 ml of water using a Sorvall blender (Omni-mixer 17220, Newton, USA). The homogenate was heated for 30 min in a boiling water bath (Haake Inst., Berlin, Germany). After cooling, the homogenate was centrifuged for 15 min at 25 000 × g (Heraeus Sepatech GmbH, Osterode/Harz, Germany) and the supernatant was filtered on a 0.25 μm filter (Millipore SA, Molsheim, France). Sugars were separated by HPLC using a Varian 5000 model (Vista, 5000 series, Les Ulis, France) fitted with a Polyspher CH-CA column (300 × 7.8 mm. Merck, Darmstadt, Germany) set at 80 °C and a differential refractometer detector (Knauer GmbH, Hegaver, Berlin, Germany). The mobile phase was DDI water at a flow rate of 0.5 ml min⁻¹. Sugars were identified and quantified by comparison with authentic samples (Sigma Co., St. Louis, USA) and each determination was run in triplicate.

2.7. Organic acids analysis

Oxalic acid, citric acid, succinic acid, malic acid and fumaric acid were determined by HPLC. Samples (5 g) of freeze-dried tissues were homogenised in 50 ml of water using a Sorvall blender (Omni-mixer 17220), and the supernatant was filtered on a 0.25 μm filter (Millipore SA). Organic acids were separated using the Varian 5000 HPLC fitted with a Polyspher AOKC column (300 × 7.8 mm. Merck, Darmstadt, Germany) set at room temperature. The mobile phase was DDI water at a flow rate of 0.5 ml min⁻¹, and organic acids were detected at a wavelength of 210 nm. Acids were identified and quantified by comparison with authentic samples (Sigma Co., St. Louis, USA) and each determination was run in triplicate.
2.8. Statistical analysis

The experiment was repeated in two successive harvesting seasons (1997 and 1998) and averaged. Data were analysed statistically by determination of least significant difference (LSD at $P < 0.01$) using XLSTAT PRO® statistical software (XLStat, Paris, France).

3. Results

After 5 days of N$_2$O application, the reduction in RR of N$_2$O treated bulbs averaged 50%, while under 100% N$_2$ (0% N$_2$O) a 58% reduction of RR occurred (Fig. 1). After 10 and 15 days under N$_2$ and N$_2$O treatments, the RR of treated bulbs increased and the difference among control bulbs, N$_2$ and N$_2$O treated bulbs ranged from 17 to 25% of RR control with an average difference of 20% below the control.

Total soluble sugars of onion tissues varied slightly under different treatments (Fig. 2). In control bulbs TSS averaged 5.33% fresh weight, but in N$_2$O treated bulbs TSS averaged 7.17, 6.18 and 6.58% in 50, 80 and 100% N$_2$O treated bulbs, respectively, and TSS of onions kept under N$_2$ averaged 6.97%. Comparatively, bulbs maintained under these anoxic conditions showed a slight and significant accumulation of soluble sugars. Similar results were found in 50 kPa N$_2$O treated bulbs. Under N$_2$, citric acid concentration remained almost the same during the treatment, ranging from 32 to 36 mg 100 g$^{-1}$ fresh weight, whereas under N$_2$O, citric acid levels exceeded 40 mg in almost all cases, increasing over time (Fig. 3). Citric acid concentrations seemed to increase as a function of N$_2$O concentration. The concentration of citric acid in control bulbs averaged 18.9 mg whereas in treated bulbs it averaged 38.7, 46.1 and 47.1 mg 100 g$^{-1}$ fresh weight after 5, 10 and 15 days, respectively.

The increase in succinic acid was very slight during the first five days of treatment (Fig. 3). On the other hand after 10 and 15 days under N$_2$O, succinic acid increased sharply by two or three times the control. After 5 days, control bulbs averaged 0.21 mg 100 g$^{-1}$ fresh weight of succinic acid and the concentration in treated bulbs averaged 0.22, 0.56 and 0.58 mg after 5, 10 and 15 days, respectively.

Fumaric acid showed a similar pattern to the two previous acids, although the difference among treatments was less apparent (Fig. 3). Concentrations in control bulbs averaged 0.68 mg 100 g$^{-1}$ fresh weight whereas in treated bulbs concentrations averaged 1.08, 1.19 and 1.16 mg 100 g$^{-1}$ fresh weight after 5, 10 and 15 days, respectively.

Malic acid concentrations were similar, with a slight increase occurring after 10 days of treatment.
with 80 and 100% N₂O (Fig. 3). Control bulbs had average malic acid concentrations of 102 mg 100 g⁻¹ fresh weight when N₂ and N₂O treated bulbs averaged 99, 122 and 119 mg after 5, 10 and 15 days, respectively. Oxalic acid, which is a major acid in onion tissues, increased slightly in treated bulbs (Fig. 3). The control bulbs averaged 37 mg 100 g⁻¹ fresh weight when treated bulbs averaged 53, 52 and 56 mg after 5, 10 and 15 days, respectively.

There was no effect of N₂O on sprouting in any treatment (data not shown). There was a large
variation in sprouting of control and treated bulbs, ranging from 41 to 55% after 120 days of storage. \( \text{N}_2 \text{O} \) reduced incidence of fungal rots on onion bulbs (Fig. 4a–c). Bulbs kept under \( \text{N}_2 \text{O} \) for 5 days had less rots than control bulbs, but those maintained under \( \text{N}_2 \) developed more than 25% rots, possibly due to the selective effect of \( \text{N}_2 \) on micro-organisms that develop rapidly during or after such treatment (Fig. 4a). After 10 and 15 days of treatments (Fig. 4b and c), similar patterns of rot development occurred, but bulbs kept under \( \text{N}_2 \) for 10 days had less rots than those treated for 5 and 15 days.

4. Discussion

The potential of \( \text{N}_2 \text{O} \) as a postharvest compound has been suggested in several investigations, but no information is available on the effect of \( \text{N}_2 \text{O} \) on respiration of whole fruit and vegetables. The restoration of tissue respiration could be due to acclimatization of bulbs to the anoxic atmospheres and the progressive diversion of catabolism to the anaerobic pathway. However, anaerobic catabolism remained low compared with previous results where \( \text{CO}_2 \) production under partial or total anoxia did not exceed 0.5 mmol kg\(^{-1}\) h\(^{-1}\) after 24 h (Benkeblia et al., 2000).

According to Sowa and Towill (1991), \( \text{N}_2 \text{O} \) caused a reversible, dose-dependent, partial inhibition of oxygen utilisation by mitochondria isolated from cell suspension cultures of the grass \( \text{Distichlis spicata} \). Sowa et al. (1987) reported that \( \text{N}_2 \text{O} \) affected \( \text{O}_2 \) utilisation by both bean seed (BS) and bovine heart submitochondrial particles when either succinate or reduced cytochrome \( c \) were used as substrates. BSH particles exhibited reversible, dose-dependent partial inhibition of respiratory activity when exposed to \( \text{N}_2 \text{O} \). On the other hand, respiration of BS particles was stimulated by low levels of \( \text{N}_2 \text{O} \), while higher concentrations were inhibitory.

Although carbohydrates constitute 60–80% of onion bulb dry matter, their changes under atmospheric stresses is not documented. Respiratory stress caused by ionising treatment did increase RR and soluble sugars catabolism (Benkeblia et al., 2002). Organic acids, including tricarboxylic acids of the Krebs cycle, are widely distributed in plants, although their localisation within tissues is
poorly understood due to a lack of data on their metabolism and fluxes between the different tissue parts. Unfortunately, no data are available on accumulation of different organic acids in onion tissues. Accumulation of malic acid could be due to its metabolic formation during the glycolytic pathway where it is the result of the transformation of succinic acid which was highly accumulated, compared to normal levels. It seems that stress effect on cellular catabolism induced by N\textsubscript{2}O is similar to that induced by anoxia. Onion catabolism seems to be reduced by such treatments rather than being diverted to the fermentative pathway because there was no high production of CO\textsubscript{2} following N\textsubscript{2}O treatments.

There is little information on the effect of N\textsubscript{2}O on germination or sprouting. Benkeblia et al. (2001) showed that N\textsubscript{2}O shocks at different concentrations, 25, 50, 80 and 100\% for 2 days and 100\% for 4 days, had no effect on sprouting of bulbs stored at 18 °C under air, similar to the present results. Although different from sprouting, germination of lychee and longan seeds stored in 100\% N\textsubscript{2}O was reduced at the same rate as those stored in air (Sowa and Roos, 1991).

Rotting of onion bulbs during storage is caused by numerous bacteria and fungal species (Snowdon, 1991). N\textsubscript{2}O is known to have some antibacterial or antifungal effects. Enfors and Molin (1977) found that N\textsubscript{2}O was a potent growth inhibitor of chemically induced germination of Bacillus spores. At low pressure N\textsubscript{2}O inhibited growth of Escherichia coli, Saccharomyces cerevisiae and Tetrahymena thermophila (Thom and Marquis, 1984). Inhibition of postharvest decay of numerous climacteric and non-climacteric fruit by N\textsubscript{2}O has been studied by Qadir and Hashinaga (2001a). Regardless of the physiological nature of the fruit or group of fungi, N\textsubscript{2}O delayed appearance of the disease and reduced lesion growth rate, the response being dose and time dependent. Qadir and Hashinaga (2001b) evaluated N\textsubscript{2}O as a potential fungistatic or fungicidal agent in vitro on twelve postharvest fungi exposed to 10 to 80 kPa N\textsubscript{2}O and 20 kPa O\textsubscript{2}. Results showed that fungi could be divided into N\textsubscript{2}O high, medium and low-sensitive groups. The high-sensitive group included Botrytis cinerea responsible for the brown stain of onion, a disease rarely observed. The low-sensitive group included Fusarium oxysporum, a major fungal pathogen of Alliums causing Fusarium basal rot. The present results, similar to those of Benkeblia et al. (2001), show that N\textsubscript{2}O applied at different concentrations reduced incidence of rots, especially in bulbs pretreated with 100\% N\textsubscript{2}O for 4 days. This delay in the appearance of rots could be attributed to the direct inhibitory effect of N\textsubscript{2}O on fungal growth of the pathogen, and/or, a possible increased resistance of host tissue to decay.

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References


