THE EFFECT OF NITROUS OXIDE TREATMENT ON THE PHYSIOLOGY AND QUALITY OF BANANAS

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ABSTRACT

One factor which affects the profitability of marketing bananas is that they have to be transported over long distances. Because of this, they have to be specially treated to delay ripening so that they survive long shipment and reach the consumer in the most palatable and appealing state possible. One such treatment involves the use of nitrous oxide at 20ºC. N_2O is a non-contaminating gas which delays ripening by inhibiting ethylene synthesis. The duration and intensity of inhibition depend on the dose and the length of the treatment. This inhibitory effect can be reversed before the bananas are put on the consumer market. The quality of bananas which had been previously treated with N_2O and then allowed to ripen is very close to the quality of bananas that have ripened naturally.

Key words: Bananas, ripening, N_2O, nitrous oxide, ethylene inhibition

INTRODUCTION

The banana (*Musa acuminata* L.) is a climacteric fruit. After harvest, bananas go through a pre-climacteric phase, followed by phase of increased ethylene production as the ripening process proceeds. Because bananas usually have to be transported over long distances, they have to be specially treated to delay ripening so that they survive long shipment and reach the consumer in the most palatable and appealing state possible. Among the methods which have been used are cold storage, CA storage, or inhibition of ethylene synthesis or activity. Recent research on ethylene inhibitors has been very promising. One agent which has been studied is nitrous oxide, a non-contaminating gas. N_2O is produced by aerobic denitrifying soil bacteria and inhibits ethylene synthesis and activity in vascular plants (Gouble et al., 1995). N_2O is non-toxic and is used as an surgical anaesthetic and as a safe

food additive (Benkeblia and Varoquaux, 2003). N_2O has already been used with some success to delay ripening in some fruits and vegetables, including tomatoes, avocados, and onions (Gouble et al., 1995; Benkeblia and Varoquaux, 2003).

The aim of this study was to determine how N_2O affects the physiology and storability of bananas at different doses and treatment times. The parameters examined included ethylene synthesis, respiration, and fruit quality.

MATERIAL AND METHODS

The bananas used in this study were of the cultivar 'Cavendish' and had been harvested in Tenerife on the Canary Islands. Individual bananas of uniform size and free from visual defects were selected and treated for three minutes with a fungicidal solution (1 g Benlate, 3 g Dithane, and 250 µl Tween 80 per litre). After air drying, the bananas were divided into treatment lots of at least twelve bananas each and placed into sealed glass jars. The bananas were kept at 20°C in a flow-through system through which water-saturated air flowed at a rate of 1-2 litres per hour per fruit. N_2O was introduced at four concentrations: 20, 40, 60, and 80% in air. Treatment was carried out for four different durations: 3, 5, and 10 days, as well as continuously. The concentration of N_2O was measured by gas chromatography.

Ethylene synthesis and respiration were measured daily to monitor the ripening process. Ethylene synthesis was measured by withdrawing 1 ml of the headspace gas from each jar and injecting it into a gas chromatograph (GC6000 Vega Series 2, Carlo Erba, Italy).The temperature of the column was lowered to 80°C to separate ethylene from N_2O. Results were recorded as litres of ethylene produced per hour per kilogram of fresh fruit. CO_2 was measured with infrared gas analyzer (Cristal 300, COSMA, France).

Fruits were classified into five different ripening stages based on ethylene synthesis and respiration as presented in the following table:

Ripening Stage		
E1	pre-climacteric	fully grown, green
E2	early climacteric	increase in ethylene synthesis and respiration
E3	peak climacteric	maximum ethylene synthesis and respiration
E4	late climacteric	fully, ripened. one day after climacteric peak
E5	post-climacteric	over-ripened. four days after climacteric peak

At each ripening stage, two bananas of each treatment lot were selected to test fruit quality parameters and chemical properties. Pulp and peel flesh tissue were collected, frozen in liquid nitrogen, and stored at –80ºC until they could be tested for ACC. Weight loss was monitored by direct weighing and compared to the E1 stage. Color was evaluated with a CR-300 colorimeter (Minolta, Japan). Peel and pulp texture were evaluated with a manual fruit firmness tester (EFFEGI: FT 327), fitted with an 8 mm diameter tip. Starch content was measured by iodine staining.

To measure soluble solids, pH and acidity, 15 g of pulp were blended for two minutes in 15 ml of deionised water and centrifuged for fifteen minutes at 5000 x g. Measurements were carried out on the supernatant.

Free ACC was extracted and quantified by conversion of ACC to ethylene (Mansour et al., 1986; Lizada and Yang, 1979). M-ACC was measured in the same way, but from the hydrolysed extract (Hoffman and Yang, 1980). ACC oxidase activity (ACO) was determined *in vivo* (Mansour et al., 1986).

Data were statistically elaborated by ANOVA, and the results were evaluated by a least significance test at $P \leq 0.05$.

RESULTS AND DISCUSSION

In air at 20ºC, bananas normally begin to ripen after a lag phase lasting from five to twenty days, depending on the age of the fruit and shipment time. The bananas used as controls in this experiment varied widely in terms of the duration of the lag phase. This lag phase is considerably longer in bananas treated with N_2O, and increases with higher doses and higher exposure times.

Treatment for three days with 20% N_2O had no significant effect on ethylene synthesis and respiration. Treatment with 40 or 60% N_2O reduced ethylene synthesis and respiration, resulting in significant prolongation of the lag phase (Fig. 1 and 2).

Treatment for ten days resulted in significant prolongation with all concentrations of N_2O tested. The higher the concentration, the longer the lag period. Beyond a certain point, increasing the N_2O concentration no longer prolonged the lag phase. For example, the lag phase after treatment with 80% N_2O was not significantly longer than after treatment with 60% N_2O, although other physiological effects were seen, such as peel browning.

The length of the lag phase also increased with increasing duration of the treatment. This can be seen in Figure 3, in which the effects of treatment with 40% N_2O for three, five and ten days as well as continuous treatment are presented.

While N_2O treatment prolonged the lag period, it did not have a significant effect on the level of ethylene synthesis or respiration during the subsequent climactic phase (Fig. 3). The effect of N_2O on ripening is completely reversible, and once reversed, the ripening process follows its normal course. Treatment with N_2O did not significantly affect the fruit quality and chemistry parameters of the treated bananas.

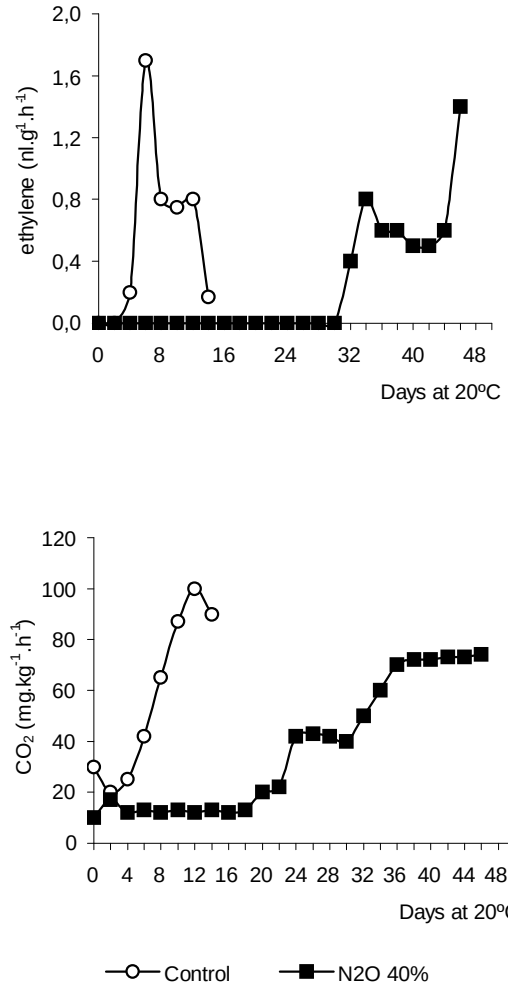

Figure 1. Ethylene and CO_2 production in control and fruit treated with a continuous treatment with 40% N_2O and kept at 20ºC. The end of each curve corresponds to ripened fruit (day 14 for the control and day 46 for N_2O treatment)

During the ripening process, ACC oxidase activity peaked during the early climacteric phase (Fig. 4). The occurrence of this peak was delayed with all of the treatment doses and durations tested. The levels of both free and conjugated ACC was the same in treated bananas as it was in the controls. This suggests that N_2O does not inhibit ACC synthase activity.

N_2O is a potent inhibitor of ethylene synthesis and respiration which has been used to delay ripening in climacteric fruits including tomatoes and avocados. We found that treatment with N_2O at 20ºC also delays the onset of climacteric ripening of bananas without affecting the fruit quality parameters

of the ripened fruit. The length of the lag period increases with increasing doses of N_2O and with increasing treatment times. It also depends on the type and developmental stage of the fruit. N_2O treatment is a promising option for ensuring that bananas survive long shipment and reach the consumer in the most palatable and appealing state possible while reducing losses and increasing profits for the distributor.

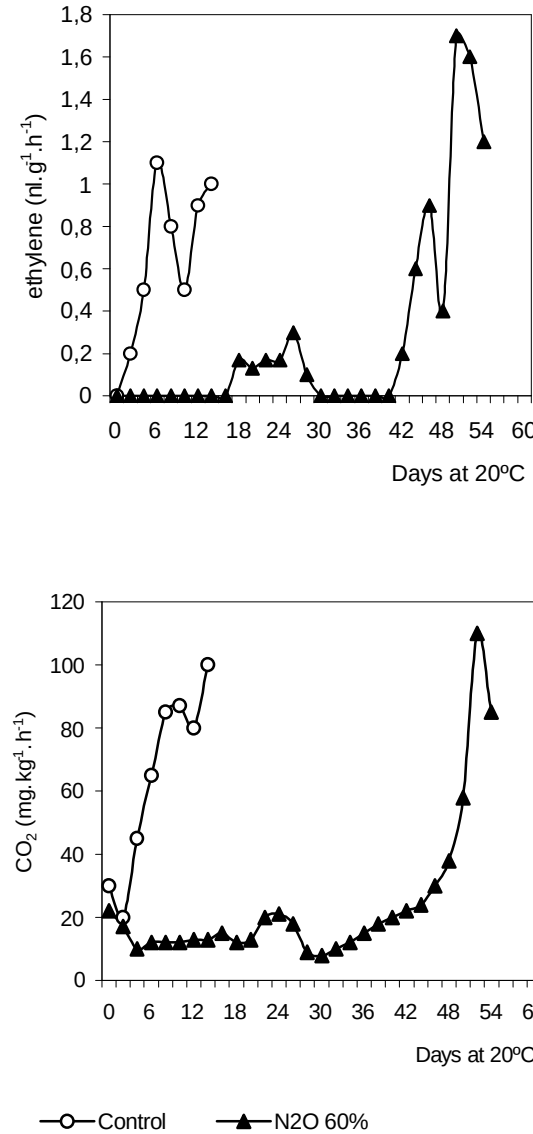

Figure 2. Ethylene and CO_2 production in control and fruits treated with a continuous treatment with 60% N_2O and kept at 20ºC. The end of each curve corresponds to ripened fruit (day 14 for the control and day 54 for N_2O treatment)

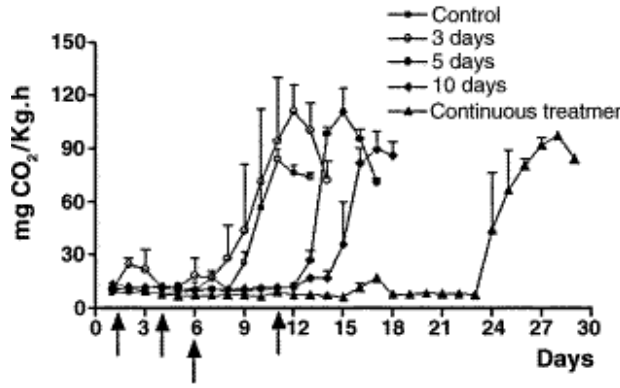

Figure 3. Respiration rates of banana fruit stored at 20ºC following application of 40% N_2O for 3, 5, 10 days and in continuous treatments. The arrows represent the times that N_2O flushing was started and halted for each treatment. Results are means for three independent experiments ± S.D

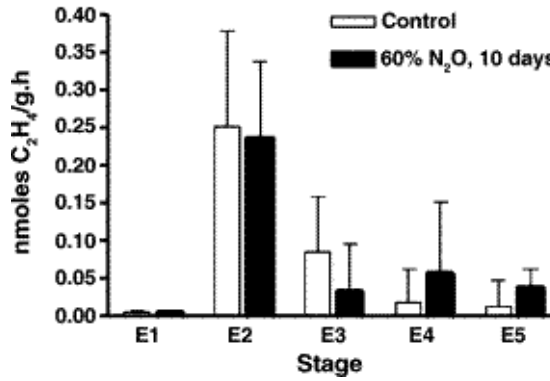

Figure 4. Effect of flushing 60% N_2O for 10 days on ACO activity in pulp during ripening, in banana fruit stored at 20ºC. Results are means for two independent experiments ± S.D

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WPŁYW TRAKTOWANIA PODTLENKIEM AZOTU NA FIZJOLOGIĘ I JAKOŚĆ BANANÓW

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STRESZCZENIE

Problemy w handlu bananami spowodowane są tym, że owoce te zwykle wymagają transportu na dalekie odległości. Aby po długim transporcie owoce dotarły do konsumentów w jak najlepszym stanie, wymagają one specjalnego traktowania opóźniającego dojrzewanie. Banany traktowano podtlenkiem azotu (N_2O) w temperaturze 20ºC. N_2O jest gazem nietoksycznym i niezanieczyszczającym środowiska, który opóźnia dojrzewanie poprzez hamowanie syntezy etylenu. Efekt jest zależny od dawki i czasu traktowania. Wpływ na hamowanie produkcji etylenu i dojrzewanie jest odwracalny. Jakość bananów traktowanych przed dojrzewaniem N_2O jest porównywalna z jakością owoców nietraktowanych, dojrzewających w sposób naturalny. Traktowanie N_2O umożliwia kontrolowanie dojrzewania owoców po zbiorze, podczas transportu i przechowywania.

Słowa kluczowe: banany, dojrzewanie, N_2O, podtlenek azotu, hamowanie produkcji etylenu