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DEFENSE RESPONSES IN CHERIMOYA FRUIT PRETREATED WITH HIGH CO_2 LEVELS

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Summary

In a previous work we showed that pretreatment with 20% CO_2 plus 20% O_2 for 3 days effectively retains the quality of cherimoya (*Annona cherimola* Mill.) fruit [6]. In the work described here we analyzed the potential of such pretreatment to induce typical defense systems in cherimoyas stored at low temperature. At the end of treatment γ-aminobutyric acid (GABA) content was observed to be higher in treated as compared to untreated fruit. Chitinase activity was higher in treated than in untreated fruit. CO_2 pretreatment was accompained by a delay in the ethylene production.

Introduction

Low temperature storage is considered to be the most effective method for maintaining the quality of most fruit and vegetables. Unfortunately, for chilling injury-sensitive commodities such as tropical and subtropical fruit, low temperature storage is often responsable for substantial postharvest losses. Several technologies, as modified atmospheres (MA) or controlled atmospheres (CA) - with high CO_2 and low O_2 concentrations - have been shown to control some physiological disorders, thereby extending the postharvest life of fruit and vegetables [8,14]. In cherimoya (*Annona cherimola* Mill. cv. 'Fino de Jete'), controlled atmosphere storage and pretreatments with high levels of CO_2 are beneficial for maintaing fruit quality [10]. In addition to being a host competitive postharvest technology, CA is an alternative to chemical fumigation [9, 15], and has been reported to be used commercially in insect control [2,4]. Rapid GABA accumulation has also been shown to be involved in the defense against phytophagous insects [11]. Several authors have also reported speedy and substantial rises in γ-aminobutyric acid (GABA) levels [1,3] as an adaptive response to stress conditions. In this context, specific pathogenesis-related proteins (PR-proteins) exhibit direct or indirect antimicrobial defense activity, although certain PR-proteins as chitinases has been found in a wide range of plants under any number of circunstances [16].
The aim of the work discussed here was to evaluate how high CO_2 levels enhance the cherimoya fruit defense system during storage at low temperature. In this study, we evaluated the effect of high CO_2 pretreatment on the titers of GABA and analyzed the activity of chitinase. To obtain more information regarding the physiological effect of high carbon dioxide treatment ethylene production was also determined. Our results indicate that high CO_2 pretreatment is associated with a higher chitinase activity and

transient increase in GABA. These protective events in treated fruit may have enabled cherimoya fruit to overcome low temperature storage.

Materials and Methods

Materials

Cherimoyas (*Annona cherimola* Mill. cv. 'Fino de Jete') were harvested in Almuñecar (Granada, Spain). One day after harvest, fruit were selected so as to obtain a healthy uniform population. Treated fruit were stored in two 20-L respiration chambers at 6°C and ventilated with a 180 mL min^{-1} continuous flow of 20% CO_2-20% O_2. After three days under 20% CO_2, treated fruit were analyzed and transferred to continuous humidified air flow for a futher 20 days. Untreated (control) fruit were stored at 6°C in a respiration chamber under a continuous flow of air. During low temperature storage, untreated and CO_2-treated cherimoyas with identical chronological ages were periodically collected, peeled, chopped and frozen in liquid nitrogen. The mesocarp tissues were stored at -80°C for further assays. Three individual fruit replicates were evaluated per day.

Measurements of Physiological Parameters

Ethylene Production

Ethylene production was checked immediately after removal of the fruit from the air or controlled atmosphere by introducing individual pieces into an air-tight glass container and measuring gas concentration in the head-space after 1 h. 1-mL aliquots were injected into a gas chromatograph (model 3700; Varian, Walnut Creek, CA) and ethylene was detected by a flame ionization detector on Porapak Q, with He as the carrier gas (30 mL min^{-1}).

γ-Aminobutyric Acid Determination

The procedure described by [3] with minor modifications, was used to prepare an aqueous fraction for determination of GABA. GABA was determinated on the basis of the increase in A_{340} after 30 min following factory recommendations for commercially available GABAse (Sigma), a spectrofotometric-coupled enzyme assay system for GABA.

Chitinase Extraction and Activity

Frozen mesocarp cherimoya tissues (1-g fresh weight) was homogenized at 4°C in 5 mL of 100 mM sodium acetate buffer, pH 5.0 and 2 % (w/v) PVP and centrifuged at 27,000*g* for 30 min at 4°C. In the clarified supernatant, chitinase activity was assayed using a commercial blue enzyme substrate CM-Chitin-RBV solution (Loewe). Enzyme activity was assayed by incubating a standard reaction mixture containing 30 μL of crude enzyme extract, 200 μL of aqueous CM-chitin-RBV (2 mg mL^{-1}) and 100 mM sodium acetate buffer, pH 5.0 to yield a final reaction volume of 0.8 mL. The supernatant containing degraded polymers was diluted (1:1, v/v) with nanopure water and absorbance was measured at 550 nm against a blank reaction (incubation mixture with HCl-treated crude extract). Specific enzyme activity was defined as "absorbance at 550 nm h^{-1} mg^{-1} of total protein".

Results

Effect of High CO_2 Pretreatment on Ethylene Production

Ethylene production is very low in general during storage at 6°C. Levels were significantly lower in fruit treated with 20% CO_2 than in fruit under ambient atmospheric conditions (data not shown). The slight increase in ethylene production recorded throughout storage at low temperature was delayed in treated fruit.

γ-Aminobutyric Acid Titers

Figure 1 shows the levels of GABA during storage at low temperature in untreated and CO_2-treated fruit. Whereas insignificant variations in GABA titers were observed in air-stored fruit during the first 3 days of storage at 6°C, in treated fruit the levels of GABA increase 9-fold by the end of CO_2 pretreatment. After transfer to air, GABA content dropped sharply to levels similar to those observed in untreated fruit on the ninth day of storage. Moreover; as storage progressed GABA titers were found to rise in both untreated and treated fruit.

Changes in Chitinase Activity

Changes in chitinase activity, expressed as per cent of the activity recorded in fruit after harvest (19.53 ± 0.4 $A_{550\,nm}$ h^{-1} mg^{-1} total protein), were determined in untreated and high CO_2-treated fruit during storage at 6°C (Fig. 2). At the end of CO_2 pretreatment, chitinase activity was found to be considerably higher than in fruit just harvested as well as in untreated fruit. Furthermore, enzyme activity in treated fruit dropped continually, although the levels of activity in CO_2-treated fruit were higher than in control fruit stored in air.

Discussion

CO_2-rich atmospheres have been reported to be effective in retaining fruit quality by delaying senescent changes and inhibiting decay [5,15]. Moreover, controlled atmospheres have been reported to effectively kill certain insects in grain, dried and fresh fruit and vegetables without detrimental effects on the commoditity [10]. Our working hypothesis was that high CO_2 controls fruit quality during storage at low temperatures by activating the defense systems in the fruit. In this sense we have analyzed GABA titer patterns in treated and untreated fruit stored at low temperature. This compound has been reported to accumulate rapidly in soybean leaves tranferred abruptly to a lower temperature, and in response to mechanical damage [13]. Our results confirmed that GABA accumulated by the end of CO_2 pretreatment declined sharply after transfer to air. Any further increase in GABA titer in either untreated and treated fruit may be related to rises in titratable acidity, associated with the metabolism

of cherimoya fruit.
In order to analyze high CO_2-activated resistance also determined chitinase activity in this work. This protein is a specific PR-protein, typically associated with the protective response to biotic and abiotic stress [7]. We observed that chitinase activity differed between untreated and treated fruit, while both groups had very low levels of ethylene production, with the lowest values corresponding to treated fruit. Furthermore, the exposure of fruits to CO_2-rich atmospheres (concentrations ranging from 5 to 30%) results in a considerable reduction in ethylene production [12]. Although we do not know whether chitinase protein is related to low temperature damage, it is clear that pretreatments with high CO_2 levels does cause a transient activity increase that is not observed in fruit stored in air.

Conclusions

In conclusion, the activation of some defense mechanisms such as increased GABA and chitinase activity are involved in the effect that high CO_2 levels have on cherimoya fruit stored at low temperature. These results might explain the potential success of CO_2-rich atmospheres as a postharvest quarantine treatment for pathogen suppression or insect control.

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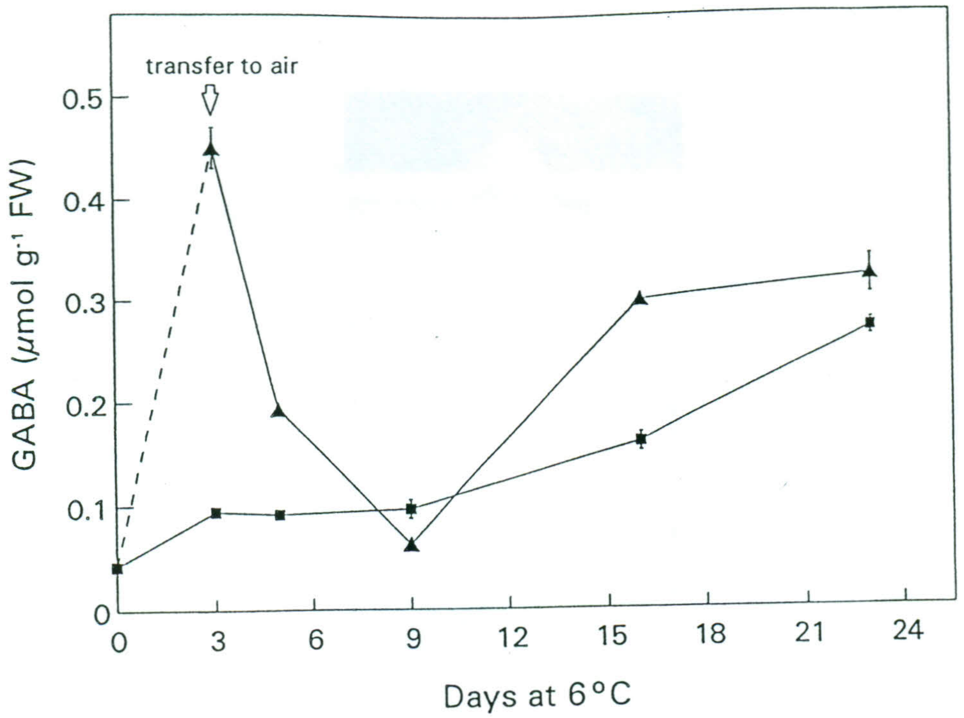

Figure 1. Changes in GABA levels in untreated (■) and CO_2-treated (▲) cherimoya fruit during storage at low temperature. Data are averages of two separate experiments (n = 6) and SE are shown by verticals bars. FW, Fresh weight.

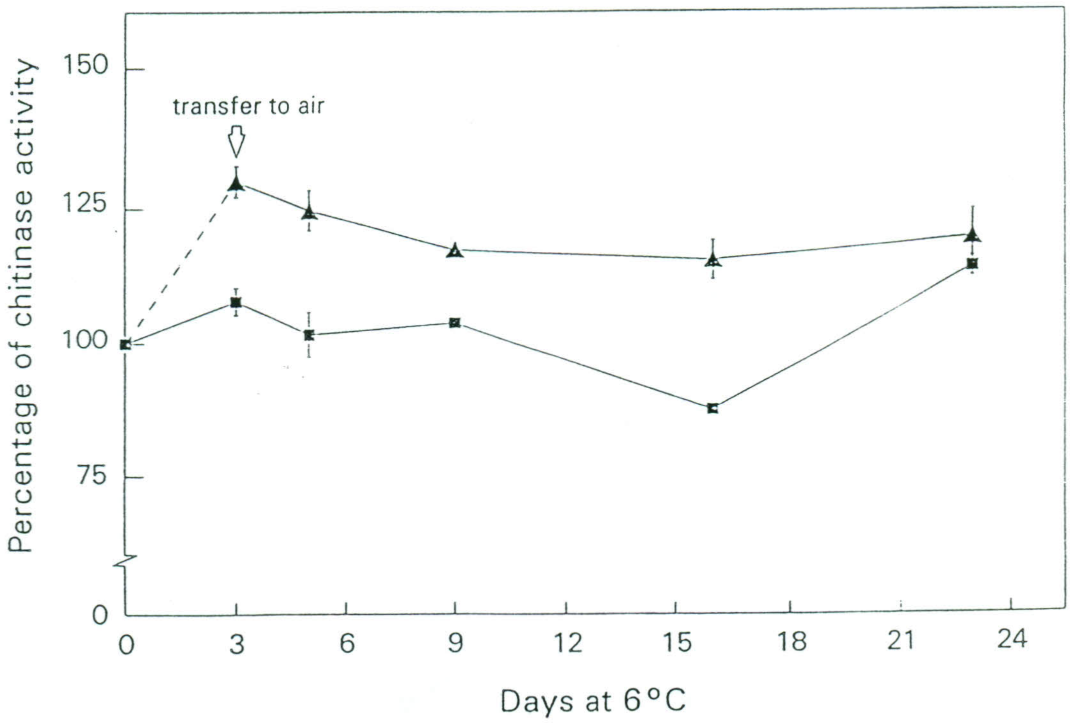

Figure 2. Chitinase activity patterns in untreated (■) and CO_2-treated (▲) cherimoya fruit during storage at 6°C. Data are averages of two separate experiments (n = 6) and SE are shown by verticals bars where they exceed the size of the respective symbol.