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**Mechanisms of Action and Uses of
Anaerobiosis and Anaerobic Intermediates
for the Post Harvest Regulation of Fruit
Quality**

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**MECHANISMS OF ACTION AND USES OF ANAEROBIOSIS AND ANAEROBIC
INTERMEDIATES FOR THE POSTHARVEST REGULATION OF
FRUIT QUALITY.**

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ABSTRACT

The use of pretreatments with anaerobic conditions, or the natural metabolic product acetaldehyde (AA) to stimulate fruit metabolism, constitutes a method to enhance fruit sensory quality.

We were able to show that applied AA can influence ripening in grapes and oranges, non-climacteric fruits, and bring about an increase in the sugar/acid ratio of early picked grapes and oranges. In oranges AA caused also degreening of the peel associated with an increase in CO₂ evolution and a reduction in acidity. In red tomato fruits the increase in the sensory quality achieved by AA vapors is attributable to gluconeogenesis processes which cause a reduction in organic acids and an increase in total and reducing sugars.

Although AA enhanced the ripening processes, it is not associated with an increase in ethylene production. On the contrary, AA caused a significant reduction in the ethylene evolution in grape berries by inhibiting of the conversion from ACC to ethylene. In whole tomato at the breaker or turning stage, AA caused inhibition of fruit ripening as expressed by less development of color.

Postharvest application of AA vapors or different anaerobic conditions of N₂ or CO₂ for 24 h increased the production of aroma volatiles in all the fruit tested. Different volatiles accumulated in the various fruits which may be attributed to their genetic differences. Volatile esters such as ethyl acetate and ethyl butyrate, produced following the treatments, can enhance fruit sensory quality if they exist in moderate levels as in feijoa, but higher levels could produce off-flavors, as in strawberry. The anaerobic treatment of N₂ atmosphere for 24 h had the potential to increase the strength of the commercially important flavor fractions in aqueous orange essence and essence oil obtained from treated fruit. AA was found to be a natural compound which has a fungicidal effect. Treatments with AA vapors for 24 h were effective in the inhibition of fungal development in grapes and strawberries. The mode of action suggested that AA acts primarily on the cell membrane, leading to loss of its integrity, and subsequently causing the death of the fungi.

OBJECTIVES OF THE RESEARCH

In harvested fruit, complete and satisfactory ripening is not always attainable, even when ethylene is applied. In some non-climacteric fruits, notably citrus and grapes, ripening processes are discontinued after harvest. Our main objective was to improve the fruit quality of citrus and grapes that are picked early in the season. An increase in the sugar content, or a decrease in the acidity, which lead to an increase in the sugar/acid ratio, can ready early-picked fruit for the fresh market and for processing. Climacteric fruit (tomato) was chosen for comparison purposes.

Another objective was to study the metabolic basis for the glucogenic effect of AA. We explored the possibility that pretreatment with anaerobic conditions or with AA may stimulate ripening processes or, alternatively, provide substrate precursors for the production of quality constituents such as aroma volatiles. In addition, AA treatments are already known for their fungicidal effects and we intended to study the fungicidal effect on the fruits we treated with AA or anaerobic conditions.

It was intended to carry out research only on citrus, grape and tomato fruits. However, as the induction of aroma volatiles was found to be predominant, we extended the research to two other aromatic fruits: strawberry - a non-climacteric fruit, and feijoa - a climacteric fruit.

DESCRIPTION OF COOPERATION

During the second year Dr. Pesis from Israel and Dr. Shaw from Florida met together twice, in November 1987 in Florida, and in November 1988 in Israel. They conducted experiments using oranges in the early harvesting season and studied the specific conditions of each country. In addition, they exchanged information and discussed the results obtained in the first two years of the project. Dr. Frenkel from Rutgers University visited Dr. Pesis, in Israel in July 1988, and they exchanged information and discussed the continuation of the research program. Dr. Frenkel visited Dr. Pesis again in November 1989, when they discussed the final and overall results.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

The use of pretreatments with anaerobic conditions, or the metabolic product AA to stimulate fruit metabolism, was an opportunity to add a new dimension to the regulation of ripening and, furthermore, constitutes a method to enhance fruit sensory quality.

Fruit ripening and the associated processes can be regulated by ethylene in climacteric, but not in non-climacteric, fruit. In this work we showed that applied AA can influence ripening in grape, a non-climacteric fruit, and bring about an increase in the sugar/acid ratio of early picked grapes (14% total soluble solids), which enhances their sensory quality. AA caused a significant reduction in the ethylene evolution in grape berries by inhibition of the conversion from 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. The reduction in ethylene production by grapes can explain the firmer berries obtained after AA treatments, which may increase the fruit's resistance to fungal attack. In early picked 'Shamouti' oranges, another non-climacteric fruit, AA vapors caused yellowing of the peel in addition to an increase in CO_2 evolution which led to a decrease in fruit acidity. Thus, this postharvest application of AA vapors

can extend the harvesting season for grapes and oranges.

Exposure of whole tomatoes to 0.2-0.6% AA vapors for 6-24 h prior to storage, inhibited fruit ripening, as expressed by less development of color. Inhibition of color occurred whether fruits were at the breaker or turning-pink stage. However, the highest concentration of AA and the longest duration of treatment caused some damage to the fruits, in the form of a hollow around the stems. Application of N_2 atmosphere or CO_2 in different concentrations (50-90%) for 24 h prior to storage, caused different production of endogenous acetaldehyde and ethanol, which was correlated with the color inhibition. The higher the endogenous AA and ethanol levels produced, the greater the inhibition of ripening. Regardless of the anaerobic treatment applied, the fruits did not suffer any injury, so this pretreatment can be used to increase tomato shelf-life.

In tomato fruits, the increase in sensory quality caused by AA vapors is attributable to gluconeogenesis processes which caused a reduction in organic acids and an increase in total and reducing sugars. AA may influence the level of fructose 2,6-bisphosphate and subsequently the glycolysis-gluconeogenesis balance and the resultant sugar accumulation and fruit quality.

Postharvest application of AA vapors or different anaerobic conditions of N_2 or CO_2 for 24 h increased the production of aroma volatiles in all the fruit tested. In hand-picked feijoas, which are highly acidic, these treatments led to the accumulation of AA, ethanol, ethyl acetate and ethyl butyrate, which increased fruit aroma and flavor. A sensory panel judged the treated fruits as sweeter than the control. In feijoas, the anaerobic treatments were more effective in ester biosynthesis probably because under those conditions AA is formed endogenously and immediately converted into esters. Fruits from the N_2 treatment maintained the best appearance after storage of 13 days at 20°C. The anaerobic conditions, and particularly the N_2 treatment for 24 h, can serve therefore as a potentially practical tool in preserving and enhancing the sensory quality of feijoa fruits. In another very aromatic fruit, strawberry, a brief treatment with

5000 ppm AA for 1 h or with 1500 ppm AA for 4 h resulted in superior taste, associated with a moderate increase in volatile production, minimal off-flavor and minimal microbial decay. However, at higher concentrations it caused injury to the berries.

Volatile ester production was much lower in oranges than in the aromatic fruits. However, also in Hamlin, Pineapple and Valencia oranges that were treated with AA or anaerobic conditions, changes in seven volatile components during 8 days' storage at 20°C were found. The flavor evaluations of control versus treated juice showed no consistent changes from any of the treatments. However, the flavors of aqueous essence and essence oil from N₂-treated Valencia oranges were significantly different from comparable flavor fractions from control fruit when evaluated in a bland orange juice base. The anaerobic treatment of N₂ atmosphere with <1% oxygen for 24 h has the potential to increase the strength of the commercially important flavor fractions in aqueous orange essence and essence oil obtained from treated fruit.

The inability to effectively control postharvest decay of fruits and vegetables by nontoxic chemical methods and the refusal to accept commodities treated with toxic fungicides, have stimulated interest in biological methods for decay control. AA is a natural volatile compound found in fruits and vegetables, and thus the use of volatile compounds produced by the fruit itself may be of great significance. Treatment with AA vapors for 24 h was found to be effective in the inhibition of fungal development in grapes and strawberries. Treatment of grapes with AA vapors at 20° or 0°C reduced significantly the decay caused by the fungi Botrytis cinerea, Rhizopus stolonifer, Aspergillus niger and Alternaria alternata. Exposing the fungal mycelium to AA resulted in increased leakage of electrolytes, reducing sugars and amino acids. This suggests that AA acts primarily on the cell membrane, leading to loss of its integrity, which causes the fungus death. AA can cause damage to the fruits at high doses. However, at the right concentrations and with sufficient precautions, AA can be an ideal postharvest-decay-control treatment for grapes and strawberries.

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