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# STABILIZATION OF PECTINMETHYLESTERASE OF TOMATO FRUIT CAUSED BY ETHYLENE AT DIFFERENT TEMPERATURES[1]

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## 1. INTRODUCTION

Ethylene indirectly affects many enzymatic processes in plant tissue. It has been reported that ethylene can protect the activity of zymase in solution (NORD, *9*). More recently, it was reported that ethylene treatment can stabilize the activity of yeast alcohol dehydrogenase in solution (*3*). Ethylene has also been shown to stimulate the activity of L-phenylalanine ammonia lyase (PAL) in excised plant tissues (*2*), in pea seedlings (*7*), in citrus fruit peel (*10*) and in carrot tissues (*11*). Since ethylene is the most potent abscission agent known, and was shown by HERRERA and HALL (*5*) to affect the activities of the enzyme involved in the abscission of the cotton leaf, it was deemed desirable to develop this basic information as a background to study the effects of ethylene upon certain specific enzymes involved in the ripening process.

GERTMAN and FUCHS (*4*) reported chages in PME activity caused by ethylene applied at different temperatures in both purified enzyme solution and in avocado fruit tissue. Avocado fruit is different from many other fruits in that during ripening its PME activity decreases with advancement of the softening process (*14*). In view of these findings, we investigated whether ethylene might have any effect on the enzymatic activity of tomato PME.

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## 2. MATERIALS AND METHODS

Comercial tomato PME was utilized in initial experiments. A solution of 1.0 mg/ml of PME in 10 per cent NaCl solution was placed in a vacuum flask. The flask top was fitted with a two-hole stopper with inlet and exhaust ports, and gas mixtures of various concentrations of ethylene in nitrogen were passed over the enzyme solution. The sidearm of the flask was fitted with a serum cap and enzyme solution aliquots of 2.0 ml volume were withdrawn using a glass syringe. PME enzymatic activity of the aliquots was determined by the method of HILLS and MOTTERN (*6*) from the amount of 0.02N NaOH required to maintain a 0.5 per cent solution of citrus pectin at pH 8.0 for 5 minutes. One unit of PME activity is defined as the milequivalents of NaOH required per minute per ml of PME solution.

The same system was utilized to study the effects of acetone on PME stability. Acetone was added to the enzyme solution to a final concentration of 10 per cent, and compressed air was passed over the solution. Aliquots were removed and assayed as above.

Further investigations were conducted *In vitro* to test the effects of ethylene on the multiple forms of PME. A series of flasks were fitted as described earlier and enzyme solutions were placed in the flasks. Incubation temperatures were 6°C and 25°C. Enzyme aliquots were removed for assay at 0, 1, 2, 4, 6, 7, 8, 10 and 12 hours during incubation.

To one-half the flasks containing enzyme solutions a flowing gas mixture of 100 $\mu$ 1/1 ethylene in nitrogen was administered, and remaining flasks were treated with nitrogen gas alone. After a 6-hour incubation the gas flow treatments were reversed and the flasks treated with ethylene in nitrogen were purged with nitrogen only, while those treated with nitrogen gas only were now administered ethylene and nitrogen. The experiments were terminated after 12 hours incubation.

Confirmatory of the above procedure, further experiments were performed to investigate the reversibility of temperature effects on PME activity. Flasks were incubated with constant gas flows of either nitrogen or 100 $\mu$ 1/1 ethylene in nitrogen. After a 6-hour period, flasks which were incubated at 25°C were transferred to 6°C and those incubated at 6°C were transferred to 25°C for 6 hours additional incubation.

In other *In vivo* experiments, pericarp tissue disks (2.5 cm dia.) from tomato fruits at 60 per cent of development were vacuum infiltrated with an aqueous solution of 50 mg/l ethephon. Control disks were infiltrated with distilled water. Disks were then placed in glass containers ventilated by an air stream free of ethylene and $CO_2$ at a flow rate of 1 1/hr. The containers were then incubated at 25°C and 6°C for three days. Each treatment was replicated 6 times. $CO_2$ and ethylene evolution rates were monitored daily by flow-through cell infrared gas analysis and flame ionization gas chromatography, respectively. Fruit disks were removed daily for assay of PME activity by the method given in an earlier experiment. The experiment was terminated prior to any increase in $CO_2$ evolution.

## 3. RESULTS AND DISCUSSION

The effects of ethylene concentration on PME activity are summarized in Table 1. It was found that a concentration of 1 $\mu$ 1/1 ethylene in the atmosphere above the enzyme solution was the lowest concentration which would effectively reduce the activity of PME *In vitro*. When the enzyme was incubated in an atmosphere of 100 $\mu$ 1/liter, activity was considerably depressed. Table 2 ilustrate the effects of

TABLE 1 - Effect of ethylene concentration on PME activity. The activity of the control treatment was 76 units/mg protein and was regarded as 100% of activity

| Ethylene concentration µl/liter in $N_2$ | Relative enzymatic activity |
|---|---|
| 0 | 100.0 |
| 1 | 95.7±1.40 |
| 10 | 91.5±1.00 |
| 100 | 86.8±0.85 |
| 1000 | 82.3±4.23 |

Data in temperature and acetone on the stability of PME. At 25ºC, when acetone was present, the activity of PME decreased after six hours of incubation. At 6ºC the activity of PME decreased; whereas, in the presence of acetone, no such decrease in activity was observed.

TABLE 2 - Effect of temperature and acetone on the stability of PME activity

| Temperature | Acetone | PME Activity (units/mg protein) Incubation time | |
|---|---|---|---|
| | | 3 hr | 6 hr |
| 25°C | - | 78.20±0.40 | 78.00±0.46 |
| 25°C | + | 76.80±1.13 | 74.00±0.80 |
| 6°C | - | 70.40±0.90 | 65.00±0.40 |
| 6°C | + | 71.40±1.43 | 75.00±0.40 |

The effect of ethylene treatment and alteration of incubation temperature on the activity of the enzyme is shown in Figure 1. The activity of PME incubated in ethylene was much lower than that in nitrogen. This effect of ethylene on PME activity was found to increase with time. The effect of ethylene was a reversible

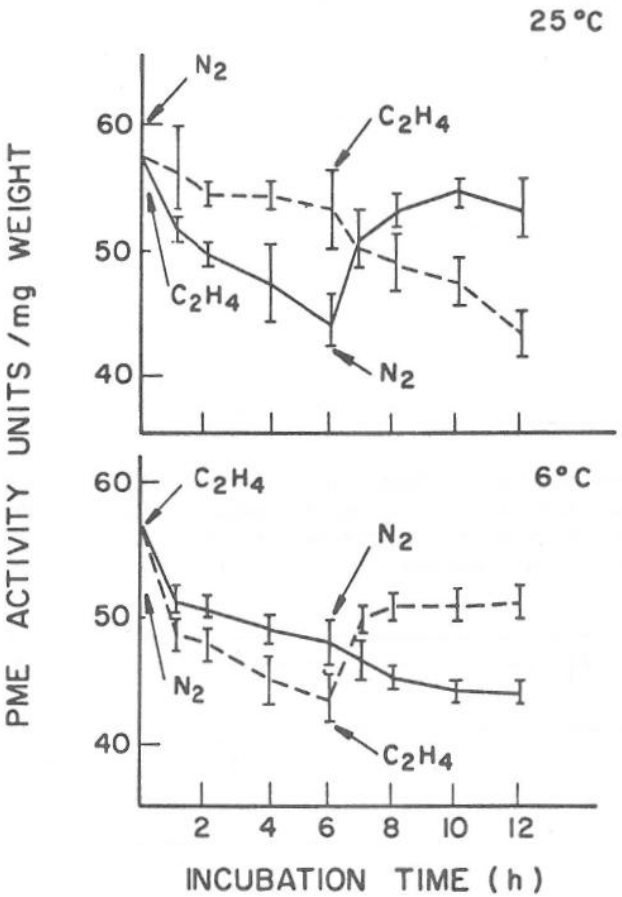


FIGURE 1 - Effect of temperature and alteration of incubation gas of PME solution on the activity of the enzyme. A. Flasks containing enzyme solution were incubated in nitrogen or ethylene for six hours and than transferred to ethylene or nitrogen atmosphere at 25°C. B. Flasks containing enzyme solutions were incubated in ethylene or nitrogen for six hours in ethylene or nitrogen for six hours and than transferred to nitrogen or ethylene atmosphere at 6°C. The time at which nitrogen and ethylene were applied is indicated by arrows.

process. When the flasks which were incubated with ethylene were administered a nitrogen atmosphere alone, PME activity was restored to its initial rate. This is in accordance with earlier work of GERTMAN and FUCHS (*4*). At 6°C, ethylene did not have the same effect. It seems that at low temperature ethylene had a protective effect against the suppression of PME activity.

As may be noted in Figure 2, the effects of both temperature and ethylene on PME activity were reversible phenomena. Ethylene had a greater effect on PME activity when incubation was initiated at 6°C than at 25°C.

When treated tomato disks were kept at 25°C the decrease in PME activity in the fruit was markedly accelerated (Figure 3). On the other hand, when treated fruit disks were incubated at 6°C, PME activity in the fruit was significantly higher than in untreated disks at the same temperature or in disks held in a 25°C regime.

Ethephon application markedly increased the rate of ethylene evolution as shown in Figure 4. Ethylene evolution of ethephon-treated disks at 25°C increased until day 2, after which it declined, while disks held at 6°C showed a slow general decline in ethylene evolution rate. Disks at all temperatures and treatments showed an overall rate of decline in $CO_2$ evolution with time (Figure 4).

Although the effect of ethylene on fruit ripening has been defined, the mechanism of its action in plant tissue is still unclear. Three theoretical lines have been suggested to explain how this hormone exerts its regulatory effect. Ethylene may control protein synthesis through the control of nucleic acid synthesis; or, it may interact with membranes thus altering their functions in some way; or, it may interact directly with macromolecules (proteins or nucleic acids) and in this way alter enzyme activity (*1*).

Data presented in this section seem to demonstrate that ethylene can interact with PME and in some way control its activity. It has been suggested by TAKEMORI *et alii* (*13*) that organic solvents added to dilute enzyme solutions can stabilize enzyme activity by maintaining the native structural conformation of the protein. In this study, both acetone and ethylene were found to protect against the inactivation of PME at low temperature. GERTMAN and FUCHS (4) suggested that ethylene, being a non-polar hydrocarbon, may interact with hydrophobic bonds in protein or may influence the water structure around the protein (*12*). In such a way, PME enzymatic activity may be altered.

## 4. SUMMARY

The effects of ethylene concentration on pectinmethylesterase (PME) were studied. Commercial tomato PME was utilized in initial experiments. A solution of 1.0 mg/ml of PME in 10% NaCl was placed in a vacuum flask and gas mixtures of various concentrations of ethylene were passed over the enzyme solution.

It was found that a concentration of 1 $\mu$ 1/1 ethylene was the lowest concentration which would effectively reduce the activity of PME. In further experiments, flasks were incubated with constant gas flows of either nitrogen or 100 $\mu$ 1/1 ethylene in nitrogen. After a 6-hour period, flasks were removed and placed in a water bath at the alternate temperature. Data presented showed that a concentration of 100 $\mu$ 1/1 ethylene in the atmosphere above the enzyme had a stabilizing effect on PME at 6°C while it apparently suppressed PME activity at 25°C; and, that tomato fruit disks treated with ethephon had a similar effect.

The effects of both temperature and ethylene on PME activity were reversible phenomena. The ethylene being a nonpolar hydrocarbon, may interact with hydrophobic bonds in protein or may influence the water structure around the protein, in such a way that enzymatic activity may be altered.

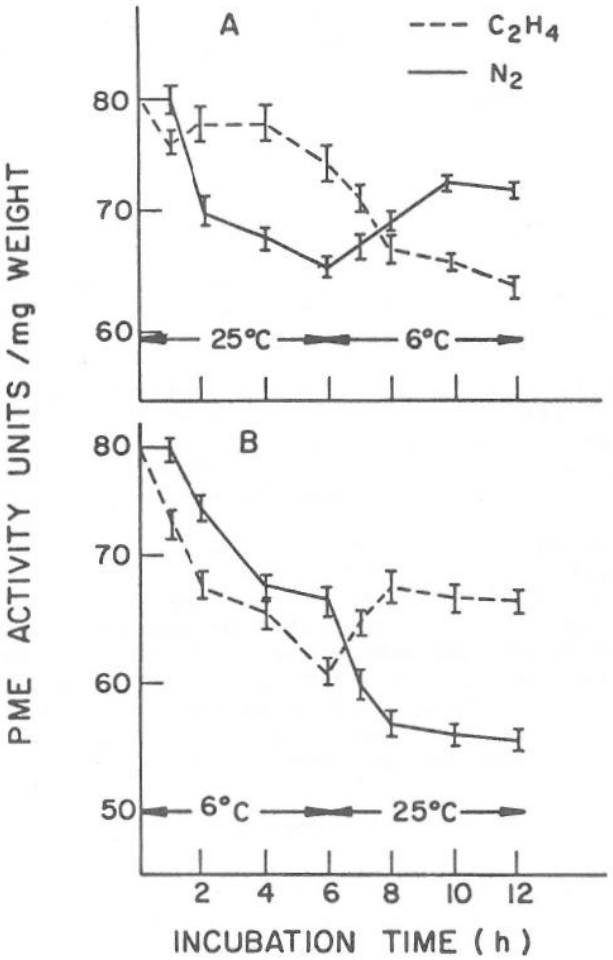


FIGURE 2 - Effect of ethylene and alteration of incubation temperature of PME solution on the activity of the enzyme. A. Incubation started at 25°C and than the solutions were transferred to 6°C. B. Incubation started at 6°C and than the solutions were transferred to 25°C.

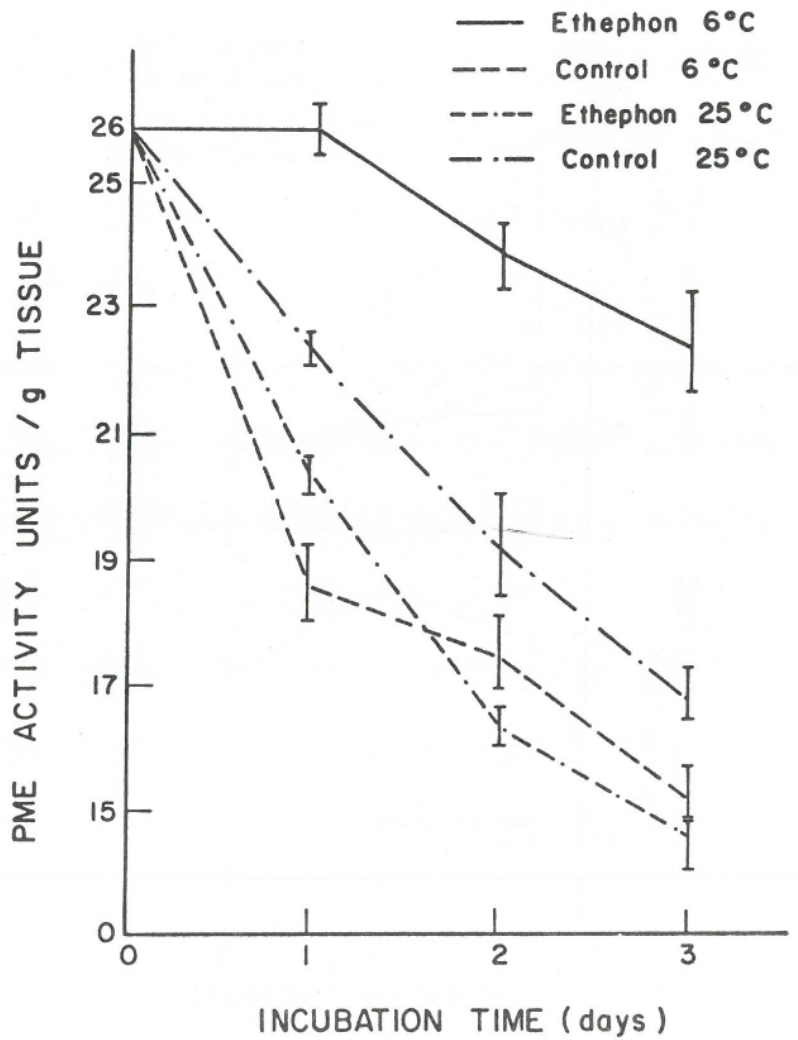


FIGURE 3 - Effect of Ethephon treatment on PME activity in tomato fruit disks.

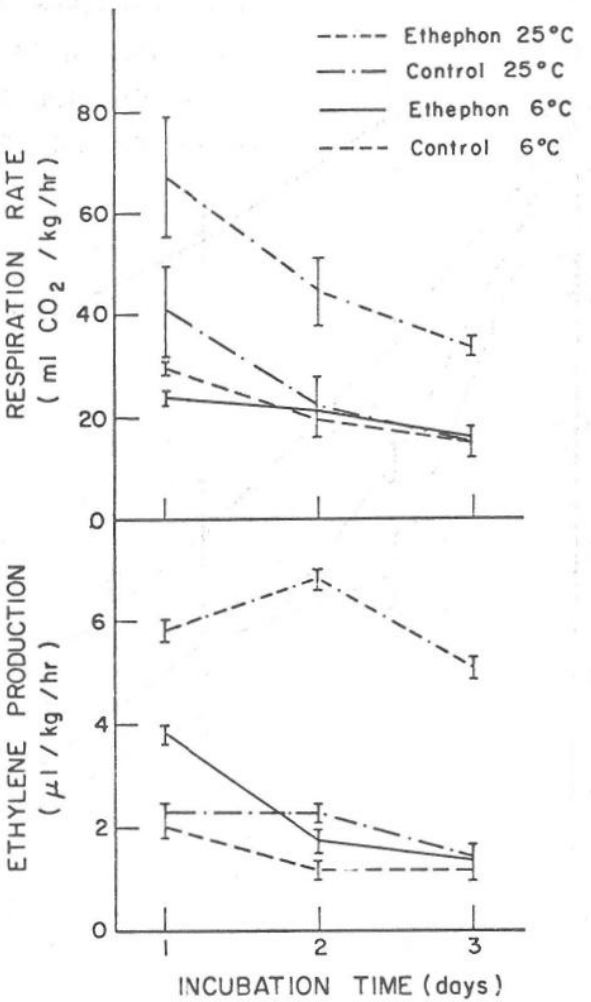


FIGURE 4. Average rates of $CO_2$ and $C_2H_4$ production by Ethephon treated and untreated disks of tomato incubated at different temperatures.

## 5. RESUMO

Este trabalho teve como objetivo estudar o efeito do etileno sobre a atividade enzimática da pectinametilesterase (PME).

Utilizou-se nos ensaios iniciais a PME comercial, cuja solução continha 1,0 mg/ml de PME em 10% de NaCl. Dentro de um sistema de incubação, aplicaram-se na solução enzimática diferentes concentrações de etileno. A mais baixa concentração que efetivamente reduziu a atividade da PME foi a de 1 $\mu$l. Em outros ensaios, quando essa mesma solução enzimática foi incubada a 6ºC, na presença de 100 $\mu$l de etileno e/ou em atmosfera que continha apenas nitrogênio, verificou-se que o etileno reduziu a atividade da PME, em relação ao nitrogênio, quando a temperatura de incubação foi mantida em 25ºC. Entretanto, o etileno, a 6ºC, teve um efeito restaurador da atividade enzimática perdida pela inativação natural causada pela baixa temperatura.

Foram obtidos resultados semelhantes com a aplicação de discos de frutos de tomateiro infiltrados com solução de ethephon.

Procurou-se dizer que tanto o efeito do etileno como o da temperatura baixa, para reduzir a atividade da PME, são fenômenos reversíveis. Possivelmente em razão de ser um hidrocarbono não-polar, o etileno pode interar-se com as ligações hidrofóbicas das estruturas da PME, ou mesmo alterar as moléculas de água em torno dessas estruturas e, dessa maneira, regular e atividade enzimática.

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