

ABSTRACT

1 - Aminocyclopropane - 1 - Carboxylic Acid (ACC)

Oxidase from *Carica papaya* : Isolation,

Characterization, Role in Ethylene Biosynthesis and fruit

Ripening.

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1-Aminocyclopropane-1-carboxylic acid (ACC) oxidase catalyses the conversion of ACC into ethylene, a phytohormone associated with the ripening process of climacteric fruits. Most of the studies done on the enzyme were carried out using *in vivo* techniques due to its difficulty of extraction. It was not until 1991 that an *in vitro* isolation was done successfully by Ververidis and John, using the melon fruit.

This thesis is an embodiment of the analysis and description of many *in vitro* assays that were conducted on the enzyme extracted from *Carica papaya* fruit. The enzyme exhibited a K_m of $37 \mu\text{M}$ and was inhibited by *n*-propyl gallate (0.24 mM); sodium dithionite (0.220 mM); sodium metabisulphite (0.240 mM) and cobalt sulphate (0.100 mM). The enzyme activity increased with ripening then declined as the fruit became over-ripe.

The enzyme is very labile in nature, losing total activity within 4 days when stored at 14 ° C. Storage of the crude homogenate at -15 ° C prolonged its shelf life beyond an 8 day period. In the initial extraction process, the papaya tissue had to be freeze-dried with liquid nitrogen to maintain the enzyme activity. Isolation and purification were subsequently achieved using ammonium sulphate precipitation, followed by gel filtration using Sephadex G 100 - 120 and then by anion exchange chromatography using DEAE - Sephadex anion exchanger 40 - 120 μ . Gel electrophoresis of the purified enzyme gave a single band which corresponded to a M_r of 27.5 kDa.

The enzyme also displayed an optimum temperature and pH of 30 ° C and 7.5 respectively. Interestingly, the enzyme can also utilize a second substrate, ACC methylester, an analogue of ACC.

The amino acid content of the enzyme was also determined which showed a relatively high percentage of valine (12.69 %) and it also contained cysteine residues (5.87 %). The latter made the enzyme highly sensitive to sulphhydryl-reactive reagents such as dithiothreitol. At low concentrations (3 mM), this compound enhanced the enzyme activity.

Some compounds that were present in the assay medium also enhanced the enzyme activity; these included bicarbonate ion (30 mM) , oxygen and iron II ions (80 μ M).

ACC oxidase activity in the fruit of Carica papaya is relatively high. As a result of this it was not necessary to subject the fruit to ethylene prior to enzyme assay.