

A NEW METHOD FOR THE DETERMINATION OF ACC

C.T. PHAN and D. LANNELUC-SANSON
Département de Sciences Biologiques
Université de Montréal, C.P. 6128, Succ. A

Introduction

The importance of ethylene in plant physiology is not to be demonstrated any longer (1). Consequently its estimation, as well as the determination of its proposed precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), must be sensitive, accurate and reliable. If ethylene can be determined very accurately by gas chromatography, the same cannot be said of ACC. The main method for the estimation of ACC which is at present widely used is that Lizada and Yang (2); it consists of releasing ethylene from ACC by a chemical reaction, and the estimation of this ethylene by gas chromatography. According to the authors of the method, a conversion rate of ACC to ethylene of 70 per cent is considered excellent, and in the literature, one can find conversion rates ranging from 30 per cent to values higher than 100 per cent (3). These latter values are an indication that the reaction, especially in biological extracts, is not stoichiometric. Added to this, the difficulty of sampling gas mixtures renders the method unreliable.

All these uncertainties have urged us to look for a method that could assay ACC itself. ACC being an amino acid, two main avenues are possible: 1. its analysis by ion-exchange (by the means of an amino acid analyzer), 2. its derivatization and analysis of the derivative by high pressure liquid chromatography (HPLC). After having tried several methods, we have adopted the derivatization with phenylisothiocyanate (PTC) followed by the separation of the derivative by reverse-phase HPLC.

Material and Methods

The apples used were of the cultivar McIntosh, harvested in an experimental orchard of Agriculture Canada in Freligsburg, Québec, Canada.

In this work, extraction of ACC was performed according to Lizada and Yang (2). The extract was then passed through a cation-exchange column, and the amino-acids recovered by elution with IN NH₄OH. After removal of this solvent by evaporation, the amino-acid solution was divided into three aliquots. The first aliquot was processed as indicated by Lizada and Yang for the determination of ACC. The second one was processed through an amino-acid analyzer. And the third one was derivatized and analyzed by reverse-phase HPLC.

The derivatizing reagent, phenylisothiocyanate, was purchased from Aldrich, and the analysis was made on a Waters' C₁₈ reverse-phase column eluted with various mixtures of acetonitrile and sodium acetate (4). The detection of the derivatized amino-acids was achieved by a UV-detector set at 254 nm.

Results and Discussion

A linear response for standard ACC was observed in the range of 66 to 1320 picomoles, and actual results were in agreement with theoretical calculations (correlation coefficient = 0.9999).

A comparative trial was made, with the same extract analyzed by the three methods. The results are gathered in table 1.

Table 1. Compared accuracies of three methods of estimating ACC

Sample	L.Y. (I)	A.A.A. (II)	D-HPLC (III)	$\frac{I - II}{II} \%$	$\frac{I - III}{III} \%$
1	4.1	6.7		-39	
2	6.0		10.4		-42
3	1.2	1.7	1.8	-29	-33

- Remarks: 1. L.Y. = Lizada and Yang's; A.A.A. = amino-acid analyzer; D-HPLC = derivatization + HPLC
2. ACC-quantities expressed in nanomoles

The amino-acid analyzer yielded results quite comparable with that of the derivatization-HPLC technique (both more accurate than Lizada and Yang's). However, on the A.A.A. chart, the ACC-peak was fused - as a shoulder - with that of another amino-acid; this is quite predictable as ACC, being a mono-carboxylic mono-amino acid, it behaves in the same manner as the numerous other mono-carboxylic mono-amino acids. On a reverse-phase column, its very specific 3 carbon atom-ring places it apart from most of the other amino acids: ACC-peak is well individualized, and well separated from the other peaks, and co-chromatography of ACC and an apple extract yielded one peak for ACC, with no interference.

This new technique thus appears sensitive and reliable.

References Cited

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