Measurement of Allicin with N-Ethyl Maleimide

Sir:

A new method to measure allicin quantities in the alliinase reaction mixtures was studied. This method was an improved one firstly discovered by Schwimmer.¹⁾ His method, a quantitative adaptation of spray reagent for chromatographic identification of thiosulfinate²⁾ is based on the reaction of thiosulfinate with N-ethyl maleimide in alkaline isopropyl alcohol. Our method was carried out as follows:

Ten ml of garlic enzyme reaction mixture was extracted three times with 10 ml portions of diethylether. The ether layers were removed, combined and evaporated to dryness at room temperature, and the residue was taken up in isopropyl alcohol. After dilution to an appropriate volume, 1 ml of isopropyl alcohol solution of the ether-extracted material was added to 1 ml of cold 0.05N ethyl maleimide dissolved in cold isopropyl alcohol and finally 3 ml of isopropyl alcohol. The intensity of the yellow color was measured at 355 m μ (Bausch-Lomb colorimeter). By the Schwimmer's original method, the reddish pink color was measured at 515 m μ , and the colors were varied from reddish pink to yellow. With the lapse of time, the absorbance at 515 m μ was decreased. Schwimmer's calibration curves were not linear especially at the point of 1.5 μ moles of thiosulfinate. In our method, the intensities of the reaction color 20~30 min. after the addition of the reagent were directly proportional to the quantities of allicin.

In the original method, the reaction mixtures were extracted by benzene, but, in our method, the extractions were carried out by ether. The reasons were as follows: 1)

1) S. Schwimmer and M. Mazelis, *Arch. Biochem. Biophys.*, **100**, 66 (1963).

2) J. F. Carson and F. F. Wong, *Nature*, **183**, 1673 (1959).

FIG. 1. Calibration Curve of Allicin by the Improved N-Ethyl Maleimide Method, 20~30 min. after the Reagent Addition.

FIG. 2. Change of Absorbance at 355 m μ , 2.9 μ moles Allicin was Used.

Benzene-extracted solution being evaporated at room temperature, the evaporation velocity was so slow that we were afraid of allicin changing to diallyldisulfide. 2) The color intensities carried out by the original method were so weak that we could not measure

allicin quantities. 3) In our country, when Yoshimura studied the detection of allicin in *Allium* plants, allicin extraction from *Allium* plants was carried out by ether, therefore we followed Yoshimura's allicin extraction method.³⁾

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3) M. Yoshimura, *Vitamins* (in Japanese), **14**, 633 (1958).