Use of *o*-Phthaldehyde Derivatives and High-Pressure Liquid Chromatography in Determining the Free Amino Acids in Cocoa Beans

The free amino acids in Ecuadorian cocoa beans were qualitatively analyzed, using the formation of precolumn *o*-phthaldehyde derivatives. The amino acids were extracted from cocoa beans and subjected to high-pressure liquid chromatography amino acid analysis. This shows the utility of the technique to a solid food matrix.

Recently the opa derivative and high-pressure liquid chromatography (LC) have been used to determine amino acids (Hill et al., 1979; Lindroth and Mopper, 1979). The technique has successfully been applied to biological and environmental samples. We report here the first use of this technique on a solid food sample. The free amino acids were extracted from a cocoa bean sample, using the procedure of Rohan and Stewart (Rohan and Stewart, 1966), and derivatized with *o*-phthaldehyde. The resulting fluorescent product was analyzed by LC. The amino acids were identified and compared to those seen by Rohan and Stewart (1966).

EXPERIMENTAL SECTION

The free amino acids were extracted by using the same general procedure developed by Rohan and Stewart (1966). Twenty grams of ground Ecuadorian cocoa beans was blended with 200 mL of water for 5 min, with the suspension filtered through Whatman 41 or equivalent. A 100-mL aliquot of the filtrate was passed through a 1 × 20 cm column of Ag 50W-X8 (Bio-Rad Laboratories). The resin was washed with 20% and 80% 2-propanol in water. Amino acids were then eluted with 4 N NH_4OH. The eluant was collected until the eluant reached a pH of 10 and evaporated to dryness under vacuum. This residue was then used for further analysis.

This residue was dissolved in 100 mL of water and adjusted to pH 6.5–7.0. A 400-μL aliquot of this solution was mixed with 100 μL of opa reagent and shaken. A 5-μL portion was then injected onto the LC (see Figure 1).

The LC procedure employs chromatography on a reversed-phase column, RP-18, 4 mm × 25 cm (E. M. Labs), with a mobile phase consisting of H_3PO_4, HOAc, DMF, and MeOH in varying portions for a gradient run. Solvent A was 0.01 M H_3PO_4/0.2 M CH_3COOH/CH_3OH/DMF (40:40:19:1) at pH 5.5 ± 0.2. Solvent B was the same four components (10:10:75:5) at the same pH. Solvent A was used alone for 5 min at a flow rate of 1.0 mL/min; then solvent B was mixed, using curve 5 of the Waters Model 660 solvent programmer for 35 additional minutes. Several other mobile phases have been used in the separation of derivatized amino acids (Hill et al., 1979; Lindroth and Mopper, 1979). It should be noted that the use of differet pHs in the mobile phase can affect the elution order of individual amino acids. The chromatogram was compared with a chromatogram obtained by the injection of a standard amino acid mixture.

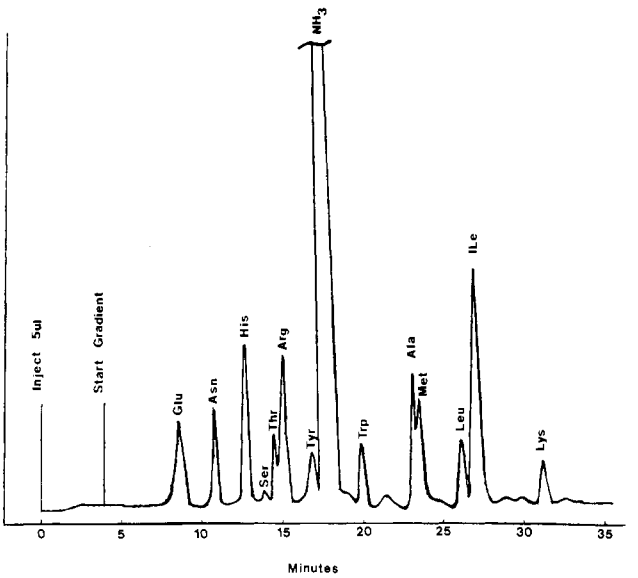

Figure 1. Amino acid opa derivative of free amino acids from cocoa beans.

DISCUSSION

The data obtained compare favorably to the qualitative data of Rohan and Stewart (1966). Preliminary precision studies show a reasonable coefficient of variation (Cv) of about 10% for both standards and sample (n = 6). The fluorescence of the derivative is subject to time decay; therefore, work is continuing on this topic to arrive at a maximum allowable delay time before analysis. This preliminary work shows the utility of the precolumn derivative in the amino acid analysis of food. Further studies are in progress on the qualitative and quantitative aspects of this assay.

LITERATURE CITED

Hill, D. W., Walters, F. H., Wilson, T. D., Stuart, J. D., *Anal. Chem.* **51**, 1338–1341 (1979).
Lindroth, P., Mopper, K., *Anal. Chem.* **51**, 1667–1674 (1979).
Rohan, T. A., Stewart, T., *J. Food Sci.* **31**, 202–205 (1966).

W. Jeffrey Hurst*
Robert A. Martin, Jr.

Hershey Foods Corporation
Technical Center
Hershey, Pennsylvania 17033

Received for review November 2, 1979. Accepted March 20, 1980.

0021-8561/80/1428-1039$01.00/0