Figure 7. Effect of acidulation ratio on conversion

six rods were used instead of the 10 rods used in previous runs. A lower heating jacket temperature of 325° F. was used instead of 350° F. The drying air rate was increased from 0.365 cu. ft. per minute to 0.512 cu. ft. per minute, and the drying air temperature was decreased from about 160° to 73° F. The opposing effects of changes in these operating variables resulted in no distinguishable changes in the product, except for higher moisture and free acid content. This could be corrected either by increasing the temperature, increasing the residence time, increasing the amount of drying air or its temperature, or by decreasing the acid and rock feeding rates. Moisture content between 3 and 4% gave optimum results and satisfactory free acid contents.

Figure 7 indicates the effect of the variation of the acidulation ratio on the conversion obtained in various runs. The particle size analysis of the rock used and the product triple superphosphate are given in Table V. Agglomeration of fine particles in the product of these runs prevented correct indication of the degree of grinding.

A residence time in the mill of about 45 minutes was indicated during run 10 by introducing ground charcoal with the rock feed and subsequent examination of the product for color.

The product was examined for storage characteristics after one month and two months. It was found free flowing and had excellent physical condition.

Literature Cited

(1) Association of Official Agricultural Chemists, "Official Methods of Analysis," Washington, D. C., 1950.
(2) Berlin, L. E., Mikerov, V. A., Zuchkoy, E. N., *J. Chem. Ind. (Moscow)* **5**, 48–51 (1933); *Chem. Abstr.* **26**, 1498 (1932).
(3) Bridger, G. L., *Tenn. Valley Authority, Chem. Eng. Rept.* **5**, 1949.
(4) Dragunov, S. S., *Fertilizers and Crops (U.S.S.R.)* **2**, 409 (1930); *Chem. Abstr.* **25**, 2247 (1931).
(5) Inskeep, G. C., Fort, R. W., Weber, W. C., *Ind. Eng. Chem.* **48**, 1805 (1956).
(6) Knox, W. H., Jr., U. S. Patent **2,160,700** (May 30, 1939).
(7) Marshall, H. L., Rader, L. F., Jacob, K. D., *Ind. Eng. Chem.* **25**, 1253 (1933).
(8) Martinez, F., M. S. thesis, Iowa State University, 1959.
(9) Meyers, H. H., U. S. Patent **1,-475,959** (1923).
(10) Nunn, R. J., Dee, T. P., *J. Sci. Food Agr.* **5**, 257 (1954).
(11) Parrish, P., Ogilvie, A., "Calcium Superphosphate and Compound Fertilizers," 2nd ed., Hutchinson's Publications, London, 1946.
(12) Phillips, A. B., Young, R. D., Lewis, J. S., Jr., Heil, F. G., J. Agr. Food Chem. **6**, 584 (1958).
(13) Rounsley, R. R., Ph.D. thesis, Iowa State University, 1957.
(14) Rounsley, R. R., Boylan, D. R., J. Agr. Food Chem. **6**, 677 (1958).
(15) Sauchelli, V., "The Chemistry and Technology of Fertilizers," Reinhold, New York, 1960.

GROWTH REGULATOR RESIDUES

Determination of Beta-Hydroxyethylhydrazine in Pineapples

METHRATTA P. THOMAS and
HENRY J. ACKERMANN

Olin Research Center,
Olin Mathieson Chemical Corp.,
New Haven 4, Conn.

A microanalytical method for the isolation and determination of beta-hydroxyethylhydrazine (HEH) in pineapples is described. The method consists of blending and extracting the HEH from the fruit with water. After the interfering pigments in the extract are removed with ion exchange resins, the HEH is reacted with cinnamaldehyde to produce a yellow color. The intensity of the color is proportional to the concentration of HEH, and is measured at 420 mμ. The amount of HEH in the sample is then calculated from a standard curve.

I T IS of economic importance to the pineapple growers to be able to make their plants fruit uniformly, and at a predetermined date. This can be achieved by spraying the plants with chemical agents which induce flowering and thus control the date of harvest (7). One of the very promising chemicals in this respect is beta-hydroxyethylhydrazine (Omaflora, Olin trade name) (3, 4, 6). The method presented was developed to determine microgram quantities of Omaflora in the presence of pineapple.

Experimental

Apparatus. The apparatus included chromatographic tubes with Teflon stopcocks, Waring Blendor, International chemical centrifuge, and Beckman Model B spectrophotometer.

Reagents. Deionized Water. Distilled water was deionized using Amberlite MB-3 resin.

Dilute HCl. Prepared by mixing 30 ml. of concentrated HCl and 70 ml. of deionized water.

Ion Exchange Resins. Dowex 1-X8 and 50W-X8, 50- to 100-mesh, were used in the investigation. The Dowex 1 resin, obtained in the Cl⁻ form, was converted to the OH⁻ form according to the procedure recommended by the

manufacturer (2). It was found preferable to remove the fines from the resins, and also not to keep the OH⁻ form of Dowex 1 beyond 3 or 4 days.

CINNAMALDEHYDE REAGENT. Prepared by mixing 95 ml. of denatured alcohol (formula 2B), 18.5 ml. of deionized water, and 6 ml. of concentrated HCl in a brown bottle, cooling the mixture to room temperature, and then adding 2 ml. of Eastman Grade trans-cinnamaldehyde (Distillation Products Industries, Rochester 3, N. Y.). The reagent was prepared fresh daily.

BETA - HYDROXYETHYLHYDRAZINE. Olin Mathieson beta-hydroxyethylhydrazine (HEH), refined grade, containing 95% HEH, was used for the investigation (5). Solutions for the standard curve were prepared in dilute HCl, whereas those for the recovery standards were made in deionized water.

Preparation of Standard Curve. About 0.1 gram of HEH was accurately weighed into a 100-ml. volumetric flask, and the solution was made up to volume with dilute HCl. Appropriate aliquots of this solution were diluted with dilute HCl to prepare standards containing 0.1 to 2 μg. of HEH per ml. From each standard, 6-ml. aliquots were pipetted into 50-ml. volumetric flasks. Into each flask, 18 ml. of the cinnamaldehyde reagent were added and allowed to react for 30 minutes. A reagent blank was prepared by mixing 6 ml. of dilute HCl and 18 ml. of the reagent. At the end of the reaction period, the absorbances of the solutions were measured at 420 mμ against the reagent blank in 5-cm. cells. The absorbances were then plotted against the concentrations of the corresponding standards to obtain the standard curve. The slope of the line gave the absorbance due to 1 μg. of HEH in 1 ml. of dilute HCl. Data for preparation of the standard curve are given in Table I.

Extraction, Isolation, and Determination of HEH. The pineapple was cut lengthwise through the center to obtain pieces which contained representative amounts of shell, flesh, and core in relative proportions. About 200 grams of the pieces were weighed and sliced into small fragments. For recovery stand-

ards, a known quantity of HEH was added to the sample. The sample was then blended with 100 ml. of deionized water in a Waring Blendor. The blend was then filtered and washed quantitatively with deionized water, using a chemical centrifuge lined with a strip of Whatman No. 1 filter paper. The filtrate and the washings were collected in a 600-ml. beaker.

About 80 grams of the OH⁻ resin were slurried with deionized water and poured into a 40 × 600-mm. chromatographic tube which contained a plug of glass wool and some water. The resin was allowed to settle and then washed with deionized water until the washings were neutral to "Hydrion" paper.

A second 20 × 400-mm. chromatographic column was prepared similarly, using 20 grams of Dowex 50W. The column was washed with dilute HCl until the washings were visually colorless, and then rinsed with deionized water until the effluent was neutral.

The filtrate and the washings from the centrifuge were passed quantitatively through the OH⁻ resin, and then through the H⁻ resin column. The H⁺ resin column was then washed with deionized water until the washings were neutral and colorless. A positive air pressure of about 10 inches of Hg was applied to the columns whenever necessary. The HEH under these conditions remained on the H⁺ resin. It was then eluted with dilute HCl, and 100 ml. of the eluate were collected in a volumetric flask. The eluate was filtered through a Whatman No. 5 filter paper to remove the cloudiness, and 6 ml. of the filtrate were reacted with 18 ml. of reagent for 30 minutes. The absorbance of the solution was then measured against a reagent blank prepared as described previously. The apparent HEH in the eluate was then calculated using the slope of the standard curve. A pineapple blank was determined by repeating the procedure without adding any HEH to the sample. Consequently, the net recovery of HEH was calculated.

Results and Discussion

Solutions of HEH decompose gradually. Hence, they are prepared fresh daily. Acid solutions are more stable than the aqueous ones.

Cinnamaldehyde and HEH react in acid medium to produce cinnamaldehyde hydrazone which, under the conditions used in the investigation, is stable for at least 2 hours.

C_6H_5CH=$CHCHO$ +

$H_2NNHCH_2CH_2OH$ $\xrightarrow{H^-}$

C_6H_5CH=CH—CH=N—

$NHCH_2CH_2OH$ + H_2O
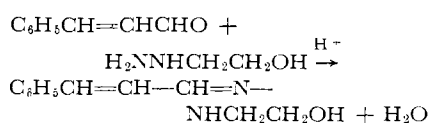
The hydrazone solution has a maximum absorbance at 390 mμ. At this wavelength, the reagent used has a high back-

ground absorbance which is avoided by choosing 420 mμ for the experimental measurements. The standard curve shows that the solutions at 420 mμ obeyed Beer's Law.

The ion exchange resins, when washed after standing for some time, give a yellow effluent. They are washed in the column as described in the text immediately before they are used. Fresh columns are prepared for each run.

Recoveries are reproducible if the duration of analysis is kept consistent. Amounts as low as 0.015 p.p.m. HEH have been added to pineapples, and an average of 79.4% recovery has been obtained (Table II). In such cases, the HCl eluate from the H⁺ column is collected in 25-ml. fractions. The HEH is found in the second fraction. Blanks for such recoveries are run in a corresponding manner.

Table II. Blanks and Recovery Values Obtained with Whole Fresh Pineapples

Blanks, P.P.M. HEH	Recovery HEH Added, p.p.m.	Recovery HEH Found, %
0.025	0.040	78.8
0.030	0.045	75.6
0.025	0.071	79.3
0.015	0.625	79.8
0.020	0.640	78.8
0.015	0.710	76.6
0.025	0.725	76.4
0.025	0.800	75.5
0.015	0.845	79.8
0.015	1.450	78.2
	1.460	77.3

Literature Cited

(1) Collins, J. L., "The Pineapple," pp. 152–4, Interscience, New York, 1960.
(2) "Dowex: Ion Exchange," p. 53, The Dow Chemical Co., Midland, Mich.
(3) Gowing, D. P., Leeper, R. W., Botan. Gaz. 123, 34–43 (1961).
(4) Gowing, D. P., Leeper, R. W., Science 122, 1267 (1955).
(5) Olin Mathieson Industrial Chemicals Division Bulletin on "Mathieson B-Hydroxyethylhydrazine."
(6) Olin Mathieson International Division Agricultural Bulletin, No. 44.

Table I. Data for Standard Curve[a]

Solution	HEH, μg. per Ml.	Absorbance
1	0.140	0.040
2	0.474	0.145
3	0.790	0.240
4	1.320	0.400
5	1.580	0.480
6	2.050	0.620
7	2.370	0.720
8	2.370	0.722

[a] Slope of the line = 0.30.