ISOLATION AND DETERMINATION OF STARCH IN PLANT TISSUE

The removal of starch from plant tissues by dispersion in various reagents has often been employed in the quantitative estimation of this substance (5). Recently Denney (1, 2) and Hopkins (3) have reviewed those methods wherein mineral acids or salt solutions are used as the dispersing agents. We have found that hot dilute ethanol can also be used for this purpose after the plant tissue has been suitably pretreated with an acid-alcohol reagent. In the course of a microchemical study on the reserve carbohydrates of apple wood spurs, it was noticed that after the tissue had been treated with a boiling solution of 1 per cent. nitric acid in 85 per cent. ethanol the starch granules could be dispersed in boiling 20 per cent. ethanol. A procedure employing this neutral and salt free extraction has been developed. Its essential features are presented below:

0.500 to 2.500-gm. samples (depending upon the anticipated starch content) are weighed into 40-cc. alundum crucibles. The latter are placed in a Soxhlet extractor and extracted for 24 hours with a 2:1 benzene-ethanol mixture. The crucibles are then taken from the extractor and the major portion of the solvent removed by suction. The contents of a crucible are transferred to a 300-cc. flask and covered with 180 cc. of the ethanolic-nitric acid reagent prepared by diluting 10.65 cc. of concentrated nitric acid, sp. gr. 1.40, to 1 liter with 85.1 per cent. ethanol by volume. This mixture is then boiled under reflux for 30 minutes. The residue remaining in the flask is now recovered by filtration through the original alundum crucible and washed free of acid with 75–90 cc. of hot 95 per cent. ethanol. The residue is partially dried by suction and again introduced into a 300-cc. flask. 100 cc. of 20 per cent. ethanol are then added and the contents of the flask boiled under reflux for 20–25 minutes. The insoluble matter left in the flask is now removed by filtration and washed with 40–50 cc. of boiling 20 per cent. ethanol. The filtrate and washings are combined. This solution contains the starch polysaccharides present in the original tissue.

If it is desired to isolate the starch polysaccharides the 20 per cent. ethanol extract is concentrated in vacuo to about 25 to 30 cc. This concentrate is poured, with stirring, into 10 volumes of a 1:1 acetone-ethanol mixture, thereby precipitating the polysaccharides which are then recovered by centrifugation and filtration.

For the estimation of the starch content of the original tissue the 20 per cent. ethanol extract is evaporated on a hot plate to a small volume (10 cc.) and 100 cc. of 2.5 per cent. hydrochloric acid added. The solution is then boiled under reflux for 2.5 hours, cooled, neutralized, and made to volume.
Glucose is determined on an aliquot portion and expressed as starch in the usual manner.

It has been observed that with certain types of plant tissue, the 20 per cent. ethanol extract contains in addition to the starch polysaccharides some non-starch polysaccharides. In such a case, a fractionation of the extract by a method such as proposed by Small (4) is recommended before proceeding with either the isolation of the polysaccharide from the extract by precipitation with the acetone-ethanol mixture, or the hydrolysis of the extract to reducing sugars.—Carl Niemann, R. H. Roberts, and Karl Paul Link, Biochemistry Research Laboratory, Department of Agricultural Chemistry and Department of Horticulture, University of Wisconsin, Madison, Wisconsin.

LITERATURE CITED