THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued quarterly by the Agricultural Experiment Station of the University of Puerto Rico, for the publication of articles by members of its personnel, or others, dealing with any of the more technical aspects of scientific agriculture in Puerto Rico or the Caribbean Area.

Vol. XL April 1956 No. 2

Identification of Sugars Present in Fruit of the Acerola (*Malpighia punicifolia* L.) by Paper Chromatography

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INTRODUCTION

Nothing is found in the literature regarding the sugars present in the fruit of the acerola. The identification of these sugars is of great importance for the study of the reactions which take place when the juice is flash-pasteurized and canned.

Paper chromatography is an important tool in analytical chemistry and has been used by many investigators to identify the sugars present in various plant materials. The qualitative paper-chromatography method of William and Bevenue (1)[2] was employed for the work on the acerola.

Before applying it as described below, some preliminary work had been done at this Station to identify the sugars present in the acerola. The work was initiated using the method of Moore and Link (2), but no definite results were obtained.

A phenyl osazone derivate was prepared using the sugar extract of the fruit. This osazone was identical with the one obtained from dextrose, levulose, or mannose. The Seliwanoff (3) and cobaltous chloride (4) tests were positive for levulose. However, the quantitative determination of levulose using the Jackson-Mathews modification of the Nyns selective method (5) indicated that it comprises only about 50 percent of the sugar present in the fruit.

Some work was also done on the determination of reducing and total sugars in acerola juice using the Lane-Eynon method (6). The statistical analysis of the results revealed that, if sucrose is present in the juice, it must be in quantities so small that it cannot be determined by this method.

PROCEDURE

Three hundred grams of acerolas were chopped and quickly added to sufficient 95-percent ethyl alcohol to give, with the water naturally present

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[2] Numbers in parentheses refer to Literature Cited, p. 89.

in the fruit, an approximate alcohol concentration of 80 percent. One to two grams of $CaCO_3$ were added and the mixture was heated for 1 hour on a steam bath. The supernatant liquid was filtered and the residue was covered with 80-percent ethyl alcohol, reheated for 30 minutes on the steam bath, and filtered. The filtrates were then combined.

An aliquot containing 4 gm. of sugars was evaporated on a steam bath to remove the alcohol. The concentrate was filtered through a mat of Celite analytical filter-aid and the filtrate was made to 100 ml. with distilled water.

To remove the substances that interfered with the identification of sugars by paper chromatography, the solution was passed first through a column containing the cation-exchanger Amberlite IR-120 and then through a column containing the anion-exchanger Amberlite IR-4B. The pH of the eluate was 6.8. The deionized solution was then ready to be chromatographed.

A penciled line was drawn parallel to, and about 8 cm. from one end of the paper (Whatman No. 1, 18½ inches x 22 inches). Division marks about 2 cm. apart were made on this line. The other end of the paper was trimmed with pinking shears to give a saw-toothed effect.

Three-percent solutions of galactose, dextrose, mannose, sucrose, sorbose, levulose, arabinose, ribose, fucose, raffinose, xylose, lyxose, maltose, and lactose were prepared to be used as standards.

Applications of known sugar solutions (5 λ) and acerola extracts (10 λ) were made on the division marks (2 cm. apart) on the paper.

Three chromatograms were irrigated with a mixture of 8 parts of ethyl acetate, 2 parts of pyridine, and 1 part of water. After 16 hours the chromatograms were ready to be dried in the hood and sprayed.

The spray reagents used were resorcinol, aniline, and 3,5 dinitro salicylic acid. Resorcinol is specific for the detection of ketoses and polysaccharides which contain ketose sugars. Aniline gives a purple color with pentose and a brown color for the other sugars, and 3,5 dinitro salicylic acid is used for the detection of aldoses, pentoses, and some disaccharides, but does not detect levulose-containing sugars such as raffinose and sucrose.

RESULTS

The spots obtained with the acerola extract corresponded to dextrose, levulose, and sucrose. However, since levulose and sorbose have such very close Rf values in this solvent, the work was repeated using another solvent mixture consisting of 4 parts of butanol, 1 part of acetic acid, and 5 parts of water.

Two phases were formed. After the mixture had been shaken and al-

lowed to stand several hours, the H_2O-rich phase was added to the bottom of the chromatographic cabinet and the alcohol-rich phase was added to the troughs. The spots obtained with this solvent mixture and the same spray reagents mentioned above confirmed the presence of levulose, dextrose, and sucrose in the acerola.

SUMMARY

By means of paper chromatography it proved possible to identify the sugars in the fruit of the acerola. Dextrose, levulose, and sucrose were found to be present.

RESUMEN

Por medio de la cromatografía de papel fué posible identificar los azúcares presentes en la acerola. Dextrosa, levulosa, y sacarosa fueron los azúcares que se encontraron presentes.

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