Determination of Polysaccharides in Sucrose Crystal

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A group of polysaccharides isolated from the raw-sugar crystal was determined by spectrophotometry. The major polysaccharides were found to be glucans. Xylan and araban are present in only small amounts.

THE NONSUCROSE CONSTITUENTS in the raw sugar crystal have been identified as substances occurring naturally and those produced in processing (*2*, *3*).

This paper discusses a method developed for the determination of araban, xylan, and glucans in sucrose crystal. The material containing the polysaccharides was the methanol-insoluble portion isolated from sucrose crystal. The procedure consists of the hydrolysis of polysaccharides and the determination of the simple sugars by paper chromatography.

Table I. Analysis of 85% Methanol-Insoluble Material in Sugar Crystal

Commercial Raw Sugars	Methanol-Insol., %	Araban, %	Xylan, %	Glucans, %	
				Sugar	Methanol-Insol.
S-1	0.21	0.0024	0.0007	0.034	16.0
S-2	0.15	0.0025	0.0011	0.044	29.5
S-3	0.09	0.0013	0.0005	0.005	5.2
S-4	0.20	0.0019	0.0005	0.017	8.3
S-5	0.11	0.0013	0.0005	0.031	28.1

Experimental

Preparation of Materials. Five hundred grams of raw sugar crystal were suspended in 2500 ml. of 85% methanol. The mixture was refluxed until all the crystals were dissolved. The precipitated polysaccharides were separated from the solution by centrifuging, washing with 95% ethanol, acetone, and ether, and finally drying in a vacuum desiccator.

Hydrolysis. Three milliliters of 72% sulfuric acid cooled to 0° C. were slowly added to 0.3 gram of the precipitate. After the precipitate was uniformly dispersed, the mixture was allowed to stand at room temperature for 4 hours with occasional stirring. The mixture was diluted to 40 ml. with water. The undissolved residue was separated by centrifuging. The supernatant solution was refluxed for 5 hours to complete the hydrolysis. The solution was cooled to room temperature and diluted with water to 100 ml. Twenty-five grams of Amberlite IR-45 (OH) ion exchange resin were added, while stirring, to the solution. The mixture was slowly poured into a column, 20 mm. in diameter, containing 30 grams of Amberlite IR-45 (OH). The effluent was collected at a rate of two drops per minute. After the solution had just moved down to the top of the resin column, the column was washed with 100 ml. of water. The combined effluent and washings were concentrated to thin syrup, and the syrup was evaporated almost to dryness.

Chromatographic Separation and Spectrophotometric Measurement. PREPARATION OF SOLUTIONS. To the syrup were added 1.5 ml. of water. The insoluble matter was removed by filtration through a microfilter. The filtrate was used for the determination of pentoses without further dilution. The filtrate was diluted with water to a ratio of 1:10 for the determination of glucose. A standard solution was also prepared containing 2 μg. per μl. each of arabinose, xylose, and glucose.

SPOTTING. Sugar solutions were analyzed by spotting suitable volumes onto a sheet of paper by using an ultramicroburet. Spots of the standard sugar solution were also applied to the paper in varying amounts over a range of 2 to 60 μg.

DEVELOPMENT AND ELUTION. The chromatograms were developed with a solvent mixture of *n*-butanol:pyridine:water (6:4:3 v./v.) for a minimum of 24 hours at room temperature by the descending technique. After air-drying, the chromatograms were sprayed with aniline hydrogen phthalate reagent and oven-heated at 120° C. for 7 minutes. The brown-colored spots developed on the chromatograms were pencil-marked and cut with scissors. For each series of the simple sugars, the areas of the cut pieces were kept uniformly small. The pieces were transferred into rubber-stoppered, 3/4 × 6-inch test tubes, and the colored spots were eluted by shaking for 5 minutes with 3 ml. of anhydrous methanol.

MEASUREMENT. The absorbances of the eluates and of the known sugars from the same chromatograms were measured in a Beckman DK-2 spectrophotometer at 420 mμ. The amount of each sugar from the sample was determined by comparison with the calibration curves of the known sugars.

Results

The results obtained from five commercial sugar samples are shown in Table I.

Discussion

Identification of the polysaccharides has been reported previously (*2*, *3*). The quantitative method of analysis developed here warrants the following conclusions:

Material Prepared and Method Used for Hydrolysis. The polysaccharides are insoluble in 85% methanol and should thus be present in the precipitate. The conditions used for the hydrolysis of the polysaccharides are similar to those used for the hydrolysis of high molecular weight, linear polysaccharides. The complete hydrolysis of the polysaccharides in the sample is therefore ensured.

Chromatographic Separation. Separation of sugars by ordinary paper chro-

matography has these disadvantages: relatively large amounts of sample are required; guiding strips are necessary to locate the bands; and a long time and a large volume of solvent are required for eluting the sugars. The procedure used in this work is simpler and more reliable within the range of 2 to 60 μg. of sugars (*1*).

Spectrophotometric Measurement. The absorbance at 420 mμ used for the determination of sugar was arbitrarily selected. Due to the instability of the color resulting from the interaction of sugar and aniline hydrogen phthalate reagent, the absorbance of the solution was measured within 20 minutes after the color was developed on the chromatogram. The errors resulting from this method are very small since the separation of known and unknown sugars and the development of the color spots are done on the same sheet of paper under the same conditions. The errors which have been reported (*1*) are within ±4%.

The results indicate that the method should be suitable for the routine determination of polysaccharides in commercial raw sugar.

Literature Cited

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SUGAR CRYSTAL ANALYSES

Determination of Starch in Sucrose Crystal

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Starch in the raw sugar crystal was spectrophotometrically determined at the maximum absorption of the starch-iodine complex rather than at 700 mμ which had been used previously.

STARCH in the sugar crystal has been reported (*4*) to be one of the substances related to low filterability. However, Nicholson (*5*) found that the soluble starch had very little, if any, effect. To study further the relationship between cane starch content and filterability, a reliable method of analysis had to be developed.

There are several methods for the determination of starch, but some of them are not suitable for sugar samples. A spectrophotometric method for the determination of starch in paper, by Browning *et al.* (*2*) and modified by Harvey *et al.* (*3*), was applied to sugar crystals and was found to be satisfactory. By this method, the absorbance of the starch-iodine complex is measured at the peak rather than at 700 mμ as was done by Balch (*1*) and Nicholson (*6*). Measurement at the absorption maximum is believed to be more accurate than at an inclining point of the curve.

Experimental

Preparation of Calibration Curve. Since cane starch was unavailable, potato starch was used for the calibration curve. The procedure used was that of Harvey *et al.* (*3*). The dilutions are shown in Table I.

The absorption maximum (580 mμ) of the starch-iodine complex was measured on a Beckman DK-2 spectrophotometer.

Preparation of Sample. One hundred grams of sugar crystals were dissolved in 100 ml. of water; 240 ml. of 95% ethanol and 2 ml. of a saturated potassium chloride solution were added; and the solution was shaken vigorously to aid precipitation (*6*). After standing overnight, the mixture was centrifuged and the supernatant solution discarded. The precipitate was washed once with 70% ethanol, centrifuged, and the supernatant solution again discarded.

Twenty milliliters of water were added to the precipitate and heated on a water bath. The solution was filtered under vacuum. The residue was washed once with 10 ml. of hot water and then treated twice with 5-ml. portions of 1:1 hydrochloric acid and once with concentrated hydrochloric acid. This was followed by a final wash with about 40 ml. of hot water.

The solution was transferred to a 100-ml. volumetric flask, cooled, and diluted to the mark. After thorough mixing, about 50 ml. were centrifuged for 10 minutes.

Twenty-five milliliters of the clear supernatant solution were pipetted into a 50-ml. volumetric flask, 2.5 ml. of iodine solution (7.5 grams of potassium iodide + 5 grams of iodine per liter) were added, and the solution was diluted to the mark. The absorption maximum was measured against a reference solution (25 ml. of 1:9 hydrochloric acid + 2.5 ml. of iodine solution, diluted to 50 ml.).

The absorbance of the starch-iodine complex was compared with the calibration curve to obtain the starch content.

Table I. Dilutions[a] for Preparing Calibration Curve

Starch, Mg./Liter	Stock, Ml.	Water, Ml.	Iodine Solution, Ml.
10	5	0	5
20	10	5	5
30	15	10	5
40	20	15	5
50	25	20	5
60	30	25	5
80	40	35	5
100	50	45	5

[a] Dilute to mark in a 100-ml. volumetric flask with 1:9 hydrochloric acid.

Table II. Analysis of Sugar Crystal

Sample	Starch, P.P.M.	Sample	Starch, P.P.M.
S-1	78	S-11	20
S-2	133	S-12	30
S-3	29	S-13	69
S-4	50	S-14	113
S-5	170	S-15	141
S-6	32	S-16	90
S-7	31	S-17	65
S-8	74	S-18	83
S-9	18	S-19	90
S-10	30	S-20	98

Results

Calibration Curve. The absorption curves of the starch-iodine complex are shown in Figures 1 and 2. These curves were obtained by scanning the samples through the visible region of the spectrum. The absorption maxima at 580 mμ of the potato starch shown in