

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 (7).

Spectrophotometric Measurement. The absorbance at 420 $m\mu$ 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 (7) are within $\pm 4\%$.

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

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SUGAR CRYSTAL ANALYSES

Determination of Starch in Sucrose Crystal

Starch in the raw sugar crystal was spectrophotometrically determined at the maximum absorption of the starch-iodine complex rather than at 700 $m\mu$ 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 $m\mu$ as was done by Balch (7) 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 $m\mu$) 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.

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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 $m\mu$ of the potato starch shown in

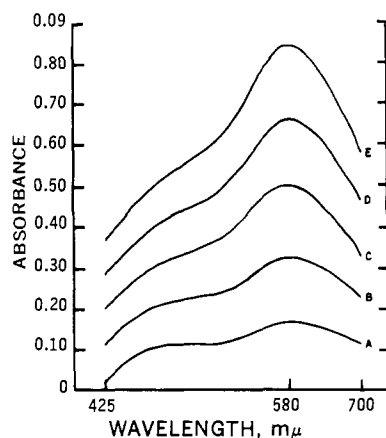


Figure 1. Absorption curves for starch-iodine complex of potato starch

(A) 10 mg./liter, (B) 20 mg./liter, (C) 30 mg./liter, (D) 40 mg./liter, (E) 50 mg./liter

Figure 1 were plotted against concentration to prepare the calibration curve.

Analysis of Sugar Crystal. The absorption maxima of the starch-iodine complex from sugar samples from several plantations were compared with and calculated from the standard curve to obtain the starch contents shown in Table II.

Discussion

A spectrophotometric method for the determination of starch in the sugar crystal has been developed. Sugar crystal samples from 20 plantations were analyzed by this method, and the starch content was found to range from 20 to 170 p.p.m. The location of the absorption maximum differed slightly with individual samples, as shown in Figure 3. This could be caused by slight differences in the cane starch from different varieties or growth locations or by other material that may be present in the solutions. Harvey *et al.* (3) showed differences also

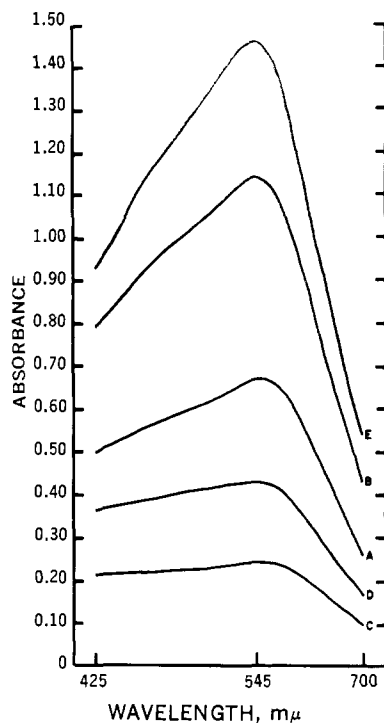


Figure 2. Absorption curves for starch-iodine complex of cane starch from sugar crystal

(A) S-1, (B) S-2, (C) S-3, (D) S-4, (E) S-5

in the peaks of corn starches. This was explained as being due to the different varieties and modifications of starch varying in their ratio of amylose to amylopectin and having a definite effect on the absorption curve.

It has been reported (3) that Beer's Law is obeyed to a concentration of 100 mg. per liter, but it was found that there is a very slight deviation from linearity as the upper limit is approached. However, this should cause no problem as the amount of sugar used can be varied to remain in the lower concentrations.

The results of analyses of sugars in Table II can be used to determine the correlation between starch content and

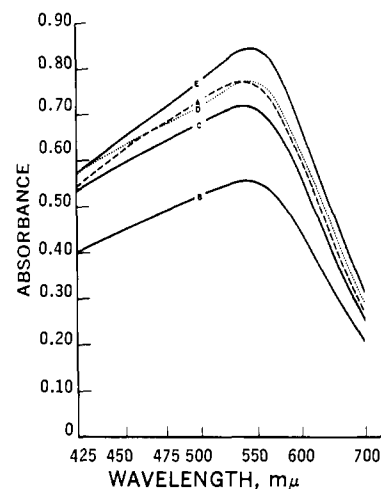


Figure 3. Absorption curves showing maxima at different wavelengths for starch-iodine complex of cane starch from sugar crystal

(A) S-16, (B) S-17, (C) S-18, (D) S-19, (E) S-20

filtration rate. This will be discussed in other papers to be published elsewhere.

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SUGAR CRYSTAL ANALYSES

Ion-Exchange Separation and Determination of Cations in Sucrose Crystal

THE EFFECT of inorganic constituents on the crystallization rate of sucrose has been investigated (3). It has also been suggested that the inorganic substances might be preferentially included by the growing sucrose crystal (5), and evidence has been obtained showing a linear relationship between color and ash of refined sugars (7).

The significance of these findings might be shown by the interrelation between inorganic constituents, rate of crystallization, inclusion, and crystal color. A systematic analytical scheme for the quantitative determination of cations in the sugar crystal was thus desirable as part of an over-all program in the study of nonsucrose constituents.

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Experimental

Separation of Known Cations. One-half milliequivalent of each uni- and bivalent chloride and 1 mg. of each trivalent chloride in 3 ml. of 0.1N hydrochloric acid solution containing one drop of 0.5N nitric acid were introduced into a 7.7 × 115 mm. column of Dowex 50W, X-12, 200- to 400-mesh