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
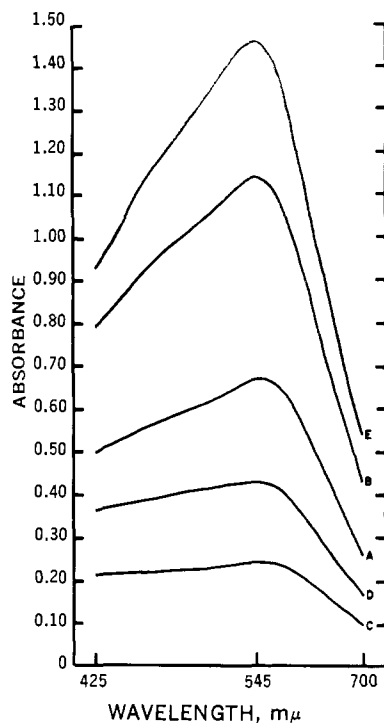

Figure 2. Absorption curves for starch-iodine complex of cane starch from sugar crystal

(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
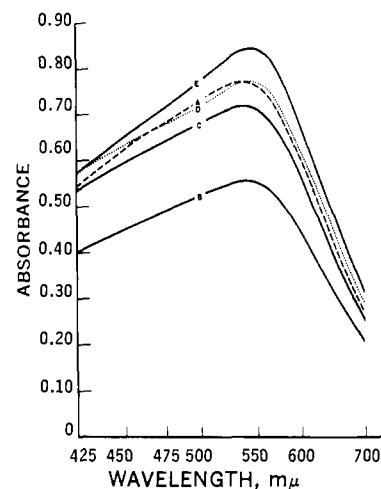

Figure 3. Absorption curves showing maxima at different wavelengths for starch-iodine complex of cane starch from sugar crystal

(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

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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 (1).

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.

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

Sodium, potassium, magnesium, calcium, aluminum, and iron present in the sugar crystal were separated on a Dowex 50W, X-12, (H) form ion-exchange column. The uni- and bivalent cations were determined by Mohr titration. The trivalent cations were determined colorimetrically with aluminon. The procedure was applied to five samples of raw sugar crystals.

Table I. Recoveries of Cations from Column

Cation	Added	Recovered	Recovery, %
Na	0.507 meq.	0.502 meq.	99
K	0.501 meq.	0.506 meq.	101
Mg	0.500 meq.	0.473 meq.	95
Ca	0.501 meq.	0.503 meq.	100
Fe	1.17 mg.	1.15 mg.	98
Al	0.97 mg.	1.00 mg.	103

Table II. Analyses of Raw Sugar Crystals

Raw Sugar from Factory	Cation, P.P.M.					
	Na	K	Mg	Ca	Fe	Al
A	19	125	34	207	12	7
B	156	296	53	49	43	84
C	8	61	7	148	3	3
D	21	73	50	89	10	8
E	8	164	11	7	9	3

(H) form ion-exchange resin. Elution with 0.1, 0.5, and 1.5N hydrochloric acid was carried out at a flow rate of 0.71 to 0.75 ml. per minute. Fractions were collected at the intervals indicated.

0.1N HYDROCHLORIC ACID   INTERVAL
     0–6 hours   0.5 hour
     6–21 hours   1.0 hour

0.5N HYDROCHLORIC ACID
     21–28 hours   1.0 hour
     28–43 hours   2.0 hours

1.5N HYDROCHLORIC ACID
     43–48 hours   0.25 hour

Figure 1. Elution curves for cations

Each fraction was evaporated to dryness on a water bath and oven-heated for 1 hour at 120° C.

Determination of Uni- and Bivalent Cations. Ten milliliters of water were added to dissolve the salts. One milliliter of 0.1N potassium dichromate was added. Titration was carried out by using a 10-ml. microburet and 0.05N silver nitrate. The end point was noted by the formation of red precipitates. From the milliequivalents of chlorides present, the amount of each cation was calculated.

Determination of Trivalent Cations. The iron and aluminum residues were dissolved in 50 ml. of 3N hydrochloric acid. Two and one-half milliliters of this solution were treated as follows: 40 ml. of water were added, then 5 ml. of 20% ammonium acetate. The pH was adjusted to 4.5 by the dropwise addition of 3N hydrochloric acid or 3N ammonium hydroxide. After 10 minutes, the absorbance was measured on a Beckman DK-2 spectrophotometer at 550 mμ for iron and 525 mμ for aluminum. The amount of cation was found by reference to absorbances of standard solutions: for iron, ferric ammonium sulfate . 24 H_2O (2.20 mg./1.0 absorbance); for aluminum, aluminum

potassium sulfate . 24 H_2O (0.08 mg./1.0 absorbance).

Recoveries of Known Cations. The elution curve for the six known cations is shown in Figure 1. Based on this curve, the following intervals were selected for the separation of the cations:

ELUTE WITH 0.1N HYDROCHLORIC ACID

     0–0.5 hour   Discard
   0.5–5 hours   Sodium
     5–21 hours   Potassium

ELUTE WITH 0.5N HYDROCHLORIC ACID

     21–28 hours   Magnesium
     28–43 hours   Calcium

ELUTE WITH 1.5N HYDROCHLORIC ACID

43–44 hours   Iron
44–45.25 hours   Fractions collected at 0.25-hour intervals
45.25–48 hours   Aluminum

The period between the end of iron and the beginning of aluminum is somewhat short. Additional fractions were therefore collected. Evaporation of the fractions containing iron showed a dark brown residue which was readily noticeable. The iron fractions were combined for a single determination. The remaining fractions were combined for a single aluminum determination.
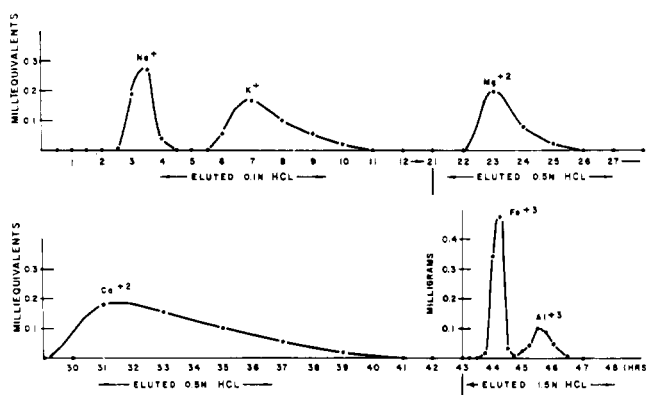
Cations in Raw Sugar Crystals. Fifty grams of raw sugar crystals washed free from molasses with a saturated solution of refined granulated sugar were ignited at 420° C. for 16 hours. Twenty milliliters of 6N hydrochloric acid were added. The solution was evaporated to dryness on a water bath, and the residue was oven-heated at 140° C. for 2 hours. The residue was dissolved by moistening with 1 ml. of 6N hydrochloric acid and diluting to 50 ml. with water. The solution was filtered and the filtrate evaporated to dryness. Two drops of 6N hydrochloric acid, 1 drop of 0.5N nitric acid, and 5 ml. of water were added to dissolve the salts. The solution was passed into the column. Elution was carried out as previously indicated, and the amounts of cations were determined.

Results

The recoveries of the known cations are shown in Table I. The results of analyses of five samples of raw sugar crystals are shown in Table II. The quantitative determination of SiO_2 will be reported at a later date.

Discussion

The separation of sodium, potassium, magnesium, and calcium on an Amberlite (H) column has been reported by Obara and Suzuki (4). The separation of iron and aluminum from each other, however, was not reported.

By employing essentially the same technique with Dowex 50W, X-12, 200- to 400-mesh, (H) form ion-exchange resins, the separation of the uni- and bivalent cations was found to be complete. The separation of iron and aluminum was largely complete. The unresolved portions, if any, were small and did not alter the absorbances by a significant amount.

The analytical scheme of Obara and Suzuki employs titration of the effluent acid from the column to determine sodium and potassium, complexometric titration with EDTA for magnesium and calcium, and two different colori-metric schemes for iron and aluminum.

To simplify the analyses, it was desirable to reduce the number of procedures involved. Sutton and Almy (6) used an indirect chloride titration to determine magnesium and calcium after removal of the uncombined chlorides by evaporation. This method was found to be applicable for the determination of sodium, potassium, magnesium, and calcium.

Molot and Kul'berg (2) reported the use of aluminon for the simultaneous determination of iron and aluminum. Iron was determined separately by the potassium thiocyanate method. It was found, however, that the suggested wave length of 530 mμ did not correspond to the absorbance maximum of either iron (550 mμ) or aluminum (525 mμ). By combining and modifying the procedures of Molot and Kul'berg (2), Obara and Suzuki (4), and Sutton and Almy (6), a simplified analytical scheme for all six cations was obtained.

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GRAPE JUICE FLAVOR

Determination of Methyl Anthranilate in Grape Juice by Electron Affinity—Gas Chromatography

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A quick, accurate determination for methyl anthranilate in grape juice is presented. The method uses an extraction of the methyl anthranilate with benzene, separation by gas chromatography, and detection with an electron affinity detector. Recoveries of methyl anthranilate from grape juice averaged 98 ± 1.7%. A concentration of less than 0.1 p.p.m. methyl anthranilate can be determined. Comparison of the diazotization and gas chromatographic methods showed that the results of the diazotization method may be expected to be high relative to the gas chromatographic method by an amount between 0.04 and 0.11 p.p.m.

METHYL ANTHRANILATE has been considered a major flavoring constituent of Concord grape juice and is the basis for evaluating grape juice products. The presence and concentration of this compound is of importance to the processors of Concord juice and the plant breeders concerned with Concord-type grapes. The present method for determining methyl anthranilate in grape juice involves steam distillation, diazotization, and coupling with α-naphthol-2-sulfonate as described by A.O.A.C. (1) and the modification of Shaulis and Robinson (7). This method appears to be satisfactory for higher concentrations of methyl anthranilate, but is not particularly sensitive at lower concentrations. The method is empirical in the time requirements for the addition of reagents, color development, and collected volume.

Lovelock and Lipsky (5) recently described a gas chromatographic detector which is exceptionally sensitive to certain classes of compounds. This device has been designated as the election affinity detector. Lovelock (4) employed this method of detection for the determination of tetraethyl lead in gasoline, while Goodwin et al. (3), Clark (2), and Mattick et al. (6) applied this technique to the determination of residues of chlorinated hydrocarbons employed as pesticides. Methyl anthranilate showed a good response to this type of detection.

Materials and Methods

Gas Chromatographic System. A Barber-Colman Model 10 gas chromatographic instrument was employed in the analysis. The electrometer circuit

Table I. Recovery of Methyl Anthranilate from Grape Juice

P.P.M. Added	P.P.M. Recovered	Corrected P.P.M.	Per Cent Recovery
5.0	5.0	4.9	98
1.0	1.06	0.96	96
0.5	0.60	0.50	100
0.1	0.203	0.099	99
0.0	0.104	0.00	...

was modified by the addition of a 9 × 10¹⁰ ohm "Victoreen" (type RX-1, Hi Meg) resistor. This resistor was placed in the circuit to change the sensitivity of the electrometer from 10^{-9} to 3 × 10^{-10} amp. full scale. The normal high voltage power supply was disconnected from the cell and replaced by a 67.5-