Organic Acids in Sugar Beet Diffusion Juices[1]

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Research and Marketing Act funds have been allotted to the Western Regional Research Laboratory for work on the technology of sugar beets. Liaison between the Laboratory and industry has been established through the Committee on processing problems, appointed by the industry. This committe has met at the Laboratory to discuss subjects meriting investigation and has proposed four problems: (1) *study of the composition of sugar beets;* (2) *study of the chemistry of betaine and the synthesis of derivatives ivhich might have industrial possibilities;* (3) *determination of the changes in composition of processing liquors;* and (4) *investigation of the causes and amelioration of scaling.*

This paper will report results thus far obtained during the current campaign on the composition of diffusion liquors. It can be considered only preliminary. The object of this work is two-fold: to discover substances which might have by-product value and to improve technology of sugar-beet processing through application of knowledge of composition.

Materials and Methods

Diffusion juices investigated were obtained early in the campaign from Manteca, California; Centerfield, Utah; Rupert, Idaho, and Sidney, Montana. Preliminary work was done on diffusion juice prepared at the Laboratory from about two tons of beets procured from Alvarado, California. Precautions, such as concentrating and/or adding toluene, were taken to preserve the samples. On receipt they were placed in storage at 3° C.

Amino acids have been identified chromatographically by the method of Consden, Gordon, and Martin (1)[3], in which phenol-water is the developing solvent and ninhydrin the indicator. Estimation of the concentration of each of the amino acids has been undertaken by use of selected strains of micro-organisms. Quantitative determinations of chloride, sulfate, citrate, and phosphate by published methods are under way. Ion exchange resins used in this study were A293M and A300, American Cyanamid Co.; A4, A5 and A6, Chemical Process Co.; D735 and S, Permutit Corp.; IR4B and IRA400, Resinous Products Corp.; and Dowex 50, Dow Chemical Co.

It became apparent that complete analysis of many samples of diffusion juice would be impossible if conventional methods were applied; however, separation of acids on ion exchange resins, followed by fractional elution and titration of the fractions, offered a possibility of yielding the desired results. A number of resins were examined with respect to capacity and length of time required to establish equilibrium. One-gram samples of resin were equilibrated overnight with 25 ml. of 1N solutions of oxalic,

[1] Report of a study made under the Research and Marketing Act of 1946.
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[3] Numbers in parentheses refer to literature cited.

citric, or succinic acid. The supernatant liquid was titrated with standard alkali. Table 1 shows that, of the resins tested, D735 and S had high exchange capacity for both oxalic and succinic acids; the former is the strongest and the latter the weakest organic acid found in sugar beets. When resin S was in the base form, carbon dioxide formation was encountered which caused disruption of boundaries and mixing of the components of the column. Resin S was used without such difficulty in the salt form.

Table I.—Capacity of some anion exchange resins for oxalic, citric and succinic acids.

Resin	Amount of acid absorbed (m.e./g.) Oxalic	Citric	Succinic
A293M	11.0	9.5	7.5
A300	14.7	15.2	10.4
A4	11.2	8.6	6.9
A5	12.4	5.9	7.4
A6	11.3	4.0	6.1
D735	15.2	15.3	11.3
S	16.0	15.9	11.2
IRA400	5.4	5.3	5.1

Equilibrium was 98 to 99% complete within 7.5 min. when *0.2N* oxalic acid was added to any of the resins. Other tests with S and D-735 showed that adsorption of O.IA^ hydrochloric acid is sufficiently slow so that flow rates should be about 0.1 ml./cm.Vmin. with resin ground to pass a 40-mesh and to be retained on a 60-mesh screen.

The columns of resin used for this work have been 1.6 to 1.8 cm. in diameter and 30-50 cm. in;. length. The resins were ground to either 60-80 or 80-100 mesh. With the resin in the base form, sufficient acid from the diffusion juice was added to change the color of the resin for the first two cm. of depth. Initial flow rates for adsorption of the acids and subsequent elution with sulfuric acid at pH 1.5 were between 0.1 and 1 ml./cm.Vmin. This concentration of eluting acid was chosen to provide as great as possible spread between percentages of unionized acids likely to be present as determined from calculated curves of pH vs. percent ionization. The size of the fraction was between 3 and 6 ml., depending upon the flow rate.

In an attempt to achieve better separations of the acids, use was made of resin S in the chloride form washed to pH 3 with distilled water. Dilute sulfuric acid (pH 1.2 or 1.7) was the eluting medium. Flow rates were 0.1 — 0.2 ml/$cm.^2$/min. and resin size was 80-100 mesh.

Results

The composition of European-grown sugar beets has been studied extensively but quantitative information is relatively incomplete. Considerable doubt still exists whether some of the compounds reported present in beets are the result of microbial action or of processing conditions (2) . Published knowledge of the composition of sugar beets grown in the United States is less complete. To obtain information on the magnitude of the problem, the number and nature of compounds present in a diffusion juice prepared from Alvarado, California, beets was investigated. Exploratory work was done with papergrams which indicated the presence of several amino acids.

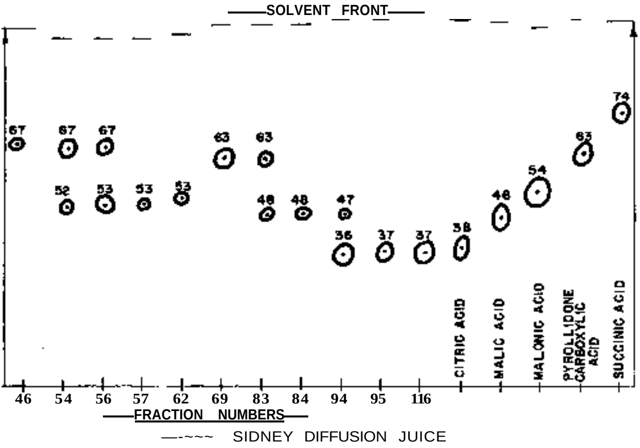

Figure 1. Papergram of certain acid fractions from Sidney, Montana, diffusion juice. Developing solvent t. butyl alcohol 1 part, benzyl 3 parts, isopropyl alcohol 1 part, water 1 part, and 1% formic acid, indicator brom cresol green. Resin in chloride form, eluting rate 0.15 ml./cm.Vmin. Numbers above the spots refer to the distance moved by the organic acid relative to the movement of the solvent.

These were identified by their growth-promoting action on selected strains of bacteria and are glycine, oc-alanine, leucine, isoleucine, valine, glutamic acid, aspartic acid, and threonine. Tyrosine and phenylalanine were present in trace quantities. The latest review available on composition (2) lists only lucine, isoleucine, aspartic acid and glutamic acid as present in beet juice.

Other organic acids have been separated by chemical means and have been identified as citric, oxalic, lactic, succinic, malic, and mucic acids. In addition the presence of glycolic, glutaric and pyrrolidone carboxylic acids is indicated from chromatograms.

Information will be published separately on a tenth acid, which has proved to be a dimer of pyrrolidone carboxylic acid through a methylene bridge on the amide groups. This acid is probably an artifact developed because of use of formaldehyde as an antiseptic during diffusion, followed by concentrating the juice to 80% refractometric dry solids. It is the only acid with the exception of mucic acid, so far isolated in the course of this work which has not been mentioned in the literature on sugar beets.

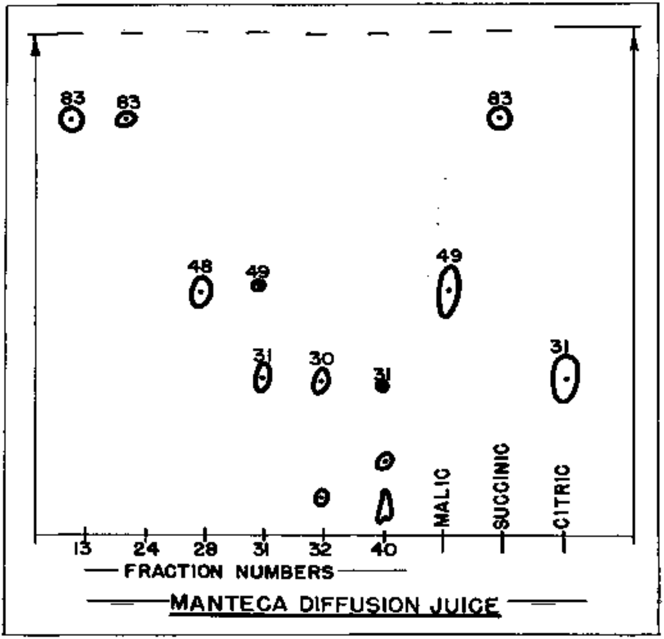

Figure 2. Papergram of certain acid fractions from Manteca, Calif., diffusion juice. Developer: chloroform 1 part, 95% ethanol 1 part, formic acid 1%. Resin in base form, flow rate 1 ml./cm.Vmin.

The effectiveness of fractionations with ion exchange resins is best illustrated in Figures 1 and 2. Lactic acid, moving to spots labeled 67 and 83 in Figures 1 and 2 respectively, is separated from citric acid. Although not shown in the figures, citric acid is separated from oxalic acid. Overlapping occurs when malic or pyrrolidone carboxylic acid is present. Separation of these acids is somewhat improved by means of resin in the chloride form. The diffusion juices contain malic, citric and oxalic acids; also, except for the juice from Manteca, there is some pyrrolidone carboxylic acid. The latter might be an artifact because of its lack of appearance in the unconcentrated diffusion juice from Manteca. Glycolic acid is apparently present in the Sidney juice. Lactic acid is present in all the diffusion juices examined. These results indicate that of the 15 non-amino acids reported present in beets (2) most are present in concentrations less than 0.05%.

Tables 2 and 3 summarize quantitative data on all the acids.

Table 2.—Concentration o£ some amino acids in sugar beet diffusion liquors.

	Manteca	Rupert	Sidney	Centerfield
		mgm/1 at 10% refractometeric dry solids		
α—Alanine	(56)[1]	present	present	20[2]
Aspartic Acid	150	79	109	63
Glutamic Acid	182	82	62	(224)
Isoleucine	73	24	28	present
Leucine	71	20	28	(13)
Threonine	52	14	19	(18)
Valine	59	13	32	(11)
Van Slyke N	310	84	67	105

[1] Figures in parentheses are single microbiological determinations. Others are an average of two determinations.
[2] Estimated from papergrams, using known amounts of alanine in the standard run on the same paper.

Values for lactic and malic acids were obtained from fractionations and might be too high because of the possible presence of other unidentified acids. For example, adipic and glutaric acids have been reported (2) and they would be probably in the same fractions as lactic acid.

Table 3.—Concentration of organic acids other than amino acids in sugar beet diffusion

	Location of sugar beet factory			
	Manteca Calif.	Rupert Idaho	Sidney Montana	Centerfield Utah
Acid anion	(mg./1 at 10% rfractometric dry solids)			
Chloride	<60	39	82	329
Sulfate	120	105	158	171
Phosphate	402	307	475	278
Oxalate	770	<31	32	92
Citrate	510	505	655	700
Lactate	1960[1]	present	70[1]	1730[1]
Glutarate	present	.....	present	..
Malate	480[1]	194[1]	312[1]	present
Glycolate	present	......	49[1]	present
Pyrrolidone Carboxylate	....	880[1]	420[1]	present
Total acid (meq/l)	73.3	39.2	63.9	60.5
Known acid (meq/l)	71.9	31.4	41.3	54.3

[1] Values obtained from titration of fractions from anion exchange columns.

To establish a limit to this work, we applied VanHook's (3) equation relating rate of crystallization of sucrose to concentration of impurities. A relatively high value of 2 was assumed for the constant *i* and a 25% decrease in rate of crystallization of sucrose in molasses was set as the minimum effect to be considered. Resulting calculations gave a concentration limit for substances in the original diffusion liquor near 0.005%. Substances below that limit will not be investigated unless they possess promising biochemical properties or are present to a greater extent in one of the juices under examination.

Discussion

Examination of the results indicates that no compound found has extraordinary value as a by-product. The production of some organic acids in factories using ion exchange treatment might warrant further study.

Discussion of the application of these results to the technology of sugar beets will be reserved for a later paper. It is apparent that of the compounds investigated the presently applied method of defecation could remove only oxalate and some citrate, phosphate and sulfate. Practically all of the other compounds investigated in the course of this work would go through the processing procedure with little or no change. There might be other methods, in addition to ion exchange, which would remove more of the non-sucrose impurities from diffusion juice.

Considerable variation in the composition of sugar-beet juices from different growing areas is apparent. Variations in inorganic constituents probably reflect differences in soil composition, fertilizer practices, hardness of water used in diffusers, variety, and possibly climate. These variations might influence the organic constituents, because biochemists in recent years have found that some inorganic ions are essential for the functioning of many enzymatic systems while others act as poisons. Thus, magnesium ion is an activator in the phosphorylation of glucose necessary for the synthesis of starch in many plants, while fluoride acts as a poison in the carbohydrate metabolism of some forms of life, preventing oxidation of glucose. If the inorganic constituents change sufficiently to influence enzyme systems in the plant, then the concentration of some of the organic constituents is likely to vary. A significant result of this analytical work might be provision of a clue to improvement of fertilization practices which would eliminate certain non-sucrose impurities or increase the amount of desirable by-products.

Summary

Preliminary analyses for acids in sugar-beet diffusion juices from four growing areas have been presented. Variation in concentration of all the organic compounds investigated, except citric acid, is marked and will in-influence attemps at recovery of by-products.

Methods of fractionating acids on ion exchange resins in the base or chloride form have been described.

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