A Method for the Determination of Organic Acids in Sugarbeets and Factory Juices by Gas Liquid Chromatography

R. M. SEQUEIRA[1]

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Introduction

In recent years, a great deal of attention has been focused on the nonsucrose constituents of beets and factory liquors with the expectation that a thorough understanding of the interrelationships between individual components would aid not only in the improvement of beet quality but also in the solution of troublesome processing problems. Of the organic nonsucrose constituents found in beet sugar operations, those containing nitrogen comprise the largest fraction (Table 1). Previous work by Spreckels research has sought to establish appropriate correlations between these known nitrogen-containing impurities and factory operations (3)[2]. Besides the nitrogen-containing fraction of beets and liquors, another important organic nonsugar fraction consists of nonnitrogenous organic acids. Not only are these acids of interest as components of beets, but also their formation through sucrose or juice decomposition during processing is of great importance. In order to study these acids, an analytical procedure based on ion exchange and gas chromatographic techniques has been developed for the quantitative determination of the commonly occurring organic acids found in beets and juices. This procedure is described herein.

Method

Reagents. Rohm & Haas Co. Amberlite IR-120 cation exchange resin, 20-50 mesh is converted to the hydrogen form. A 1-pound batch of resin (supplied in the sodium form) is loaded into a 55 mm diameter chromatography column and washed successively

Table 1.—Nonsucrose Components of Sugarbeets (4)*.

Component	% of Nonsugar fraction
Inorganics	22
Organics	
Nitrogenous:	
Betaine	8
Amino acids	8
Proteins	26
Organic acids	19
Carbohydrates	8
Others	9

*Derived from data given in Reference 4.

[1] Research Associate, Spreckels Sugar Company, Woodland, Calif.

[2] Numbers in parentheses refer to literature cited.

with 2 liters of 6N hydrochloric acid and sufficient distilled or deionized water until the pH of the effluent is the same as that of the wash water (pH 5-6). The resin is stored in a moist condition in a closed container. When required, the necessary volume of resin is measured into a graduated cylinder.

Dowex 1X2 resin, 50-100 mesh (supplied in the chloride form), is converted to the formate form in 1 lb batches in a 55 mm chromatography column by first washing it with 2N sodium hydroxide until the effluent is free of chloride ion. (Test by acidifying a few drops of the effluent with concentrated nitric acid and adding a drop of 1% silver nitrate solution. Absence of a white silver chloride precipitate indicates that the conversion is complete.) The column is then washed successively with 2 liters of water, followed by sufficient 3N formic acid to convert the resin to the formate form (pH of effluent <2), and finally washed with water to a pH of at least 5. The resin is stored moist in a closed container.

Diazomethane is prepared from N-methyl-N-nitroso-p-toluenesulfonamide ("Diazald," available from Aldrich Chemical Co.) by reaction with an alcoholic solution of potassium hydroxide (1). The final ethereal distillate of diazomethane is stored in a rubber stoppered flask in a freezer.

Myristic acid used as an internal standard is 99.5% pure and is obtained from K&K Laboratories, Plainview, N. Y. A solution of myristic acid containing about 5.000 mg/ml is prepared in methanol, and 1 ml of this solution is used in each sample as an internal standard. Other chemicals are of analyzed reagent grade purity and are used without further purification.

Sample preparation. Beets or cossettes (approx. 100 g) are extracted with 200 ml of hot water in a Waring Blendor and filtered through Pyrex wool or coarse filter paper. The resulting cloudy solution contains a considerable amount of suspended solids and is further clarified by centrifugation or filtration as necessary. Substitution of up to about 50% of the water used in the extraction with ethanol is sometimes useful in aiding the clarification. Dissolved solids and sugar contents of the final extracts are determined and about 100 g (carefully weighed) of the extract is taken for the analytical sample.

Other juices and molasses samples are made up to about 5° Brix directly with distilled water, filtered if there are any suspended solids, and 100 g samples are taken as before.

Isolation of Organic Acids. The sample is stirred with 15 ml of IR-120 cation resin in the hydrogen form for a minimum of 15 minutes at room temperature and is then decanted from the resin. The resin is washed several times with distilled water and the

washings added to the sample. The sample is neutralized to pH 6-7 by addition of a few pellets of potassium hydroxide. It is then passed through a 12 x 150 mm column of Dowex 1X2 anion exchange resin (formate form) at a rate of about 5 ml/min. The anion column is washed with 50-75 ml of distilled water, and the eluant and washings are discarded. The acids are eluted from the column with 100 ml of 3N formic acid and the eluant is collected in a 250 ml flask. The eluant is evaporated by rotary evaporation under aspirator vacuum at 50-60°C. Chloroform (15 ml) is added to the moist residue and is evaporated on a steam bath in the hood.

Derivatization. Exactly 1 ml of the standard myristic acid solution is added to the sample. Ethereal diazomethane is added dropwise until the yellow color persists. The mixture is allowed to stand for a few minutes and then excess ether and diazomethane are removed by rotary evaporation at room temperature. The sample is transferred to a small capped vial for storage and is ready for analysis by gas chromatography. Strict quantitative transfer is not necessary due to the presence of the internal standard. Note that diazomethane is exceedingly reactive and toxic. All operations involved with its preparation and use should be carried out in a well ventilated hood, preferably behind a suitable shield.

Gas Chromatography. An Aerograph Model 1520B gas chromatograph equipped with dual flame ionization detectors, Disc integrator, and Pyrex glass inserts in the injection ports is used. Approximately 5 μl of sample is injected followed by an immediate injection of 5 μl of water to reduce methanol tailing. Relevant parameters are:

Injector temperature: 220°C
Detector temperaure: 240°C
Detector flow rates: Hydrogen and air at 25 ml/min
Program: 70-190°C at 4°/min
Carrier Gas: Prepurified N_2 at 25 ml/min
Columns: Dual 1/8" x 6 ft 5% ethylene glycol succinate (EGS) on 80/100 mesh Chromosorb W, acid washed and silanized.

Calculations. The total amount of each acid in the sample is calculated from the following formula:

$$W_a = \frac{A_a/A_s}{K/W_s}$$

where A_a = area of peak for acid a
A_s = area of peak for internal standard
W_a = weight of acid a in sample
W_s = weight of internal standard added
K = relative weight response factor.

The response factor, K, is determined by derivatizing known weights of individual acids and myristic acid, followed by injection on the gas chromatograph. The areas are measured, and the response factor is calculated from the following formula.

$K = \frac{A_a/A_s}{W_a/W_s}$ where the symbols have the same relevance as before.

Discussion

Of the organic acids present in beets and processing streams, those of interest here are: lactic, glycolic, succinic, malic, aconitic, tartaric, citric, and 2-pyrrolidone-5-carboxylic (PCA). Although PCA is a nitrogen-containing acid, it is determinable by the procedure described and has, therefore, been included.

The cation exchange step removes not only the metal ions from solution, but also amino acids, proteins, and other interfering materials. Since these remain on the resin and can be removed later with an appropriate solvent, a subsequent determination of the amino acid composition is also possible.

The anion exchange step separates the remaining organic acids of interest from the sucrose and other neutral, water-soluble materials which are present. The acids remain on the column while the other neutral components are washed off with water. In practice, some colored matter is also retained by the column and is partially removed with the acids when they are eluted with 3N formic acid. This has not been found to interfere with the analysis.

Concentration of the final eluant should be as rapid as possible. Temperatures in excess of 60°C during evaporation can lead to large losses of lactic acid. Lyophilization of the eluant was examined in lieu of rotary evaporation but was found to be relatively lengthy and resulted in loss of lactic acid from the sample. Chloroform evaporation of the formic acid concentrate serves to remove the last traces of formic acid from the sample *via* an azeotrope (5).

In the derivatization step the use of diazomethane offers clear cut advantages over other methods. It reacts quantitatively and practically instantaneously with organic acids to form the corresponding methyl esters which have sufficient volatility for analysis by glc (2). The reaction is clean, yielding only nitrogen as a byproduct, and the reagent is unaffected by the presence of water or alcohol. Furthermore, ether, which is used as the solvent, is easily removed at low temperatures thus avoiding evaporative loss of the lower boiling esters.

During gas chromatography, elution of individual esters is in the order of increasing boiling points. Standard response factors and relative retention times (T_r) are given in Table 2. Overall recovery of acids through the isolation and derivatization steps is greater than 92%, except for lactic acid which can sometimes be inadvertently lost through evaporation. Recoveries of acids added to previously analyzed molasses are indicated in Table 3 and a glc trace is shown in Figure 1.

Table 2.—GLC parameters of methyl esters.

Acid	bp	T_r	K
Lactic	145	0.178	0.294
Glycolic	151	0.266	0.110
Succinic	193	0.578	0.518
Malic	242	1.34	0.302
Aconitic	270	1.58	0.049
Tartaric	282	1.84	0.134
Citric	287	2.00	0.363
PCA	181/25 mm	2.62	0.384

Table 3.—Acid recovery from spiked molasses.

Acid	mg added	mg found	Recovery %
Lactic	30.1	28.8	96
Succinic	20.2	19.2	95
Malic	15.1	14.8	98
Tartaric	19.7	18.2	92
Citric	21.8	22.2	101
PCA	18.8	20.0	110

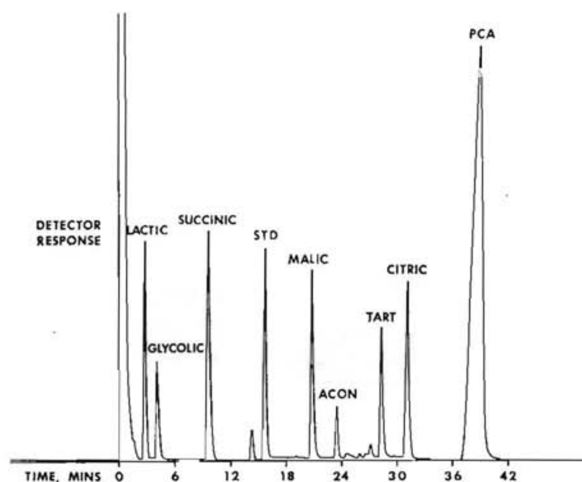

Figure 1.—A glc trace of recovered acids.

Summary

A convenient analytical method for the simultaneous quantitative determination of a number of organic acids commonly present in sugarbeets and factory juices was developed. The method is based on prior isolation of the acids by ion exchange procedures with subsequent determination of their methyl esters by gas chromatography.

Literature Cited

(1) deBoer, Th. J. and H. J. Backer. 1954. *p*-Tolysulfonylmethylnitrosamide. Org. Syntheses. 34: 96-100.

(2) Konig, H. 1967. Separation and detection of aliphatic dicarboxylic acids and hydroxy acids by gas chromatography. Fresenius' Z. Analyt. Chem. 231: 121-136.

(3) Miller, P. H. 1969. Trends in nonsucrose constituents of central California beets. I. Analysis of Steffen factory liquors. J. Am. Soc. Sugar Beet Technol. 15: 595-606.

(4) Silin, P. M. 1958. Technology of beet-sugar production and refining. (English translation 1964, Office of Technical Services, U.S. Dept. of Commerce, Washington, D.C.). p 43ff.

(5) Warner, B. R. and L. Z. Raptis. 1955. Determination of formic acid in presence of acetic acid. Anal. Chem. 27: 1783-1784.