Simultaneous Determination of Phosphoric Acid And Organic Acids in Beet Process Juices and Molasses

J. F. T. OLDFIELD, R. PARSLOW, and M. SHORE[1]

Received for publication November 1, 1972

Introduction

The changes in the concentrations of individual organic and inorganic acid anions in sugar beet juices at all stages of processing can be used to study many of the reactions involved in the production of beet sugar. A knowledge of these changes provides a sound basis for the specification of operating conditions and will also give an indication of possible sugar losses.

The extent of sugar losses due to bacterial action and inversion are indicated by the production of lactic acid during diffusion (4)[2] and lactic and saccharinic acids during carbonatation (7, 5). The decrease in the total amount of acids, resulting from the complete or part removal of some as their calcium salts but offset in part by the production of others as a result of degradation reactions, gives a measure of the base available for the production of carbonate ions during second carbonatation.

The requirement for soda ash addition at second carbonatation to maintain the required acid/base balance at thick juice may be deduced, taking into account the base lost as ammonia, from the change in the balance due to acids gained, as a result of glutamine degradation and invert destruction, and lost as carbon dioxide during evaporation (3, 14).

The loss of oxalate and citrate from juice as their insoluble calcium salts is shown by the decrease in their concentrations during evaporation and pan boiling. These precipitated calcium salts form the major portion of the scales deposited in evaporator tubes and pans and are one cause of turbidity in white sugar (6).

The determination of individual acids by specific methods, with or without the aid of ion exchange cleanup procedures, is very time consuming and methods which measure several acids simultaneously have been devised—the partition of organic acids on silicic acid columns (7), the gas liquid chromatographic (GLC) separation of methyl esters of organic acids (15), and the GLC separation of trimethylsilyl (TMS) esters and ether esters of organic acids (8, 1, 9, 11, 10).

[2]Numbers in parentheses refer to literature cited.

The preparation of TMS esters and ether esters is simpler than the preparation of methyl esters; moreover it has been reported (12) that the TMS ether esters are superior to methyl esters for GLC separation of hydroxycarboxylic acids.

Trimethylsilylation of acids as their precipitated lead salts (1, 9, 11) is not suitable for beet juices due to the solubility of lead salts of lactic acid and pyrollidone carboxylic acid, but the TMS derivatives of organic acids can be readily prepared from their ammonium salts, as can derivatives of some inorganic acids (2). An ion exchange/drying procedure for the isolation of the dry ammonium salts of the acids from beet juices has been developed and after trimethylsilylation the resultant derivatives of the acids are determined by GLC.

It proved possible to determine simultaneously, with good precision and in a relatively short time, the major organic acids and phosphoric acid in beet juices.

Method

Reagents

Cation exchange resin Zerolit 225 14-52 mesh 8% DVB
Anion exchange resin Zerolit FFip 54-100 mesh 7-9% cross linkage
2N AR hydrochloric acid.
N solution AR sodium hydroxide
N solution AR sodium carbonate
5% w/v solution AR ammonium carbonate
0.5% w/v solution AR tartaric acid used as an internal standard.
N,O - bis (trimethylsilyl) acetamide (BSA)

Apparatus

A) *Ion-exchange*

125 mm x 15.0 mm diameter glass ion exchange columns.
Rotary evaporator.
2 cm^3 test tube fitted with B14 ground joint and stopper.
Vacuum desiccator with P_2O_5 drying agent.

B) Gas Chromatography

The gas chromatograms were obtained using a Hewlett-Packard 5750 gas chromatograph equipped with dual columns and dual flame ionisation detectors. The samples were injected, with a 1μl capacity SGE microsyringe, into the empty first 60 mm section of a 6 ft. x 4 mm ID glass column packed with 10% SE52 silicone gum coated on 85/100 mesh, acid washed, dimethyldichlorosilane treated, Chromosorb W.

The column oven was temperature programmed from an initial temperature of 75°C to a final temperature of 250°C at a rate of

6°C/min. The injection port temperatures and flame ionisation detector block temperatures were 250°C and the dry oxygen-free nitrogen carrier gas flow rate was 54 cm^3/min. The detectors were fed with hydrogen at 11 psi (flow rate 45 cm^3/min) and oxygen at 33 psi (flow rate 500 cm^3/min).

The electrometer was set on Range 10^3, equivalent to 10^{-9} amp full scale deflection with an attenuation setting XI, and attenuation settings from X2 to X16 were used when recording the elution chromatograms.

Ion exchange procedure

Preparation of resins

The ion exchange columns contain only 10 cm^3 of resin and, for routine determinations, it is more convenient to regenerate the resins in bulk than to regenerate the individual columns.

A stock quantity of 1 to 5 litres of cation exchanger is converted to the hydrogen form in a large glass column by regenerating with 2 bed volumes of 2N hydrochloric acid. The resin is then washed free from acid and chloride with deionised water until the washings are negative to the chloride test with silver nitrate solution.

Resin previously used for the analysis of raw juice samples is washed free from precipitated material by decantation before regeneration.

A stock quantity of 1 to 5 litres of new anion exchanger is regenerated with about 50 bed volumes of N sodium hydroxide solution until virtually chloride free, *i.e.* until the effluent produces only a faint turbidity with silver nitrate solution acidified with nitric acid. The resin is then washed with 3 bed volumes of water followed by 3 bed volumes of N sodium carbonate solution. The sodium carbonate solution is applied until the output pH is the same as the input pH. The quantity used may be reduced by gassing the effluent with carbon dioxide to pH 11.0 and recycling it to the input. The resin is finally washed with 3 bed volumes of water.

Used resin is regenerated, after washing free from ammonium carbonate, with only 2 bed volumes of sodium hydroxide solution followed by 3 bed volumes N sodium carbonate solution.

Separation of acids from beet process juices

To 50 cm^3 of raw juice, thin juice or diluted thick juice at 15° Bx or 5 cm^3 of diluted molasses at 15° Bx, containing not more than 6.5 meq of total acids, an internal standard 5 cm^3 of 0.5% tartaric acid is added. The mixture, diluted to 200 cm^3 with deionised water to avoid the evolution of CO_2 in the anion exchanger (which would break up the resin bed and cause acid leakage due to channeling), is applied to 10

cm³ of Zerolit 225 cation resin in the H+ form in a glass column followed in series by a second column containing 10 cm³ of chloride-free Zerolit FFip anion resin in the CO_3^{--} form such that the acid effluent from the cation exchanger passes directly onto the anion exchanger. About 50 cm³ of deionised water is run through the columns; the cation exchange column is then discarded and the anion exchanger is washed sugar free, as shown by the Molisch test, before eluting the acids as their ammonium salts with 250 cm³ of 5% ammonium carbonate solution at a rate of 3 cm³/min. The resin must be sugar free before elution as the TMS ether of fructose has a similar retention time to the TMS ester of citric acid. Freedom from interfering peaks is shown in Fig. 1, which is a complete method blank produced by omitting the juice sample.

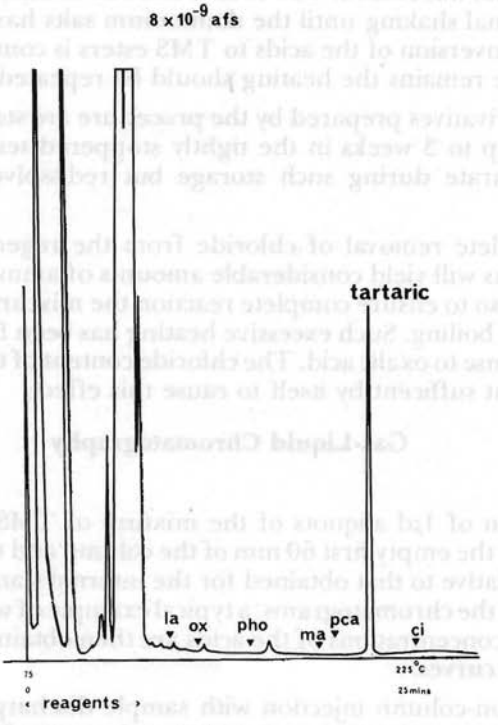

Figure 1.—Complete method blank

Preparation of TMS derivatives

Ammonium carbonate will react to form a TMS derivative (2) which has the same retention time as the lactic acid TMS ester so, to avoid interference, the excess ammonium carbonate in the eluate must be completely removed by boiling at atmospheric pressure until the

volume reduces from 250 cm^3 to 75 cm^3. The ammonium carbonate is broken down and the resultant ammonia and carbon dioxide are boiled off.

The concentrated eluate is transferred quantitatively to a 250 cm^3 round-bottom flask which is then coupled to a rotary evaporator and its contents evaporated to dryness at 60°C under reduced pressure. The residue of dry ammonium salts is dissolved completely in 5 cm^3 of deionised water and a 0.2 cm^3 aliquot of the solution transferred to a 2 cm^3 test tube fitted with a B14 ground joint which is then placed in a vacuum desiccator over P_2O_5 for about 3 hours until all the water has been removed. To the residue, which is equivalent to 2 cm^3 of the 50 cm^3 juice sample, is added 300 μl of N,O - bis (trimethylsilyl) acetamide (BSA) and the tube stoppered. The reaction mixture is heated gently, but not boiled, using a low flame on a micro burner until evolution of gas (ammonia) has ceased. The tube is allowed to stand for 5 minutes with occasional shaking until the ammonium salts have dissolved. At this stage conversion of the acids to TMS esters is complete but if any solid residue remains the heating should be repeated.

The derivatives prepared by the procedure are stable and may be stored for up to 3 weeks in the tightly stoppered test ube at -10°C; crystals separate during such storage but redissolve readily when warmed.

Incomplete removal of chloride from the regenerated ion exchange resins will yield considerable amounts of ammonium chloride at this stage so to ensure complete reaction the mixture would have to be heated to boiling. Such excessive heating has been found to cause a loss in response to oxalic acid. The chloride content of the applied juice sample is not sufficent by itself to cause this effect.

Gas-Liquid Chromatography

Procedure

Injection of 1μl aliquots of the mixture of TMS esters is made directly into the empty first 60 mm of the column and the peak heights obtained relative to that obtained for the internal standard are determined from the chromatograms, a typical example of which is shown in Fig. 2. The concentrations of the acids are then obtained by reference to standard curves.

Direct on-column injection with sample discharge onto the column packing caused deterioration in the column performance requiring repacking after about 2 weeks use, but when the first 60 mm of the column was left unpacked this was no longer necessary.

Preparation of standard curves

5 cm^3 aliquots of 0.5% tartaric acid solution are added to increasing aliquots of a standard solution of the acids under consideration.

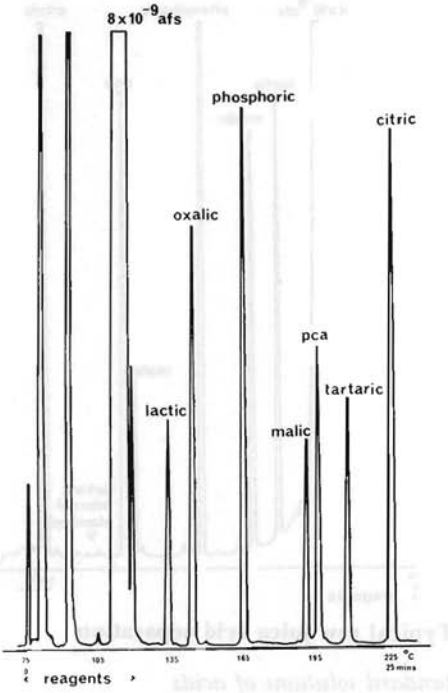

Figure 2.—Separation of acid standards (TMS ethers)

Each of these aliquots is then taken through the complete analytical procedure. A plot of relative peak height for each acid (tartaric acid = 1.0) against acid concentration is linear in the range equivalent to between 20 mg and 1000 mg/litre of juice (2000 mg/litre for citric acid and PCA) for a juice aliquot of 50 cm^3.

In general the consistency of response is good but some variations do occur so to obtain the highest accuracy calibration was carried out with each batch of juice samples. The standard curves are linear and were determined by processing the two aliquots of the mixture of standard acids giving the extreme values.

In order that the total acid applied to the ion exchange columns does not exceed 6.5 meq, while at the same time the highest acid concentration for each standard curve is greater than the maximum concentration of the same acid likely to occur in the juice sample, it is not possible to use a mixture containing equal amounts of each acid, and the relative proportions of the constituent acids in the mixture of standard acids should be similar to those in the type of process juice being analysed (*e.g.* raw juice or thin juice or molasses). Figures 3 and 4 show typical separations of the acids from raw juice and second carbonatation juice.

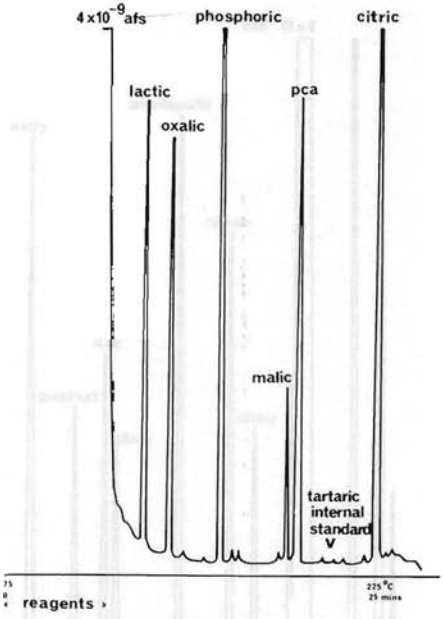

Figure 3.—Typical raw juice acid separation

Preparation of standard solutions of acids

The predominant acids of beet raw juice are citric and phosphoric acids while those of thin juice, thick juice and molasses are pyrrolidone carboxylic and lactic acids. Consequently two standard acid mixtures are required.

Standard A, used when analysing raw juice, contains:

20 mg % w/v lactic acid as lithium lactate
60 mg % w/v oxalic acid
60 mg % w/v Phosphoric acid as potassium dihydrogen phosphate
10 mg % w/v malic acid
25 mg % w/v pyrrolidone carboxylic acid
70 mg % w/v citric acid

Standard B, used when analysing thin juice, thick juice, and molasses, contains:

50 mg % w/v lactic acid as lithium lactate
10 mg % w/v glycollic acid as sodium glycollate
5 mg % w/v malic acid
160mg % w/v pyrrolidone carboxylic acid
10 mg % w/v citric acid

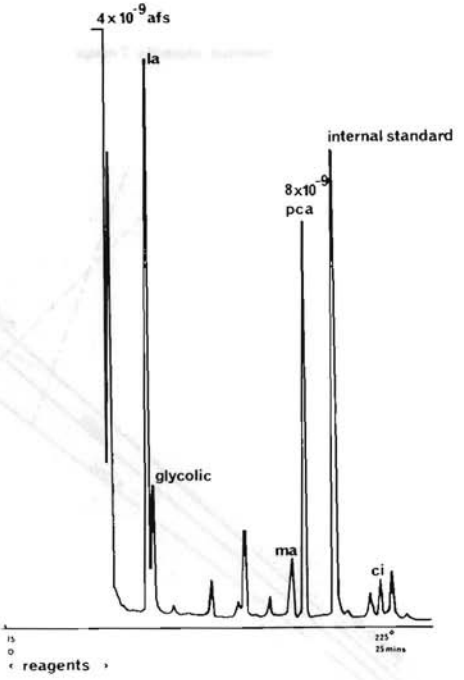

Figure 4.—Typical carbonatated juice separation

Discussion

Specification of anion exchanger capacity

To avoid losses of acids it was arranged that the capacity of the anion exchanger would be about twice the total equivalent of the acids present in the sample aliquot. Losses could not be corrected for by reference to recovery of an internal standard as the weaker acids were displaced preferentially by the stronger acids if the column capacity was exceeded.

To determine the capacity of the anion resin for acid mixtures approximating to juice composition, increasing aliquots of diluted molasses at 15° Bx, to which had been added a mixture of standard acids, were diluted to 200 cm^3 with deionised water and then applied, together with 25 mg lots of tartaric acid (as an internal standard) to sets of paired cation and anion exchangers each containing 5 cm^3 of resin.

The ammonium salts of the acids were isolated as described and the TMS esters prepared and analysed by GLC. The peak height obtained for each acid relative to that for tartaric acid was plotted against the concentration of acid; the results are shown in Fig. 5.

The response for each acid was linear up to an application of a 7 cm^3 aliquot of the molasses/standard acids mixture, which represents a

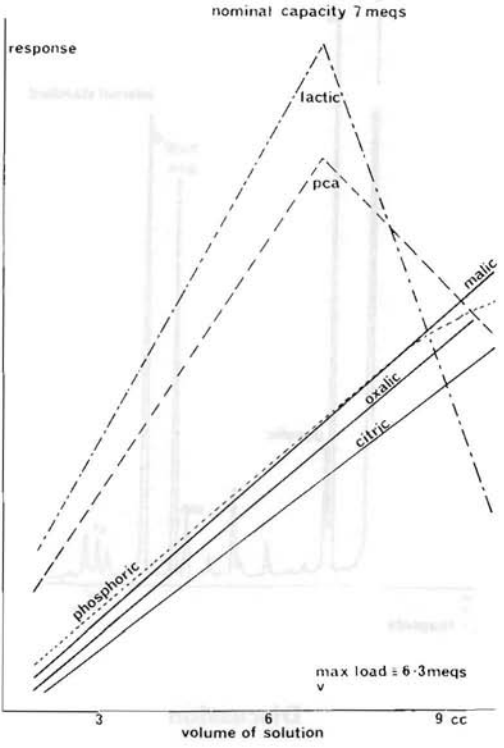

Figure 5.—Effective capacity of 5cm³ anion exchanger

total of 6.3 meq acid, and thereafter decreasing responses were obtained for lactic, pyrrolidone carboxylic, and phosphoric acids, showing leakage of these weaker acids.

To allow for the determination of juices containing unusually high amounts of acids and to allow for the occasional need to increase the sensitivity to citric, malic, and oxalic acids in clarified juices by increasing the volume of the juice sample, the use of 10 cm^3 resin columns is advocated with a maximum application of 6.5 meq of acids.

Elution of anion exchanger

Concentration of the ammonium carbonate eluate from the anion exchanger is time consuming and the minimum volume of 5% solution of ammonium carbonate eluant should be applied to the anion exchanger consistent with the quantitative recovery of the acids under determination.

7 cm^3 aliquots of 15° Bx diluted molasses with added standard acids containing 6.3 meq of total acids were diluted to 200 cm^3 with

deionised water and applied to four pairs of ion exchange columns. After washing sugar free the anion exchangers were eluted with different amounts of 5% ammonium carbonate solution. TMS derivatives were prepared and analysed by GLC; the relative response for each acid (tartaric acid = 1.0) obtained for different eluant volumes is recorded in Table 1.

Table 1.—Volume of eluant required for constant relative response.

Eluant Volume	Relative response (tartaric acid = 1.0)					
(cm^3)	Lactic	Oxalic	Phosphoric	Malic	PCA	Citric
75	1.98	0.96	1.12	1.13	1.50	0.96
125	2.75	1.08	1.36	1.42	2.31	1.13
175	2.55	1.23	1.34	1.37	2.14	1.10
250	2.55	1.37	1.40	1.43	2.01	1.12

While most of the acids were recovered using 175 cm^3 of ammonium carbonate eluant, the recovery of oxalic acid was not complete and 250 cm^3 eluant was required before a constant relative response was obtained.

There was an increased relative response for lactic acid and P.C.A. with 125 cm^3 eluant; this was due to the recoveries of these acids being more complete than the recovery of the internal standard at this low volume.

Calibration of the analytical procedure

TMS derivatives of a mixture of standard acids were prepared and then analysed by GLC. The peak area on the elution chromatogram for each ester was measured and the results, expressed in terms of area obtained per unit weight of derivative, are recorded in Table 2. The weights of the derivatives were calculated assuming that all possible reactive functional groups had reacted completely.

Table 2.—Variations in the GLC response of TMS derivatives of acids

Acid	Number of TMS groups	Calculated weight of Derivative. μg	Response $mm^2/\mu g$
lactic	2	3.12	280
oxalic	2	3.12	248
phosphoric	3	3.85	202
malic	3	3.13	224
PCA	2	2.54	189
tartaric	4	2.92	220
citric	4	3.00	225

The variation in response of the flame ionisation detector with the structure of the TMS derivatives, the uncertainty in the extent of derivative formation and possible decomposition or absorbtion taking place on the GLC column (confirmed by the values recorded in Table 2), shows that it is not possible to predict the GLC response for the TMS

derivatives of the acids. Quantitative work requires the preparation of a standard curve for each acid recording the change in response with increasing concentration relative to the response obtained for a constant addition of an internal standard.

The hydroxy acid tartaric acid, which is not present in detectable amounts in raw juice prepared from sugar beets grown in the United Kingdom as shown in Fig. 3, was used as an internal standard. The TMS derivative of tartaric acid has a convenient retention volume relative to those of the TMS derivatives of the acids contained in sugar beet process juices.

To avoid the possible introduction of errors due to small differences in the recoveries of the individual acids from the ion exchange procedure, standard curves were plotted from the results obtained by taking the mixtures of standard acids and tartaric acid through the complete analytical procedure in the same manner as the juice samples.

A plot of the relative response for each acid (tartaric = 1.0) against acid concentration was linear in the range equivalent to between 20 mg and 1000 mg per litre of juice (juice aliquot = 50 cm^3) and linearity was maintained up to the equivalent of 2000 mg per litre for citric acid and PCA.

Experimental Results

Precision of silylation and GLC procedure

A series of equal aliquots of a standard solution containing 15 mg of each standard acid were dried as their ammonium salts, reacted with BSA, and analysed by GLC. The relative responses for each acid derivative (tartaric acid = 1.0) were measured and the results recorded in Table 3.

Table 3.—Precision of silylation and GLC procedure.

Acid	lactic	oxalic	phosphoric	malic	PCA	citric
Mean relative response	2.04	1.62	2.06	1.88	1.04	1.50
Std. deviation % mean response	0.6	1.9	2.3	1.0	4.4.	2.0

The results show good precision for all the acids except PCA. The silylation reaction with PCA is more sensitive to traces of water than with the other acids, which possibly accounts for the lower precision obtained for PCA.

Precision of the analytical procedure

The precision of the analytical procedure was determined using raw juice produced in the laboratory to which had been added 500 mg

tartaric acid/litre. Laboratory raw juice contains very little lactic acid or PCA so 118 mg lactic acid/litre and 284 mg PCA/litre were added. The relative responses for each acid were measured and the results recorded in Table 4.

Table 4.—Precision of analytical procedure with raw juice.

Acid	Lactic	oxalic	phosphoric	malic	PCA	citric
Mean relative response	0.36	1.05	2.17	0.57	0.68	1.12
Std. deviation % mean response	4.8	3.1	3.2	3.3	5.2	1.8

Recovery experiments

The results of recovery experiments for beet process juices and molasses ranged from 95 to 103% as shown in Table 5.

Table 5.—Recoveries of standard acids added to process juices.

Acid	Juice	acid added range mg/l	total acid determined range mg/l	Mean % recovery
Lactic	raw juice	40-120	56-120	99.6
	dil. thick juice	130-600	394-800	98.5
	dil. molasses	1250-2500	4380-6000	100.9
Glycollic	dil. thick juice	12.5-25	75-180	96.3
	dil. molasses	125-250	470-640	97.2
Oxalic	raw juice	150-684	655-1000	101.9
	dil. thick juice	40-120	56-120	97.8
Phosphoric	raw juice	150-960	406-1180	100.6
Malic	raw juice	75-300	104-730	99.5
	dil. thick juice	12.5-25	62-75	95.0
	dil. molasses	125-250	400-666	99.0
PCA	raw juice	50-120	56-130	98.4
	dil. thick juice	400-960	1120-2150	96.8
	dil. molasses	4000-8000	11860-16200	100.1
Citric	raw juice	175-1200	535-1417	96.4
	dil. thick juice	20-120	77-160	103
	dil. molasses	200-400	540-770	101.9

The simultaneous determination of the majority of the acids present in beet juices can be carried out in about the same time as was previously required for the determination of the individual acids (4, 7, 5).

The results in Table 5 for recoveries of the individual acids at the different levels present in process juices show that the accuracy of the method is good. In Table 4 good precision is shown for the majority of the acids and although the precision is somewhat less for lactic acid and PCA it is adequate for most applications.

Summary

A combination of an ion exchange separation followed by a fast G.L.C. procedure for the identification and determination of organic acids and phosphoric acid in beet process juices is described.

Anions, separated from sugars and cationic material by an ion exchange procedure, are reacted as their dry ammonium salts, with N,O - bis (trimethylsilyl) acetamide (BSA) to form volatile trimethylsilyl ethers and ether esters which are separated by temperature programmed GLC.

The concentrations of the acids are determined from their GLC responses relative to that obtained for an internal standard which is added to the original juice. A plot of relative peak height for each acid (tartaric acid = 1.0) against acid concentration is linear in the range equivalent to between 20 mg and 1000 mg/litre of juice (2000 mg/litre for citric acid and PCA) and the concentration of each juice acid is deduced from a standard curve using the relative peak height (tartaric acid = 1.0) measured on the elution chromatogram.

Recoveries of acids added to beet process juices ranged from 95 to 103%.

Literature Cited

(1) BRUNELLE, R. L., R. L. SCHOENEMAN, AND G.E. MARTIN. J.A.O.A.C. 1967. Quantitative determination of fixed acids in wines by gas-liquid-chromatographic separation of trimethylsilylated derivatives. *50* (2) 329-334.

(2) BUTTS, W.C., AND RAINEY, W.T. Anal. Chem. 1971. Gas chromatography and mass spectrometry of the trimethylsilyl derivatives of inorganic anions. *43* (4) 538-542.

(3) CARRUTHERS, A., DUTTON, J.V., OLDFIELD, J. F.T., SHORE, M., AND TEAGUE, H.J. Int. Sug. Journal 1959. Composition of sugar beet and chemical changes occurring during processing. *61* 376.

(4) CARRUTHERS, A., AND OLDFIELD, J.F.T., Int. Sug. Journal 1956. Effect of thermophilic bacteria in sugar beet diffusion systems. *58* 48.

(5) CARRUTHERS, A., AND OOLDFIELD, J.F.T., Int. Sug. Journal 1959. Use of radio-isotopes in the sugar industry. *61* 376.

(6) CARRUTHERS, A., OLDFIELD, J.F.T., AND SHORE, M. Int. Sug. Journal 1966. Constituents of standard liquor filter cake. *68* 363-366.

(7) CARRUTHERS, A., OLDFIELD, J.F.T., SHORE, M., AND WOOTTON, A.E., Int. Sug. Journal 1954. Studies in the chemistry of sugar beet processing. *56* 218

(8) HORII, Z., MAKITA, M., AND TAMURA, T., Chem. Ind. (London) 1965. Gas-liquid-chromatographic separation of acids of the Krebs Cycle as trimethylsilyl derivatives. 1494.

(9) JOHNSON, A.R., AND FERNANDEZ-FLORES, E., J.A.O.A.C. 1969. Separation of fixed organic acids in table syrup. *52* (3) 559-564

(10) MAKITA, M., AND WELLS, W.W., Anal. Biochem. 1963. Quantitative analysis of fecal bile acids by gas-liquid chromatography. *5* 523-530.

(11) MARTIN, G.E., SULLO, J.G., AND SCHOENEMAN, R.L., J. Agr. Food Chem. 1971. Determination of fixed acids in commercial wines by gas-liquid chromatography. *19* 5, 995-998.

(12) MARTIN, G.E., AND SWINEHART, J.S.J., Gas Chromatog. 1968. Comparison of gas chromatography of methyl and trimethylsilyl esters of alkanoic and hydroxypolycarboxylic acids. *6* 533-539.

(13) ROBERTS, E.J., AND MARTIN, L.F., Anal. Chem. 1954. Identification and determination of non-nitrogenous organic acids of sugar cane by partition chromatography. *26* 815-818.

(14) OLDFIELD, J.F.T., SHORE, M., AND SENIOR, M., Int. Sugar Journal 1970. Thick Juice pH control by cation exchange. *72* 323.

(15) SEQUEIRA, R.M., J.A.S.S.B.T. 1970. ? *16* (2) 136-141.