

PRELIMINARY COMPARISON OF POLARIMETRIC AND GAS CHROMATOGRAPHIC METHODS FOR THE ESTIMATION OF SUCROSE IN SUGARCANE MIXED JUICE AND MOLASSES

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Abstract

A rapid and accurate method for the analysis of sucrose in mixed juice and molasses has been developed. Pol of factory juices and molasses can now be assessed by a specific chromatographic determination.

Introduction

The factory balance of a sugar cane mill compares sucrose, or pol, a hypothetical material considered to approximate sucrose, in the input stream (mixed juice) with the total output streams (filter cake, sugar and molasses). The important parameters that can be affected by differences between pol and various analytical methods for sucrose are:

- (1) Input tonnage of "sucrose" which in turn affects cane payments.
- (2) Recovery of sucrose i.e. $\frac{\text{tons in sugar}}{\text{tons in mixed juice}} \times 100$
- (3) Undetermined loss, i.e. sucrose in mixed juice minus total "sucrose" in output streams.

Two of the streams can be excluded from the discussion. The quantity of sucrose in filter cake is small and any difference between pol and true sucrose would have a negligible effect on the factory balance. Due to the low concentrations of impurities in raw sugar, pol and sucrose should be virtually identical. This leaves mixed juice and molasses. The history of recent analytical changes and their implications is given below.

Prior to 1972, sucrose by double polarisation was used for mixed juice and sucrose by the double reducing sugar method for molasses. The balance was thus a full sucrose balance. Although the methods used give different values to true sucrose as estimated by gas chromatography, they are generally closer to this true value than pol.

In 1972 the mixed juice determination was changed to direct pol, the molasses determination remained unchanged. Direct polarisation nearly always gives a lower value than double polarisation. Effects of this change were:

- (1) Tonnage of pol in mixed juice was lower than tonnage of "sucrose" would have been, had the change not been made. Since total cane payments for the industry are fixed, this meant that price "per ton pol" was lower than "price per ton sucrose" would have been. If the pol/sucrose ratio was constant at all mills, then no financial change would have resulted. This is in fact not so, and mills with high pol/sucrose ratios will overpay and vice versa.
- (2) The factory balance incorporated pol in mixed juice but "sucrose" in molasses. This was wrong in principle and had the effect of artificially reducing undetermined losses, to the extent that in some cases these became undetermined gains.

For the second reason given above, the molasses stream was changed to a pol basis in 1975. The balance became a full pol

balance which removed the anomalies due to different methods as far as undetermined loss is concerned. However, the assumption that there is no change in polarising properties of non-sucrose during processing is questionable.

There remains however the inequity of the varying difference between pol and sucrose in mixed juice.

As gas chromatography is an efficient and specific method for the separation of compounds in mixtures this laboratory has focussed its attention on the quantitative investigation of sucrose in factory juices and molasses.

In an earlier communication¹ on sugar analysis, relative standard deviations for sucrose in molasses of 1,0 - 1,5% were obtained for 7 replicate samples. After certain improvements to the procedure, for example juice filtration, silylation and chromatographic conditions, a gas chromatographic (gc) procedure is described for sugar juices, which can now be silylated rapidly, without drying, with a consistent relative precision of 0,2 to 0,5%.

The gc determination was used at two mills for short periods to compare pol in mixed juice to sucrose by gas chromatography. Molasses samples were also compared by the two analytical methods.

Experimental

Mixed Juice — Hourly samples, collected and analysed by the Sugar Industry Central Board, were used for gc analysis. All samples were filtered and silylated immediately. Silylated samples were stored and gc analysis carried out at Hulett's Research and Development.

Fresh juice (100 ml) was added to a 250 ml beaker containing filter aid (1 g) (Johns-Manville Celite 505). After thorough agitation the mixture was filtered through S & S No. 613 fluted filter paper into 14 mm x 15 cm test tubes. Each funnel was capped to prevent evaporation. After discarding the initial runnings an aliquot (2 ml) was pipetted and weighed into a 6 ml hypovial containing trehalose (120 - 350 mg). After sealing with parafilm, the vial was mechanically shaken for 10 minutes.

Molasses — Weekly molasses samples were obtained from Hulett's Analytical Services. A sample (1 g) was weighed into a 6 ml vial containing trehalose (300 - 350 mg) and dissolved by mechanical agitation in distilled water (1 ml).

Calibration Standard — Sucrose (120 - 300 mg) and trehalose (160 - 350 mg) were dissolved in 1,3 to 1,5 ml of water.

Silylation — An aliquot (10 μ l) was silylated in a 2 ml Hewlett Packard sampling vial by adding pyridine (0,50 ml) hexamethyldisilylazane (HMDS) (0,45 ml) and trifluoroacetic acid (TFA) (0,05 ml) and heating for 30 minutes at 65°C.

Gas Chromatography — Samples of juice or molasses together with two calibration standards were loaded into a Hewlett Packard 7671 automatic liquid sampler and injected

automatically into a Hewlett Packard 5711 gas chromatograph. (gc conditions are included in Table 1.)

TABLE 1

Gas Chromatographic Conditions for the Analysis of Sucrose in Cane Juice and Molasses

Auto Injection	H.P. 7671 A
Wash between samples	5
Replicates	3
Analysis time	5 minutes
Amount injection	0,8 µl
Chromatograph	H.P. 5711
Injector temperature	250°C
Detector temperature	300°C
Oven	265°C
Carrier flow	Nitrogen, 22 ml/min
Column	24" x 1/8" O.D. Stainless Steel, packed with UCW—982 (10%) on Chromosorb W (HP), 80—100 mesh
Detection	FID
Electrometer range	10
Integration	ISCO digital integrator Model 962
Peak width setting	1 sec
Slope sensitivity	0,1% full scale
Peak mode	tailing
Signal conditioner	40 mV

Results and discussion

Juice Storage — Initially juice samples were preserved with mercuric chloride, frozen and despatched at a convenient time to Huletts Research and Development. It was obvious from the results² that juice samples had undergone deterioration prior to freezing.

To avoid this source of error, subsequent samples were derivatised immediately and the stable trimethylsilyl — sugars chromatographed when convenient.

Silylation — In a recent communication,³ juice samples were silylated directly without prior drying. Trimethylsilylimidazole (TSIM) was the silylation reagent. This reagent could not be used with the chromatograph's autosampler as imidazole (m.p. 88°C), a by-product of the reaction, crystallised and damaged the syringe when the sampler's injection cycle was repeated some 5 minutes later.

However HMDS/TFA, a powerful silylation mixture, does not have this disadvantage and is also relatively inexpensive. Reagent equivalents necessary for complete silylation of aqueous samples have been determined (see Table 2) and the recovery results (Table 3) would indicate that the analysis is accurate.

TABLE 2

Silylation of Aqueous Sugar Solutions with HMDS/TFA (0,45 ml/0,05 ml in 0,50 ml of Pyridine)

	Molasses (mg)	Juice (mg)
Sample mass	1 000	2 000
Trehalose mass	350	350
Water added mass	1 000	—
Total mass	2 350	2 350
Silylation aliquot	20	20
Water in aliquot	8,6	14,4
Sucrose in aliquot	5,5	5,5
Hydroxyl milliequivalents	1,1	1,7
HMDS milliequivalents	4,3	4,3
Excess HMDS	300%	140%

TABLE 3

Sucrose in Mixed Juice by GC Recovery Trials

Mg Sucrose Added	Mg Sucrose Recovered	% Recovery
43,1	43,8	101,6
74,4	75,1	100,9
101,1	100,9	99,8
127,1	128,0	100,7
133,4	133,5	100,0
150,8	151,0	100,1
155,4	155,0	99,7
		100,4 ± 0,7

Chromatographic Conditions —

- (a) *Detector design:* Trimethylsilyl derivates coat the flame ionisation detector with Silicon dioxide, sensitivity drops and linearity is affected. Poor detector design adversely influenced the precision of results quoted in our original paper.¹ The precision of the analysis improved dramatically, when a gas chromatograph with rapid purging and combustion characteristics was used, ensuring longer trouble free operation.
- (b) *Column:* Retention time of trehalose, the internal standard, was reduced from 21 to 3,5 minutes by using an efficient short column at high temperature (column performance parameters are quoted in Table 4).

TABLE 4

Column performance for gc analysis of sucrose in cane juice

N	750 - 820
P.S.	97,5 - 98,5%
R	1,7 - 1,9
α	1,30

Where N = column efficiency for TMS-Sucrose
 P.S. = Peak Separation
 R = resolution, and
 α = relative retention for TMS-Trehalose relative to TMS-Sucrose

Integration of the resultant sharp peaks yielded more precise results. Gases, chromatograph, sampler, integrator and recorder were seldom switched off, increasing thermal and electronic stability. Septa were changed daily and the collector of the flame detector was cleaned daily by a simple technique using a pipe cleaner.

Procedural Errors — As the analysis is fairly complicated, the influence of each specific operation on the overall scatter was determined by a resolution of errors technique.

$$\sigma^2_{ijkl} = \sigma^2_i + \sigma^2_j + \sigma^2_k + \sigma^2_l$$

where σ^2_{ijkl} = variance of final result;

- σ^2_i = variance due to sample splitting and filtration;
- σ^2_j = variance due to weighing;
- σ^2_k = variance due to silylation;
- σ^2_l = variance due to gc procedure.

Results from the above exercise are reported in Table 5.

It is obvious that the filtration step, where originally Whatman No. 42, a relatively slow filtering paper and high dead volume receiver flasks were used, was adversely affecting precision. As extraneous matter will affect the mass of juice the following techniques for removing solids were investigated:

- (1) Hi-speed centrifuging;

TABLE 5
Resolution of errors in the gc analysis of sucrose in cane juice

	Run 1	Run 2
σ^2_{ijkl}	0,033	0,012
σ^2_i	0,025	0,007
σ^2_j	0,002	0,001
σ^2_k	0,005	0,004
σ^2_l	0,001	0,000

- (2) Screening;
- (3) Suction through cotton wool filters;
- (4) Clarification with basic lead acetate;
- (5) Filtration through S & S 613 and Celite 505 into 14 mm x 15 cm test tubes.

Filtration was found to be the most effective and rapid technique (See Table 6).

TABLE 6
Removal of extraneous material from cane juice

Technique	Rel. Standard Deviation of replicates from Mean	No. of replicates
Suction Filtering . . .	4,7%	11
Centrifuging -1. . .	1,6%	8
Screening	1,2%	8
Centrifuging -2. . .	0,9%	8
Filtering	0,7%	*

* Duplicate filtrates from 12 different juices.

Precision — By applying the improvements already mentioned to the gc technique, a mean standard deviation of

0,05 units for 24 duplicate juice samples was obtained. Data for Empangeni juice samples is included in Table 7.

TABLE 7
Precision of gc analysis of sucrose in Empangeni Factory juices, week ending 14.2.75. (Duplicate Samples.)

Primary Juice	Secondary Juice
16,76 ± 0,13	9,52 ± 0,04
17,20 ± 0,01	10,49 ± 0,11
17,86 ± 0,01	9,74 ± 0,01
17,67 ± 0,06	9,28 ± 0,01
16,17 ± 0,06	8,01 ± 0,04
16,55 ± 0,02	7,46 ± 0,02
17,74 ± 0,05	9,02 ± 0,04
15,79 ± 0,02	8,92 ± 0,05
16,11 ± 0,01	9,32 ± 0,00
18,86 ± 0,07	8,97 ± 0,06
16,50 ± 0,03	9,31 ± 0,01
17,72 ± 0,08	9,53 ± 0,11

Comparison of GC and Pol Methods —

(a) *Molasses*: Molasses samples from Mount Edgecombe and Empangeni were analysed by gc and the sucrose results compared to pol. The pol/sucrose ratio, as shown in Figure 1, can differ between Mount Edgecombe and Empangeni by about 0,06 units. These mills are fairly extreme in variation from average pol/sucrose ratios, Mount Edgecombe being low and Empangeni high.

(b) *Juice*: Two tests were carried out over a 5 day period for both Mount Edgecombe and Empangeni. Some 20 - 24 juice samples were analysed by gc and pol at each centre. Raw data and the pol/sucrose ratios for both mills are presented in Tables 8 and 9.

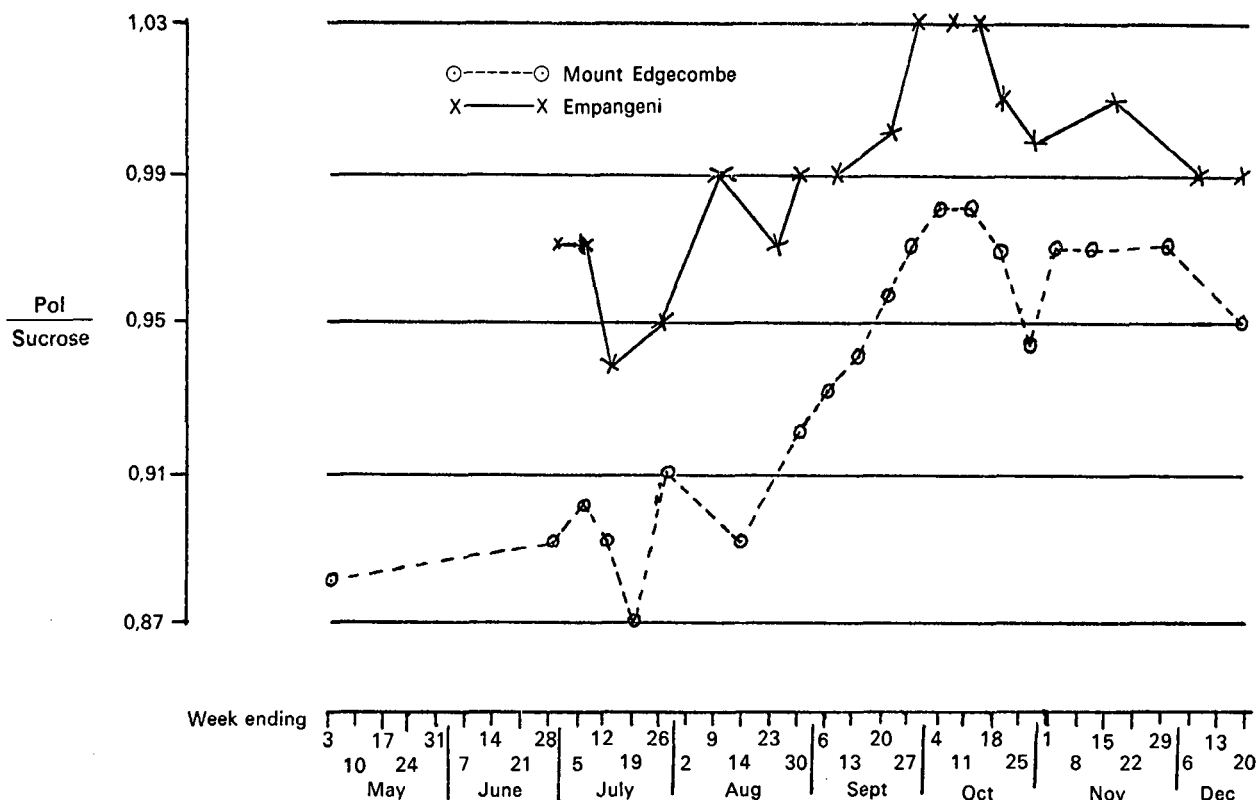


FIGURE 1 Pol/Sucrose ratios for Mount Edgecombe and Empangeni molasses samples for 1975 season.

TABLE 8

Comparison of gc and pol methods for mixed juice, Mount Edgecombe, week ending 30th October, 1975

% S (gc)	Pol	$\frac{\text{Pol}}{\text{Sucrose}}$
17,38	17,21	0,99
16,63	16,46	0,99
12,50	12,36	0,99
10,84	10,72	0,99
19,22	18,87	0,98
17,21	17,04	0,99
13,76	13,80	1,00
15,30	15,49	1,01
13,90	13,60	0,98
12,71	12,54	0,99
11,08	10,96	0,99
16,73	16,51	0,99
20,71	20,60	0,99
19,66	19,61	1,00
17,88	17,68	0,99
11,86	11,88	1,00
12,15	12,06	0,99
13,09	12,88	0,98
12,30	12,06	0,98
		$\bar{X} = 0,99 \pm 0,01$

TABLE 9

Comparison of gc and pol methods for mixed juice, Empangeni, week ending 14th December, 1975

Sucrose by gc	Pol	$\frac{\text{Pol}}{\text{Sucrose}}$
16,76	16,75	1,00
17,20	17,25	1,00
17,85	17,77	1,00
17,67	17,59	1,00
16,17	16,14	1,00
16,55	16,44	0,99
17,74	17,67	1,00
15,79	15,77	1,00
16,11	16,26	1,01
16,86	16,80	1,00
16,58	16,53	1,00
17,72	17,52	0,99
9,52	9,34	0,98
10,49	10,40	0,99
9,74	9,58	0,98
9,28	9,11	0,98
9,31	9,17	0,98
8,07	8,00	0,99
7,46	7,36	0,99
9,02	8,93	0,99
8,92	8,86	0,99
9,32	9,13	0,98
8,97	8,84	0,99
9,53	9,46	0,99
		$0,99 \pm 01$

The difference between the two analytical methods was shown by statistical analysis to be significant at the 99% level for both mills.

Although data is limited and covers only one week of the crushing season, in each case a tentative pol/sucrose ratio of 0,99 was obtained and pol in mixed juice was in error by approximately 1%. For a mill with capacity of 150 000 tons of sucrose in mixed juice per annum, this error is equivalent to 1 500 tons. At a sucrose price of approximately R100/ton this is worth R150 000 per annum.

Due to the lack of data no meaningful conclusion on the accuracy of pol in mixed juice can be made, on the other hand gc has provided the tool for an extensive investigation in the future.

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