APPLICATION OF GAS CHROMATOGRAPHY IN A PRELIMINARY INVESTIGATION INTO CHANGES IN SOME NON-SUCROSE CONSTITUENTS DURING SUGAR-BOILING

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Abstract

An accurate and precise oximation procedure has been developed for sugar factory products. The method has been applied routinely, in conjunction with silylation and gas chromatography, to demonstrate a seasonal trend in the fructose/glucose ratio in final molasses at two Huletts factories. Using chloride as a base it has been shown that reducing substances other than fructose or glucose are formed during boiling house operations and that increased fructose/glucose ratios are due mainly to a loss of glucose. There is no apparent seasonal trend in amino-nitrogen levels but levels decrease significantly between syrup and molasses. The influence of the major optically active impurities on the pol measurement is discussed briefly and a tentative correlation established between pol and sucrose, glucose and fructose. There is an increase in optically-active substances between syrup and molasses.

Introduction

Although non-sucrose impurities enter the factory in mixed juice and are largely beyond the mill's control, further non-sucrose may be formed during processing altering the initial impurity composition. Radio-active tracer studies[1] have indicated the partial decomposition of sucrose during molasses formation.

The main optically-active non-sucrose constituents are fructose and glucose. Cane juice is subjected to complex reaction conditions in the sugar factory and innumerable permutations of the various interactions between glucose and fructose and other impurities can occur. Apart from decomposition, fragmentation and condensation reactions, the alkalinity and temperature changes occurring during process will cause isomerisation between glucose and fructose. Binkley[2] has shown that mannose in molasses originates mainly from fructose.

Irvine[3] demonstrated a varietal and seasonal difference in the fructose/glucose (F/G) ratios for Louisiana cane juices (0,51 - 0,83), – with early-maturing varieties showing higher F/G ratios than later maturing varieties. Reported results for South African juices have generally indicated F/G ratios greater than unity.[4,5]

Carruthers[6] used paper chromatography to determine the origin of invert in molasses and concluded that factory conditions were so overriding that the initial F/G ratio in juice had little effect on the ultimate ratio in molasses. Values ranged between 0,38 and 0,93 for beet molasses.

Although fructose is considered more sensitive to low pH/high temperature conditions than glucose, and invert solutions prepared by acid hydrolysis give F/G ratios less than unity[7] there is ample evidence that both cane and beet final molasses generally contain a higher proportion of fructose than glucose [4, 8, 9, 10]. Gardiner [11], however reported F/G ratios in cane molasses between 0,73 and 0,94 and Dowling [12] noted similar ratios for refinery blackstrap molasses.

Gas chromatography (gc) provides a useful tool for the specific determination of the individual sugars in factory products. An accurate procedure for measuring sucrose by gc in mixed juice and molasses has been developed in this laboratory[13] and applied routinely.[14] Although this same silylation technique can be used to measure glucose and fructose, several assumptions regarding mutarotation equilibria are involved and quantitative evaluation is often difficult because the monosaccharides produce a large number of overlapping anomeric peaks. Consequently it is preferable to block the aldehyde or keto groups before silylation. Methyloxime or oxime derivatives are frequently used to give simpler chromatograms with increased sensitivity. We used Brobst's[15] oximation-silylation procedure, but found that sucrose hydrolysis occurred during oximation. This side-reaction was eliminated by increasing the pH of the oximation reagent.[16]

In an attempt to acquire some insight into the fate of certain non-sucrose constituents in the boiling house this modified gc procedure was applied routinely throughout the season to measure sucrose, glucose and fructose in syrup and molasses from Empangeni and Mount Edgecombe.

To enable direct comparison of the input (syrup) and output (molasses) streams chloride was monitored. Since chloride is highly soluble and unlikely to undergo chemical reaction, it is an ideal reference.

It is often assumed that amino-nitrogen is implicated in glucose losses. An automated colorimetric procedure was used to measure amino-nitrogen.

The results of our preliminary investigations into changes in some non-sucrose constituents during boiling are presented here.

As pol % molasses is widely applied in factory control, the effect of these optically-active impurities on pol is discussed.

Experimental

The syrup and final molasses samples analysed were weekly composite samples for the 1977/78 season from each factory. Syrup samples were preserved with mercuric chloride solution[17] (about five drops per litre). All samples were refrigerated (-10°C) prior to analysis.

The methods of analysis were as follows:

(i) *Refractometer brix:*
The method is detailed in the Laboratory Manual for South African Sugar Factories.[17]
– Syrup: Section 8.5.4.
– Molasses: Section 8.7.4.3.

(ii) *Pol:*
For details refer to the Laboratory Manual.[17]
– Syrup: Section 8.5.5.
– Molasses: Section 8.7.4.5.

(iii) *Reducing substances:* were determined by Lane and Eynon titration.
– Syrup: Laboratory Manual[17] Section 8.5.7.
– Molasses: S.R.I. Method.[18]

(iv) *Non-fermentable reducing substances:*
The Java Sugar Experiment Station procedure was used for fermentation.[19] Reducing sugars on the filtrate were determined using the Luff Schoorl method. (Laboratory Manual[17] Section 8.8.1.7.2.)

(v) *Sucrose:*

(a) Sucrose was determined on molasses using the Lane and Eynon titration after acid hydrolysis according to the Mackay method.[18]

(b) Sucrose on syrup and molasses samples was determined gas chromatographically as the TMS ethers using the silylation technique of Brobst and Lott.[20] Details are included in Appendix 1.

(vi) *Glucose and Fructose:*

The monosaccharides were oximated and the resulting oximes silylated prior to gc analysis using a modification of the method of Brobst.[15] Details are included in Appendix 1.

(vii) *Chloride:*

This was determined by potentiometric titration as described by Comrie.[21] Aliquots contained 5 g syrup or 1 g molasses.

(viii) *Amino Nitrogen:*

An automated adaptation of the colorimetric method described by Carruthers[22] and based on that of Eveleigh[23] was used. Details appear in Appendix 2.

Results and Discussion

Validation of sample storage precautions

Before deciding to collect syrup samples over weekly periods several 8-hourly composites (preserved with mercuric chloride) were stored at approximately 5°C and were analysed daily for fructose, glucose and sucrose. After storage periods of up to 8 days there were no significant differences in the results.

In addition, the result calculated from six 24 hour composites analysed daily, and the analysis of the corresponding weekly composite gave similar results. Six such composites were analysed.

Similar storage tests without mercuric chloride, although too few to be statistically significant, suggested that glucose and fructose levels increased slightly.

Accordingly, mercuric chloride was added to all syrup samples. It is possible to correct the results for this additional chloride. However, provided the recommended chloride dosage (i.e. about 10 mg ℓ^{-1}) is adhered to, the error incurred in the chloride determination will be less than 0,25% relative and can be regarded as insignificant. The precision of the potentiometric chloride determination was good, chloride analysis for both syrup and molasses was found to give a CV (coefficient of variation) of ± 0,3% over the season (142 duplicate samples).

Molasses and syrup samples were stored as described (-10°C) and re-analysed up to ten weeks later. Pooled CV's for original and repeat analyses are summarised in Table 1, and give an indication of the sample storage efficacy as well as the reliability of the analyses.

TABLE 1

Efficiency of low-temperature storage – precision of repeat analyses. (Storage period : 10 weeks)

Product	Samples		Sugar		
			Fructose	Glucose	Sucrose
Syrup	22	CV (%)	2,4	3,2	0,6
		t	0,084	1,037	0,552
Molasses ..	28	CV (%)	3,6	4,0	0,8
		t	1,007	3,180	0,537

CV = coefficient of variation

Statistically, the t-values imply that the pairs were not significantly different at the 95% confidence level. Glucose in molasses was anomalous in this regard. However, the 28 samples gave an overall mean of 6,04% ± 1,46 for the initial glucose analysis and 5,87 ± 1,42 for the repeat analysis. The mean difference between original and repeat results was 0,17. Although this gave a bias of almost 3% relative for repeat glucose analyses, the difference is acceptable in practice.

In contrast to these results two molasses samples were stored at ambient temperatures (6-9 months) and subsequently re-analysed. (Table 2). It is evident that little sucrose destruction occurred, but fructose and more particularly glucose losses have taken place.

TABLE 2

	Fructose		Glucose		Sucrose		Reducing Substances	
	Original	Repeat	Original	Repeat	Original	Repeat	Original	Repeat
Sample 1	9,6	9,1	5,5	4,3	26,1	26,0	19,7	18,3
Sample 2	7,7	7,4	3,2	2,6	27,9	27,6	15,1	14,2

Analytical precision –

The to-date CV's for samples massed in duplicate are presented in Table 3.

TABLE 3

Precision of g.c. analysis (samples massed in duplicate)

Product	Samples		Sugar		
			Fructose	Glucose	Sucrose
Syrup	*180	CV (%)	1,9	2,3	0,6
	**111 (OT)				0,3
Molasses ..	*136	CV (%)	1,2	1,5	0,6
	**118 (OT)				0,4

* precision tests using packed and capillary columns
** precision tests using capillary columns

Despite the two-step derivatisation (i.e. oximation and silylation) and the temperature-programmed gc separation, the sucrose precision is very similar to that obtained with the direct silylation-isothermal procedure. (i.e. CV better than 0,5%). Although the fructose and glucose precision is of the order of 2%, this is adequate for syrup and molasses samples.

Analytical accuracy –

(a) *Comparison of modified procedure with direct silylation*

A comparison of sucrose procedures:

(i) Direct silylation using isothermal gc conditions with

(ii) Oximation-silylation using temperature programming, on 30 samples of syrup and molasses showed no significant difference between the methods. (t = 0,071; pooled CV ± 0,7%). Since the accuracy of the direct silylation procedure has been established[13] it can be assumed that the accuracy of the sucrose determination is unaffected by the modified oximation procedure.

(b) *Synthetic samples –*

Mixtures containing fructose, glucose and sucrose were prepared. The sugars were estimated gas chromatographically and the expected pol calculated as described in a later section. The measured pol agreed well with the calculated pol – further evidence that the gc procedure is accurate (Table 4).

TABLE 4

Accuracy test – synthetic samples.

Gas chromatographic analysis			Pol$_d$	Pol	Brix
Fructose	Glucose	Sucrose			
6,2	4,0	30,34	25,2	25,3	39,9
10,2	7,7	30,25	22,5	23,0	47,6

(c) *Recovery trials* –

Varying amounts of fructose, glucose and sucrose were added to samples of the same molasses and percentage recoveries determined (Table 5).

The precision of the g c procedure has been recorded in Table 3. This does not represent a precision test but rather actual results obtained from a season's work (approximately 700 samples, weighed in duplicate, single silylation and injected in duplicate). In our opinion the to-date results for sucrose, glucose and fructose of 0,5%, 1,9% and 1,5% respectively are gratifying.

When a new method is published it is mandatory to test its accuracy by quantitave recovery of known concentrations of the components.

The result of 15 recovery runs for the three sugars are included in Table 5. The procedure appeared to be quantitative – recoveries for sucrose, glucose and fructose were 100,6 ± 2,9%, 100,6 ± 6,3% and 98,1 ± 8,1% respectively. However the precision of the recovery procedure at first sight appeared a little disappointing.

The overall scatter of the recovery procedure is influenced by an additional variance (equation 1) and is therefore greater than the normal gc procedure.

$$\delta^2 \text{ Total} = \delta^2 \text{ std} + \delta^2 \text{ sample} + \delta^2 \text{ (sample + sugar)} \quad (1)$$

where δ^2 Total = variance of the overall recovery procedure

δ^2 Std = variance due to the calibration standard (s)

δ^2 sample = variance in estimating sugur concentration of unknown sample.

δ^2 (sample + sugar) = variance in estimating sugar concentration of the recovery sample.

Non-sucrose changes –

The seasonal changes in non-sucrose between syrup and molasses for Empangeni and Mount Edgecombe are presented in Tables 6 and 7. Molasses results are weighted according to weekly tonnages, whereas syrup results are arithmetic means.

F/G ratio:

The F/G ratios for syrup were close to unity throughout the season, although Empangeni showed a minimum value of 0,92 in December. There was generally a large increase in the ratio of fructose to glucose between syrup and molasses. This increase showed a maximum in the period August-October at Empangeni and September-November at Mount Edgecombe. Ratios were consistently higher at Mount Edgecombe than Empangeni.

(F/G maximum: Empangeni 1,47;
Mount Edgecombe 1,64; Jaagbaan 2,41)

Note: As Jaagbaan consistently produces good performance data, molasses samples from this mill were included for comparative purposes. The relevant data are included later in this report (Table 8).

TABLE 5

Recoveries of sucrose, glucose and fructose added to molasses samples – accuracy test.

Sugar	Approx. Mol (g)	Added (mg)	Re-covered (mg)	Re-covered (%)	Approx. Mol (g)	Added (mg)	Re-covered (mg)	Re-covered (%)	Approx. Mol (g)	Added (mg)	Re-covered (mg)	Re-covered (%)	Mean
Sucrose	1,8	52,7	53,3	101,1	1,9	57,1	56,7	99,3	1,9	51,6	49,6	96,1	
	1,6	100,6	106,6	106,0	1,6	101,2	105,3	104,1	1,8	100,7	96,3	95,6	
	1,4	149,8	152,5	101,8	1,4	150,8	154,5	102,5	1,6	154,6	149,8	96,9	
	1,2	201,3	205,3	102,0	1,3	200,8	205,8	102,5	1,4	200,0	202,4	101,2	
	1,0	249,8	253,0	101,3	1,1	249,5	251,1	100,6	1,2	250,0	245,7	98,3	
													100,6 ±2,9
Glucose		*				*							
	1,9	8,8	9,6	109,1	1,8	8,8	10,3	117,1	18,5	92,4	92,1	99,7	
	1,7	17,6	16,3	92,6	1,7	17,6	16,9	96,0	17,0	179,6	166,5	92,7	
	1,5	26,4	26,2	99,2	1,6	26,4	26,0	98,5	15,5	265,0	272,3	102,8	
	1,5	35,2	36,1	102,6	1,4	35,2	35,1	99,7	14,0	352,7	350,1	99,3	
	1,3	44,0	45,1	102,5	1,3	44,0	45,4	103,2	12,6	445,9	421,6	94,6	
													100,6 ±6,3
Fructose		*				*							
	1,9	8,8	9,7	110,2	1,9	8,8	8,9	101,1	18,5	100,3	94,8	94,5	
	1,8	17,6	16,4	93,2	1,7	17,6	–	–	17,0	210,8	181,6	86,2	
	1,6	26,4	26,8	101,5	1,6	26,4	28,4	107,6	15,5	300,4	289,7	96,4	
	1,6	35,2	35,4	100,6	1,4	35,2	31,1	88,4	14,0	399,8	355,6	88,9	
	1,2	44,0	46,7	106,1	1,2	44,0	47,7	108,4	12,6	501,4	455,3	90,8	
													98,1 ±8,1

* Volumetric additions of 1% solutions
Mol = molasses

TABLE 6

Change in non-sucrose between syrup and final molasses – EM (Non-sucrose expressed per g chloride)

Product	Analysis	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Syrup	R.S. (g)	7,16	6,27	6,12	5,91	5,98	6,02	7,17	7,49
	F+G (g)	6,98	5,97	5,84	5,58	5,89	5,56	6,47	6,90
	F (mM)	19,2	16,8	16,4	15,7	16,2	15,0	17,2	19,0
	G (mM)	19,6	16,4	16,0	15,3	16,5	15,8	18,7	19,6
	Amino – N (mM)	7,0	6,7	7,5	8,2	8,2	6,9	7,2	7,4
	Amide – N (mM)	3,3	3,1	3,9	4,0	4,2	3,6	3,2	3,6
	F/G	0,98	1,02	1,03	1,03	0,98	0,95	0,92	0,97
	F+G/R. S.	0,97	0,95	0,95	0,94	0,98	0,92	0,90	0,92
	G/R.S.	0,49	0,47	0,47	0,47	0,50	0,47	0,47	0,47
	Amide/amino	0,47	0,46	0,52	0,49	0,51	0,52	0,44	0,49
	G/Amino-N	2,8	2,5	2,1	1,9	2,0	2,3	2,6	2,7
Molasses	R.S. (g)	8,80	7,39	6,92	6,77	6,37	6,73	7,26	8,24
	F+G (g)	7,66	5,87	5,58	4,93	4,93	5,14	5,63	6,42
	F (mM)	22,9	17,3	17,4	16,2	16,3	16,3	17,6	19,9
	G (mM)	19,7	15,3	13,7	11,2	11,1	12,3	13,9	15,8
	Amino – N (mM)	4,5	4,5	5,2	5,6	5,6	5,2	4,3	4,6
	Amide – N(mM)	2,0	2,0	2,5	2,6	2,6	2,4	1,8	2,1
	F/G	1,16	1,13	1,27	1,44	1,47	1,33	1,27	1,25
	F+G/R.S.	0,87	0,80	0,81	0,73	0,77	0,76	0,77	0,78
	G/R.S.	0,40	0,37	0,35	0,30	0,31	0,33	0,34	0,35
	Amide/amino	0,44	0,44	0,48	0,46	0,46	0,46	0,42	0,46
	G/Amino-N	4,4	3,4	2,6	2,0	2,0	2,4	3,2	3,4
Change (as % in syrup)	R.S.	+23	+18	+13	+15	+ 7	+12	+ 1	+10
	(F+G)	+10	- 2	- 5	- 12	-16	- 8	-13	- 7
	F	+19	+ 3	+ 6	+ 3	0	+ 8	+ 2	+ 5
	G	0	- 7	- 15	- 27	-33	- 22	-26	-19
	Amino-N	- 36	- 33	- 31	- 32	-32	- 25	-40	-38
	Amide-N	- 39	- 35	- 36	- 35	-38	- 33	-44	-42

Key: R.S. = reducing substance; F = fructose; G = glucose; Amino- N = amino-nitrogen; Amide- N = amide-nitrogen; mM = milli-moles

TABLE 7

Change in non-sucrose between syrup and final molasses – ME (Non-sucrose expressed per g chloride)

Product	Analysis	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
Syrup	R.S. (g)	11,13	11,61	10,74	9,47	8,85	6,80	6,11	6,45	7,90	8,43
	F+G (g)	10,98	11,70	10,97	8,95	8,89	6,82	5,98	5,84	7,00	7,70
	F (mM)	29,9	31,8	30,2	25,9	25,4	19,8	17,3	16,7	19,6	21,9
	G (mM)	31,1	33,2	30,7	23,8	24,0	18,1	16,0	15,7	19,3	20,9
	Amino - N (mM)	9,3	9,9	10,3	10,6	10,7	9,6	9,9	9,8	10,9	10,9
	Amide - N (mM)	4,4	4,6	5,3	5,8	5,5	4,9	5,1	5,0	5,7	5,4
	F/G	0,96	0,96	0,98	1,09	1,06	1,10	1,08	1,06	1,01	1,05
	F+G/R.S.	0,99	1,01	1,02	1,01	1,01	1,00	0,98	0,90	0,89	0,91
	G/R.S.	0,50	0,51	0,51	0,49	0,49	0,48	0,47	0,44	0,44	0,45
	Amide/Amino	0,47	0,46	0,51	0,55	0,51	0,51	0,52	0,51	0,52	0,50
	G/Amino-N	3,3	3,4	3,0	2,2	2,2	1,9	1,6	1,6	1,8	1,9
Molasses	R.S. (g)	12,43	13,45	12,93	10,84	10,55	8,32	6,76	7,24	8,43	9,68
	F+G (g)	10,73	11,49	11,20	9,11	8,45	6,35	5,05	5,43	6,32	7,89
	F (mM)	32,0	35,1	34,4	29,4	27,8	21,6	17,4	18,5	21,2	24,9
	G (mM)	27,6	28,7	27,8	21,2	19,2	13,6	10,6	11,7	13,9	18,9
	Amino - N (mM)	6,1	6,7	7,6	8,0	8,1	7,3	6,8	6,9	7,4	7,6
	Amide - N (mM)	2,6	2,2	3,7	4,0	3,9	3,4	3,0	3,3	3,6	3,7
	F/G	1,16	1,22	1,24	1,39	1,45	1,59	1,64	1,59	1,53	1,32
	F+G/R.S.	0,86	0,85	0,87	0,84	0,80	0,76	0,75	0,75	0,75	0,82
	G/R.S.	0,40	0,38	0,39	0,35	0,33	0,29	0,28	0,29	0,30	0,35
	Amide/Amino	0,43	0,33	0,49	0,50	0,47	0,47	0,44	0,48	0,49	0,49
	G/Amino-N	4,5	4,3	3,7	2,7	2,4	1,9	1,6	1,7	1,9	2,5
Change (as % in syrup)	R.S.	+12	+16	+20	+15	+19	+22	+11	+12	+ 7	+15
	(F+G)	- 3	- 2	+ 2	+ 2	- 5	- 7	-16	- 7	-10	+ 2
	F	+ 7	+10	+14	+14	+ 9	+ 9	+ 1	+11	+ 8	+14
	G	-11	-13	-10	-11	-20	-25	-34	-26	-28	- 9
	Amino - N	-34	-32	-26	-25	-24	-24	-31	-30	-32	-30
	Amide - N	-41	-52	-30	-31	-29	-31	-41	-34	-37	-32

Key: R.S. = reducing substance; F = fructose; G = glucose; Amino-N = amino-nitrogen; Amide-N = amide-nitrogen; mM = milli-moles

Reducing substances:

At Mount Edgecombe the reducing substances in syrup were due almost entirely to fructose and glucose until October. From October to the end of the season as much as 10% of the reducing substance in syrup was neither glucose nor fructose. At Empangeni, by contrast, 'other' reducing substances were present in syrup all season.

Reducing substances increased between syrup and molasses, but this increase could not be attributed to glucose and fructose. In fact, total fructose and glucose decreased during boiling.

During the latter part of the season (Aug-Jan) there was a good correlation between non-fermentable reducing substances (NFRS) and (RS-F-G):-

(RS-F-G) = 0,35 + 1,02 NFRS
(r = 0,81 for 12 pairs)

Fructose:

There was generally an increase in fructose between syrup and molasses. The increase averaged 6% at Empangeni and 10% at Mount Edgecombe. This could be indicative of sucrose inversion or glucose/fructose interchange.

Glucose:

At Empangeni the glucose in syrup comprised about 47% of the reducing substances all season, whereas at Mount Edgecombe the G/RS ratio declined throughout the season (0,51 in May to 0,44 in December).

The main non-sucrose change during boiling was the dramatic decrease in glucose between syrup and molasses. The average loss for the two mills was 19%, with the largest drop (33%) occurring in October.

Amino-nitrogen:

There was no obvious seasonal trend in amino-nitrogen levels in syrup, although levels at Empangeni seemed to peak September-October. However, approximately one third of the amino-nitrogen was destroyed between syrup and molasses, together with a slight decrease in the asparagine/amino-nitrogen ratio. Levels at Mount Edgecombe were generally higher than at Empangeni.

These trends are illustrated in Figures 1 and 2.

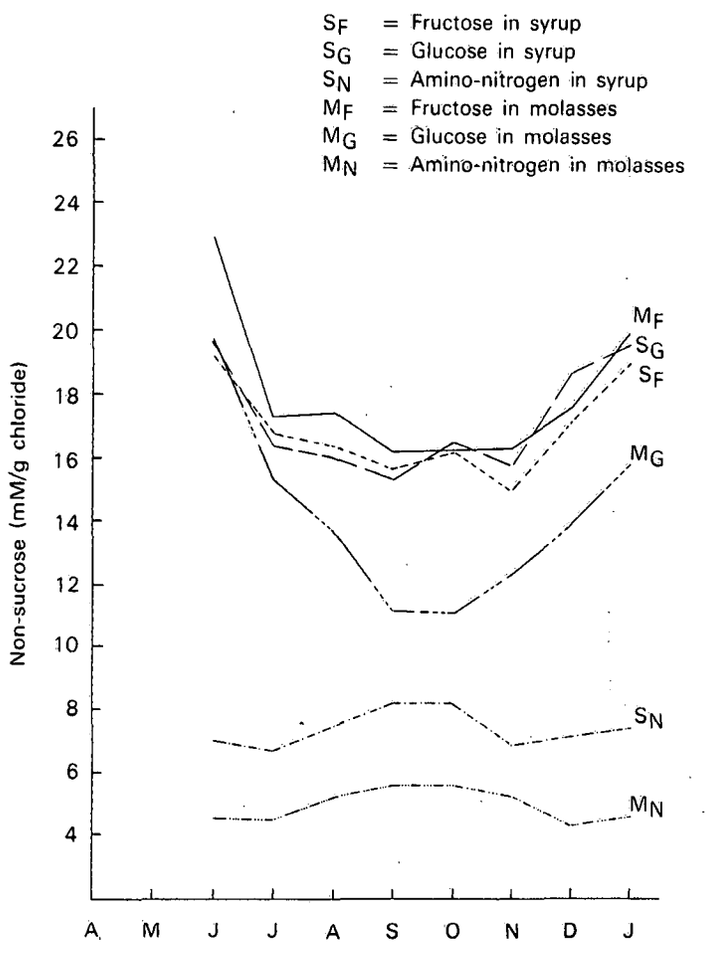

Figure 1: Comparison of non-sucrose in syrup and molasses – Empangeni (EM)

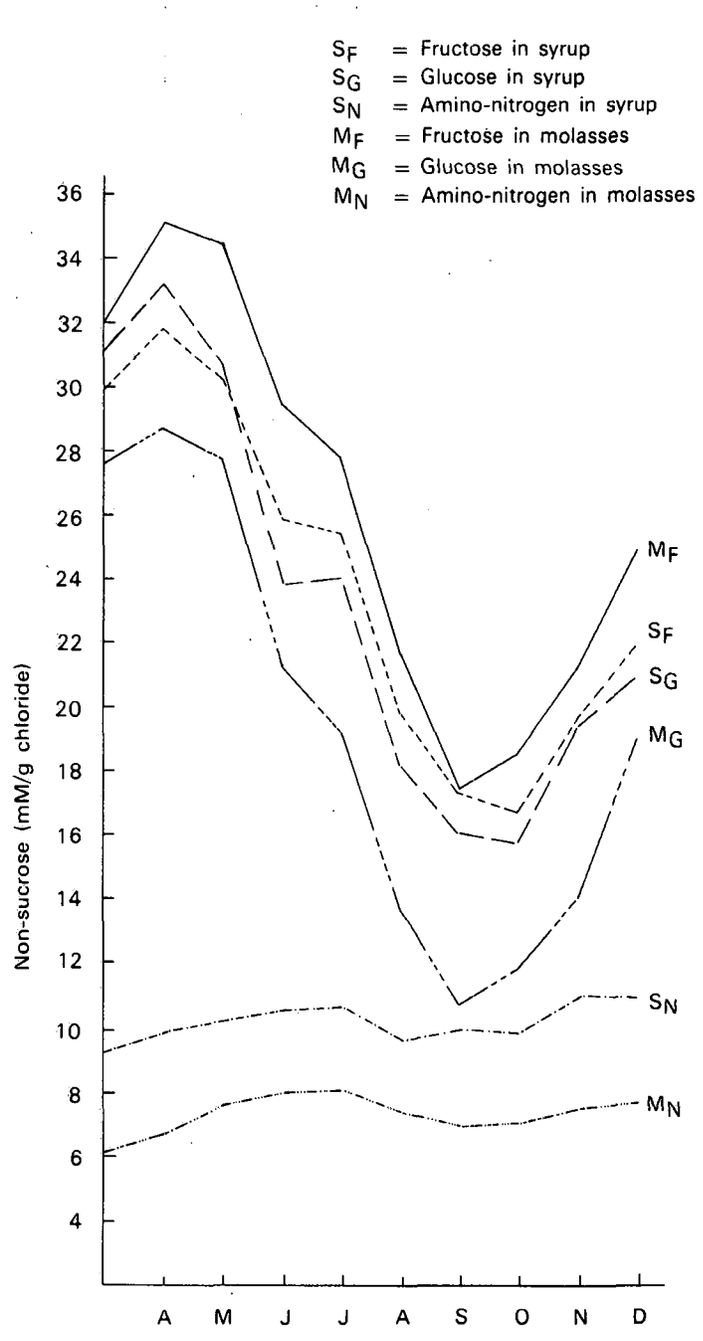

Figure 2: Comparison of non-sucrose in syrup and molasses – Mount Edgecombe (ME)

The effect of optically-active non-sucrose on pol –

The weekly pol, sucrose, fructose and glucose data have been summarised and are presented in Table 8. Jaagbaan has been included for molasses comparisons.

We have demonstrated that considerable changes in non-sucrose occurred during sugar boiling. Any changes in polarising properties during this process will affect the pol in molasses and hence the pol-based factory balance. Fructose and glucose levels are generally high in molasses and the highly laevorotatory fructose will have a depressing effect on the pol. Kort[10] found no correlation between either the level of reducing sugars, fructose, glucose or F/G ratios and the difference between sucrose and pol.

The polarising properties of the individual non-sucrose sugars have been well-documented[24] and their contribution to the pol can be derived from the following relationship:

$$Pol_{experimental} = Pol_d + Pol_x$$

where

$$Pol_d = Pol_{sucrose} + Pol_{glucose} + Pol_{fructose}$$

(See Appendix 3)

and Pol_x is the contribution to pol from optically-active impurities other than fructose and glucose.

Pol_{exp} and P_d were then subjected to regression analysis (Table 9).

TABLE 8

Syrup and final molasses analysis

Mill	Product	Analysis	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
EM	Syrup	Pol	–	–	54,7	55,9	56,9	56,8	57,3	57,9	54,3	52,2
		S_{gc}	–	–	55,6	56,3	57,2	56,6	57,3	57,7	54,7	52,4
		F	–	–	1,34	1,23	1,25	1,20	1,22	1,17	1,37	1,54
		G	–	–	1,37	1,20	1,22	1,17	1,24	1,23	1,49	1,59
		Pol_d	–	–	54,9	55,6	56,5	55,9	56,7	57,1	54,0	51,7
		Pol/S_{gc}	–	–	0,98	0,99	1,00	1,00	1,00	1,00	0,99	1,00
		Pol/Pol_d	–	–	1,00	1,00	1,01	1,02	1,01	1,01	1,01	1,01
		F/G	–	–	0,98	1,03	1,02	1,03	0,98	0,95	0,92	0,97
	Molasses	Pol	–	30,3	28,4	29,1	30,5	29,8	30,7	30,6	31,1	30,4
		S_{LE}	–	32,7	31,3	31,4	32,9	32,3	32,8	32,6	33,9	33,0
		S_{gc}	–	31,4	29,8	30,7	31,7	31,2	31,0	30,9	31,9	31,3
		F	–	8,5	8,6	6,8	7,0	6,5	6,3	6,1	6,6	6,9
		G	–	7,8	7,4	6,0	5,5	4,5	4,3	4,6	5,2	5,5
		Pol_d	–	26,1	24,0	26,3	26,6	26,0	25,9	26,3	27,1	26,3
		Pol/S_{gc}	–	0,97	0,95	0,95	0,96	0,96	0,99	0,99	0,97	0,97
		Pol/Pol_d	–	1,17	1,18	1,11	1,15	1,15	1,19	1,16	1,15	1,16
		S_{LE}/S_{gc}	–	1,04	1,05	1,02	1,04	1,04	1,06	1,06	1,06	1,05
		F/G	–	1,09	1,16	1,13	1,27	1,44	1,47	1,33	1,27	1,25
ME	Syrup	Pol	54,3	55,6	57,3	58,4	57,7	58,6	58,7	58,1	57,3	55,9
		S_{gc}	54,6	56,1	57,9	59,3	58,5	58,9	58,8	58,2	57,9	56,3
		F	1,86	1,92	1,90	1,77	1,86	1,47	1,20	1,17	1,46	1,63
		G	1,94	2,00	1,93	1,63	1,76	1,34	1,11	1,10	1,44	1,55
		Pol_d	53,7	55,1	56,9	58,2	57,4	58,0	58,1	57,5	57,1	55,4
		Pol/S_{gc}	1,00	0,99	0,99	0,99	0,99	1,00	1,00	1,00	0,99	0,99
		Pol/Pol_d	1,01	1,01	1,01	1,00	1,01	1,01	1,01	1,01	1,00	1,01
		F/G	0,96	0,96	0,98	1,09	1,06	1,10	1,08	1,06	1,01	1,05
	Molasses	Pol	23,9	22,8	22,7	24,1	23,3	25,9	29,7	27,7	25,7	28,7
		S_{LE}	29,1	28,1	28,3	29,7	28,9	29,7	32,0	30,6	29,6	31,7
		S_{gc}	27,7	27,1	27,1	28,2	27,4	28,1	30,0	28,4	27,5	30,5
		F	10,2	11,0	11,4	10,7	10,0	8,1	6,4	7,0	7,8	8,3
		G	8,8	9,0	9,2	7,7	6,9	5,1	3,9	4,4	5,1	6,3
		Pol_d	20,8	19,2	18,8	19,7	19,2	21,2	24,5	22,4	21,0	24,2
		Pol/S_{gc}	0,86	0,84	0,84	0,86	0,85	0,92	0,99	0,98	0,93	0,94
		Pol/Pol_d	1,15	1,19	1,21	1,22	1,21	1,22	1,21	1,24	1,22	1,19
		S_{LE}/S_{gc}	1,05	1,04	1,04	1,05	1,05	1,06	1,07	1,08	1,08	1,04
		F/G	1,16	1,22	1,24	1,39	1,45	1,59	1,64	1,59	1,53	1,32
JB	Molasses	Pol	–	22,3	21,7	21,8	21,3	22,2	25,9	26,5	25,4	
		S_{LE}	–	27,3	27,0	27,0	27,3	27,9	29,5	30,1	29,8	
		S_{gc}	–	26,3	26,1	26,2	26,3	27,0	27,9	28,4	27,9	
		F	–	9,1	9,6	9,3	10,1	9,2	7,7	7,7	7,7	
		G	–	5,5	5,5	5,5	5,3	4,0	3,2	3,4	3,5	
		Pol_d	–	18,3	17,4	17,9	16,7	17,7	20,0	20,7	20,3	
		Pol/S_{gc}	–	0,85	0,83	0,83	0,81	0,82	0,93	0,93	0,91	
		Pol/Pol_d	–	1,22	1,25	1,22	1,28	1,25	1,30	1,28	1,25	
		S_{LE}/S_{gc}	–	1,04	1,03	1,03	1,04	1,03	1,06	1,06	1,07	
		F/G	–	1,65	1,75	1,69	1,91	2,30	2,41	2,26	2,20	

Key: S_{gc} = sucrose determined by gas chromatography
Pol_d = Pol derived from sucrose, glucose and fructose (explanation follows in text)
S_{LE} = sucrose determined by Lane & Eynon after acid hydrolysis.
F = fructose
G = glucose

TABLE 9

Correlation between Pol and Pol_d

Mill	n(prs)	Linear regression	s.d. (slope)	r
EM	39	$P = 9,42 + 0,80\ P_d$	± 0,14	0,89
ME	39	$P = 1,13 + 1,16\ P_d$	± 0,11	0,96
JB	8	$P = -2,69 + 1,40\ P_d$ ...	± 0,32	0,97
EM + ME	78	$P = 5,07 + 0,97\ P_d$	–	0,97
EM+ ME +JB	86	$P = 5,18 + 0,96\ P_d$	± 0,05	0,97

P = measured pol
P_d = pol derived from sucrose, glucose and fructose
s.d. = standard deviation
r = correlation coefficient

A strong correlation was found for all mills (r = 0,97). This implies that the main non-sucrose contribution to pol is due to fructose and glucose and serves to underline the fact that pol is a poor estimate of sucrose for low-purity products.

The Lane and Eynon chemical estimation consistently overestimates sucrose, since it includes any non-reducing compounds which yield, after hydrolysis, substances reducing to Fehling's solution. Kort[10] found most, but not all, of this difference was due to kestoses as the major oligosaccharide impurity. Since 1% kestose will inflate the pol by 0,4 units, we can approximate the kestose content to the difference between SLE and S_{gc} (Table 10).

Pol_X can then be partially explained:

$$Pol_X = Pol_{kestose} + Pol_X{}^1$$

Data for the three mills are summarised in Table 11.

Pol_X was remarkably similar at each of the three mills and showed little seasonal variation (4,4 ± 0,7%) as did $pol_X{}^1$ (3,8 ± 0,6%.)Although pol_X and $pol_X{}^1$ were always dextrorotarory, it is highly unlikely that the non-sucrose responsible was a single constituent.

Results presented in Table 12 indicate that some of this unaccounted pol was formed during sugar-boiling.

TABLE 10

($S_{LE} - S_{gc}$) for molasses for the 1977/78 Season.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mean	s.d.
EM	–	1,3	1,5	0,7	1,2	1,1	1,8	1,7	2,0	1,7	1,4	0,4
ME	1,4	1,0	1,2	1,5	1,5	1,6	2,0	2,2	2,1	1,2	1,6	0,4
JB	–	1,0	0,9	0,8	1,0	0,9	1,6	1,7	1,9	–	1,2	0,4

TABLE 11

$Pol_{non\text{-}sucrose}$ for molasses for the 1977/78 Season.

Mill		April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mean	s.d.
EM	$Pol - S_{gc}$	–	-1,1	-1,4	-1,6	-1,2	-1,4	-0,3	-0,3	-0,8	-0,9	-1,0	0,5
	Pol_{F+G}	–	-5,3	-5,8	-4,4	-5,1	-5,2	-5,1	-4,6	-4,8	-5,0	-5,0	0,4
	Pol_X	–	4,2	4,4	2,8	3,9	3,8	4,8	4,3	4,0	4,1	4,0	0,6
	Pol_k	–	0,6	0,6	0,3	0,5	0,5	0,8	0,7	0,9	0,7	0,6	0,2
	$Pol_X{}^1$	–	3,6	3,8	2,5	3,4	3,3	4,0	3,6	3,1	3,4	3,4	0,4
ME	$Pol-S_{gc}$	-3,8	-4,3	-4,4	-4,1	-4,1	-2,2	-0,3	-0,7	-1,8	-1,8	-2,8	1,6
	Pol_{F+G}	-6,9	-7,9	-8,3	-8,5	-8,2	-6,9	-5,5	-6,0	-6,5	-6,3	-7,1	1,1
	Pol_X	3,1	3,6	3,9	4,4	4,1	4,7	5,2	5,3	4,7	4,5	4,4	0,7
	Pol_k	0,6	0,4	0,5	0,6	0,6	0,7	0,9	0,9	0,9	0,5	0,7	0,2
	$Pol_X{}^1$	2,5	3,2	3,4	3,8	3,5	4,0	4,3	4,4	3,8	4,0	3,7	0,6
JB	$Pol-S_{gc}$	–	-4,0	-4,4	-4,4	-5,0	-4,8	-2,0	-1,9	-2,5	–	-3,6	1,3
	Pol_{F+G}	–	-8,0	-8,7	-8,3	-9,6	-9,3	-7,9	-7,7	-7,6	–	-8,4	0,8
	Pol_X	–	4,0	4,3	3,9	4,6	4,5	5,9	5,8	5,1	–	4,8	0,7
	Pol_k	–	0,4	0,4	0,3	0,4	0,4	0,7	0,7	0,8	–	0,5	0,2
	$Pol_X{}^1$	–	3,6	3,9	3,6	4,2	4,1	5,2	5,1	4,3	–	4,3	0,6
Mean	$Pol-S_{gc}$	–	–	–	–	–	–	–	–	–	–	-2,4	1,6
	Pol_{F+G}	–	–	–	–	–	–	–	–	–	–	-6,8	1,6
	Pol_X	–	–	–	–	–	–	–	–	–	–	4,4	0,7
	Pol_k	–	–	–	–	–	–	–	–	–	–	0,6	0,2
	$Pol_X{}^1$	–	–	–	–	–	–	–	–	–	–	3,8	0,6

Key: S_{gc} = sucrose determined by gas chromatography; F = fructose; G = glucose
Pol_X, $Pol_X{}^1$ } See text
$Pol_k = Pol_{kestose}$

TABLE 12

Change in Pol_X during sugar boiling. (Pol_X expressed relative to chloride)

Mill	Product	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mean	s.d.
EM	Syrup ...			-0,5	0,7	1,0	-0,2	1,4	1,9	0,7	1,1	0,8	0,8
	Mol.			2,0	1,3	1,7	1,7	2,2	2,1	1,9	2,1	1,9	0,3
ME	Syrup ...	0,6	0,5	0,4	0,2	0,3	0,6	0,6	0,6	0,2	0,5	1,6	1,2
	Mol.	1,8	2,1	2,1	2,2	2,1	2,3	2,6	2,5	2,3	2,4	2,2	0,2

Conclusions

It has been demonstrated that gas chromatography can be used routinely to obtain information about specific sugars during processing.

The stability of frozen syrup and molasses samples was such that sampling frequency could be reduced. However, extended sampling frequencies will mask short term variations.

During the season the relative proportions of the various non-sucrose constituents in syrup altered. The scope of this investigation did not indicate whether these variations were evident in the incoming cane. In the coming season we hope to monitor the F/G and (F+G)/RS ratios in mixed juice and to compare these ratios with those in syrup.

There was an increase of fructose over boiling which may have been due to sucrose hydrolysis. The largest change in non-sucrose during boiling was found to be glucose losses of up to 35% between syrup and molasses.

Almost one-third of the amino-nitrogen in syrup was destroyed during boiling. No obvious correlation could be established between glucose loss and amino-nitrogen levels.

Glucose and fructose were shown to have the largest influence on pol in molasses. 'Unaccounted' pol was approximately constant at about 4 pol units for both mills throughout the season. Some of this optical-activity was generated during the boiling process.

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Appendix 1

Procedure for glucose, fructose and sucrose in factory products.

Materials:

Fructose (B.D.H. low in glucose)
Glucose (B.D.H. Analar)
Sucrose (B.D.H. Aristar)
Xylose (B.D.H. Biochemical)
Trehalose (B.D.H. Biochemical)

(Note: All reference sugars were dried in vacuo over phosphorus pentoxide)

Pyridine (Merck for Analysis)
Hydroxylamine hydrochloride (M & B. Lab. reagent)
Dimethyl-amino-ethanol (B.D.H. Lab. reagent)
Hexamethyldisilazane (HMDS) (Ohio Valley)
Trifluoroacetic acid (T.F.A.) (Pierce)
Benzoic acid (B.D.H. Lab. reagent)

Reagents:

A – Sodium benzoate:- 0,3% aqueous solution, pH is adjusted to 6,9 ± 0,1 with sodium hydroxide.

B – Hydroxylamine:- Hydroxylamine hydrochloride (2,5 g) dissolved in pyridine (100 mls).

C – Oximation reagent:- Dimethyl-amino-ethanol (270 µℓ) is added to reagent B (5 ml) just before use.

Procedure:

1. Sample preparation

Duplicate samples with internal standards are massed into dry 6 ml hypovials and sodium benzoate (reagent A) added:

	Syrup	Molasses	
Sample (g)	0,50	2,00	
Xylose (mg)	*12-20	a) 200	b) 100
Trehalose (mg)	280	520	
Reagent A (ml)	*0,5	2,0	

Notes: * Added volumetrically:- 150 - 250 µℓ of 4% xylose solution and the volume of sodium benzoate adjusted so that the total volume added is 0,5 ml.

The vials are sealed with parafilm and shaken until the sugars dissolve.

2. Preparation of reference sugar solutions.

The following sugars are massed into dry 6 ml hypovials according to the product being analysed and the corresponding amount of sodium benzoate (reagent A) added:

Prepared for:	Syrup	Molasses	
		**(a)	(b)
Xylose (mg)	*12-20	200	100
Fructose (mg)	*12-20	220-170	125-100
Glucose (mg)	*12-20	180-150	100-70
Sucrose (mg)	550-590	520-600	
Trehalose (mg)	560	520	
Reagent A (ml)	1,4	2,4	

Notes: * Added volumetrically:- 300-500 µl of either i) 4% xylose or ii) 4% glucose and 4% fructose. The volume of sodium benzoate added is adjusted so that the total volume added is 1,4 ml.

** a) is used when reducing sugar levels are 12-20% and b) for lower reducing sugar levels.

Calibration standards are prepared so that the sample range is bracketed.

The vials are sealed with parafilm and shaken until the sugars have dissolved.

3. Oximation

Aliquots (5 µl) of the reference standard and sample solutions are placed in 3 ml screw-cap vials. Oximation reagent (reagent C) (0,5 ml) is added and the vials well shaken at 80°C in an ultrasonic bath (10 mins).

TABLE 13

G.c.	Varian 2700	H.P. 5840
Column	1 metre x 3 mm OD S.S. packed with 3% OV-17 on Chromosorb W (HP) 80/100 mesh.	50 metres x 0,5 mm I.D. S.S. coated with OV-17
Injection temperature (°C)	250	250
Detector temperature (°C)	300	250
N_2 flow rate	25 ml min^{-1}	Column: 3 ml min^{-1} Vent: 200 ml min^{-1} Make-up: 47 ml min^{-1}
H_2 flow rate	33 ml min^{-1}	39 ml min^{-1}
Air	500 ml min^{-1}	240 ml min^{-1}
Injection Volume	1 µl (manual)	10 µl – split ratio 70 : 1
Initial temperature	120°C	180°C
Final temperature	275°C	250°C
Programming rate	15° min^{-1}	12° min^{-1}
Integrator	HP 3380 S	
Slope sensitivity	0,3 mV min^{-1}	0,3 (G,F) 0,75 (S)

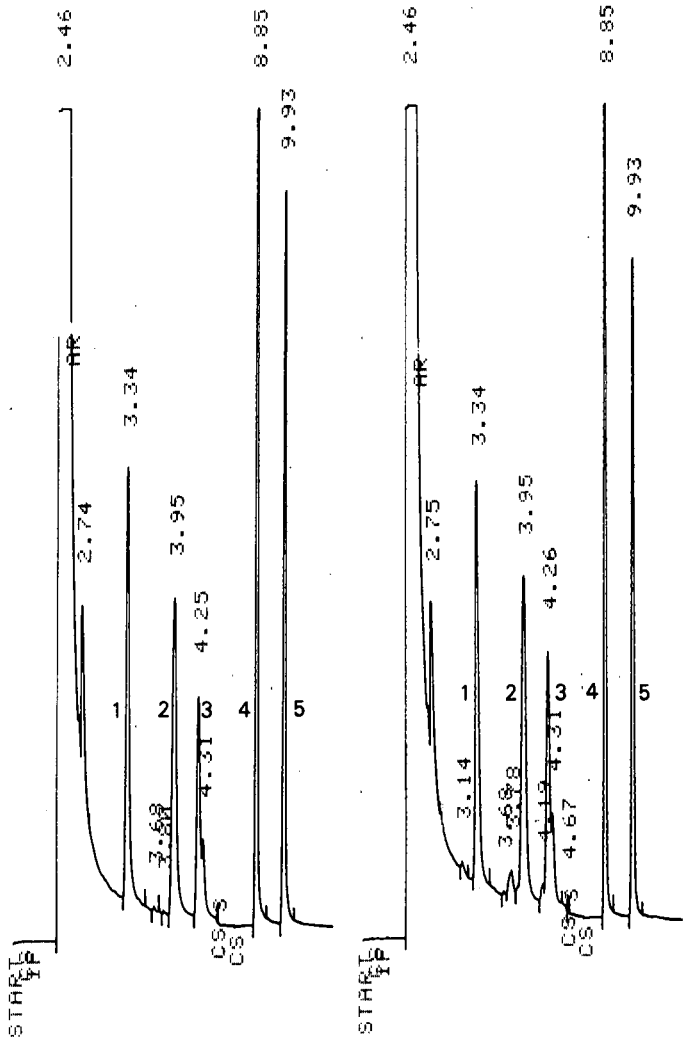
4. Silylation

The vials are cooled, HMDS (0,45 ml) and TFA (50µl) added and placed in an ultrasonic bath at 80°C (10 mins). After cooling and allowing the precipitate to settle, the supernatant is transferred to 2 ml hypovials which are then sealed.

5. G.c. separation

Separations were achieved using OV-17 as stationary phase. Columns were either packed conventionally or WCOT columns. G.c. conditions are summarised in Table 13.

Typical g.c. separations are illustrated in Fig. 3.



(a) Calibration standard (b) Molasses sample

1 = xylose (monosaccharide internal standard)
2 = fructose
3 = glucose (partially split peak)
4 = sucrose
5 = trehalose (disaccharide internal standard)

Figure 3: Typical g.c. separations

Appendix 2

Procedure for amino-nitrogen in syrup and molasses.

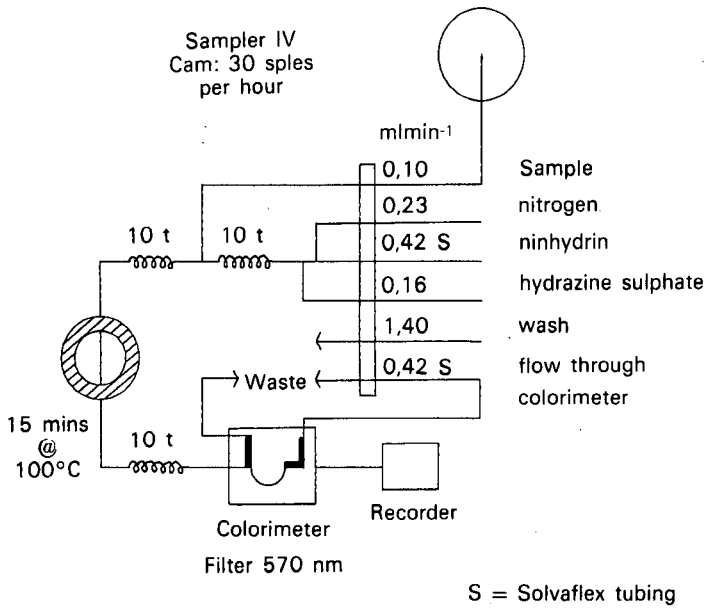


Figure 4: AutoAnalyser Manifold for amino-nitrogen

Materials:

Ninhydrin (B.D.H. Lab. reagent)
Hydrazine sulphate (M & B Lab. reagent)
Sodium acetate trihydrate (B.D.H. Lab. reagent)
Acetic acid (B.D.H. Analar)
2-Methoxyethanol (Methyl Cellosolve) (Merck for synthesis)
Aspartic acid (B.D.H. Biochemical)
Asparagine (B.D.H. Biochemical)

Reagents:

A – Sodium acetate buffer (pH 5,5):- Dissolve sodium acetate trihydrate (272 g) in distilled water (200 ml), warming if necessary. Cool and add acetic acid (glacial) (50 ml). Dilute to 500 ml. Adjust pH to 5,5 with sodium hydroxide if necessary.

B – Hydrazine sulphate (2 mM):- Hydrazine sulphate (0,1301 g) dissolved in distilled water and made up to 500 ml with distilled water.

C – Ninhydrin reagent:- Ninhydrin (1 g) is dissolved in peroxide free methyl cellosolve (10 ml), reagent A (75 ml) added and diluted to 100 ml with distilled water.

Calibration standards:

Aqueous solutions containing 1-5 g ml^{-1} of aspartic acid or asparagine are freshly prepared.

Procedure:

Syrup samples (1 g) are diluted to 200 mls with distilled water and molasses samples (1 g) to 500 mls.

A Technicon AutoAnalyser is plumbed as indicated in Figure 4.

Standards and diluted samples are placed in sample cups and analysed directly. The mean response for asparagine and aspartic acid is used for calibration.

Appendix 3

Calculation of Pol_d

For 26 g of sample dissolved in 100 mls of solution at 20°C and read in a 20 cm polarising tube:

$$Pol_d = \%\ S_{gc} + 0{,}015 \left\{ [\alpha]^{20}_{D_G} \%G + [\alpha]^{20}_{D_F} \%F \right\}$$

Values for $[\alpha]^{20}_{D_G}$ and $[\alpha]^{20}_{D_F}$

were obtained from Bates[24]

$$[\alpha]^{20}_{D_G} = 52{,}50 + 0{,}0188\ (\%G \text{ by wt}) + 0{,}00517\ (\%G \text{ by wt})^2$$

$$[\alpha]^{20}_{D_F} = -\left\{88{,}12 + 0{,}260\ (\%F \text{ by wt})\right\}\ (F < 10\%)$$

1% of the major sugars will affect the pol approximately as follows:-

Sucrose + 1,0
Glucose + 0,8
Fructose – 1,3
Kestose + 0,4