ANALYSIS OF FINAL MOLASSES FOR SUCROSE AND POL

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Abstract

Final molasses from several factories were analysed for sucrose by isotope dilution, gas chromatography and the chemical method and pol was determined after clarification with 10 g of dry basic lead acetate. The results were compared and it was concluded that, while GC is the method of choice, the chemical method, corrected for kestose, is acceptable for routine purposes. No information on sucrose content of final molasses can be obtained from pol readings.

Introduction

As a result of an investigation[1] in which several methods for sucrose determination in final molasses proposed by ICUMSA[2] were compared and after an interlaboratory study on the reproducibility,[3] the chemical method (modified Mackay Research Institute Method) was recommended for use in South Africa.

Since no correction for trisaccharides present in molasses is applied to the results obtained by this method, sucrose in molasses is always higher than the value determined by isotope dilution.[1] This and the fact that a correction for suspended solids in mixed juice was introduced two years ago lead to the anomaly that the calculated undetermined losses sometimes had negative values. It has therefore been suggested that pol rather than sucrose in molasses should be used for factory balance purposes.

This investigation was undertaken in order to test the validity of the suggestion. For this purpose samples of final molasses from six selected factories were analysed throughout last season for sucrose by chemical method, isotope dilution and gas chromatography and pol of these samples was determined under standardised conditions. In addition, the molasses were analysed for glucose, fructose, kestose and reducing sugars. The investigation was extended to cover the influence which dry basic lead acetate has on polarisation of molasses.

Experimental

Composite weekly samples of final molasses, representing the third week of every month, were collected from DL, JB, ML, TS, UF and UK. These mills represented a fair selection on a geographical basis as well as in terms of the RS/ash ratios of their molasses. Altogether 51 samples were collected from May, 1974 to January, 1975.

The samples were warmed to 50°C and thoroughly mixed before subsamples were taken for various analyses.

Sucrose by chemical method

The Mackay Research Institute inversion method[4] as modified by Beams and MacGillivray[1] was used.

Sucrose by isotope dilution

The method described by Bruijn and Carreyett[5] was used. The analysis was done in duplicate and the average value reported.

Sucrose by gas chromatography

Sucrose as a TMS derivative was determined using a capillary column coated with OV17 as described by Nurok and Reardon.[6] The analysis was done in duplicate and the average value reported.

Pol

Unless otherwise stated, 50 g of molasses were made up to 250 g with water. A 100 ml aliquot was diluted to 200 ml,

TABLE 1

Final molasses analysis for sucrose by chemical method, isotope dilution and gas chromatography and Pol.

Mill	Method	May	June	July	August	September	October	November	December	January
DL	Pol	28,30	27,55	29,00	26,70	28,45	27,70	27,65	31,05	28,70
	Chem.	33,46	32,75	33,30	32,29	32,92	32,47	32,20	33,45	31,53
	I.D.	31,15	31,04	31,44	30,64	32,29	31,72	31,42	32,26	30,56
	G.C.	29,80	30,78	30,49	30,00	31,29	30,68	30,40	31,52	29,71
JB	Pol	23,30	22,25	24,20	24,45	22,30	21,05	23,00	26,50	27,55
	Chem.	28,79	29,50	30,48	30,51	29,29	29,61	30,30	30,92	32,80
	I.D.	27,57	27,65	29,14	29,66	28,82	28,25	28,86	30,21	31,09
	G.C.	26,86	27,78	29,11	28,94	28,19	27,17	28,27	29,78	30,69
ML	Pol	32,20	26,85	26,75	28,10	28,80	29,80	31,08	31,70	29,55
	Chem.	36,77	30,90	32,11	32,46	33,50	34,30	34,78	33,88	32,26
	I.D.	32,22	31,08	31,14	32,69	32,65	33,37	33,95	34,09	31,88
	G.C.	34,55	30,65	30,78	31,99	31,82	32,51	33,19	33,97	31,19
TS	Pol	28,55	29,85	30,30	28,15	27,05	30,20	32,50	31,60	28,75
	Chem.	32,59	33,71	33,70	32,03	30,90	33,15	33,87	32,97	31,15
	I.D.	30,39	31,18	31,90	30,71	30,26	31,51	32,89	31,83	29,87
	G.C.	30,12	31,19	31,65	30,16	29,74	30,76	32,13	31,50	29,40
UF	Pol		35,85	35,10	34,15	34,60	36,00	35,40	35,70	32,85
	Chem.		37,56	38,31	37,50	36,57	39,08	38,01	37,92	35,08
	I.D.		37,42	36,51	36,64	35,95	37,67	36,65	36,12	34,47
	G.C.		37,15	37,33	36,13	35,86	37,57	36,44	36,00	34,15
UK	Pol		28,20	27,15	27,20	28,45	29,20	30,05	30,85	
	Chem.		30,65	29,82	30,25	30,70	31,25	31,26	32 43	
	I.D.		29,42	28,65	29,48	30,61	30,62	30,75	31,76	
	G.C.		29,50	28,46	28,94	29,77	29,62	30,20	31,45	

TABLE 2

Final molasses analysis for reducing sugars, fructose, glucose and kestose.

Mill	Analysis	May	June	July	August	September	October	November	December	January
DL	Kes.	0,88	0,91	0,95	1,05	1,31	1,46	1,66	1,57	1,59
	Fruct.	8,6	6,7	7,6	9,1	7,8	8,9	9,3	7,1	8,0
	Gluc.	3,7	4,2	4,7	4,9	4,1	5,3	4,6	3,9	4,5
	F / G	2,3	1,6	1,6	1,9	1,9	1,7	2,0	1,8	1,8
	R.S.	17,07	15,53	16,21	18,18	18,76	22,63	23,51	16,65	19,06
JB	Kes.	0,57	0,56	0,53	0,52	0,60	0,90	0,99	0,92	0,84
	Fruct.	8,0	8,4	7,9	8,2	7,6	8,1	7,8	5,9	6,8
	Gluc.	3,4	3,8	2,9	2,7	2,0	2,7	1,2	1,0	2,2
	F / G	2,4	2,2	2,7	3,0	3,8	3,0	6,6	5,9	3,1
	R.S.	16,94	17,74	15,76	16,92	17,43	19,20	16,91	13,50	14,99
ML	Kes.	0,64	0,56	0,62	0,66	0,68	0,83	0,86	0,90	0,69
	Fruct.	9,5	11,0	11,3	10,8	8,7	7,3	6,5	8,4	7,6
	Gluc.	7,3	12,6	9,9	8,2	3,8	4,2	2,8	4,7	4,7
	F / G	1,3	0,9	1,2	1,3	2,3	1,7	2,3	1,8	1,6
	R.S.	20,38	23,94	24,00	23,26	19,60	18,35	16,66	19,00	18,81
TS	Kes.	0,80	0,77	0,75	0,86	0,89	1,29	1,30	1,21	1,30
	Fruct.	10,1	8,8	7,9	9,6	12,2	8,0	6,9	6,8	8,4
	Gluc.	7,5	7,1	6,7	6,3	9,5	5,1	3,7	4,1	4,9
	F / G	1,4	1,2	1,2	1,5	1,3	1,6	1,9	1,7	1,7
	R.S.	20,31	18,61	17,36	19,99	20,11	19,42	17,33	16,93	18,74
UF	Kes.		0,66	0,72	0,74	0,79	0,99	0,98	1,03	0,91
	Fruct.		7,1	7,9	7,6	6,5	6,2	10,0	7,0	7,8
	Gluc.		4,0	3,9	4,2	3,7	2,7	6,2	4,0	5,0
	F / G		1,8	2,0	1,8	1,5	2,3	1,6	1,8	1,5
	R.S.		14,05	14,66	16,07	15,25	15,31	15,51	16,36	19,03
UK	Kes.		0,44	0,59	0,58	0,61	0,76	0,72	0,77	
	Fruct.		6,9	5,6	7,5	6,5	5,6	5,6	6,2	
	Gluc.		4,5	3,2	3,4	3,0	2,5	2,5	3,4	
	F / G		1,5	1,8	2,2	2,2	2,2	2,2	1,8	
	R.S.		15,71	13,51	15,61	14,62	12,79	13,20	12,45	

10 g of dry basic lead acetate were added and the clarified solution was filtered and polarised using a Saccharomat I.

Reducing sugars

These were determined by Lane and Eynon titration using an aliquot of the sample prepared for analysis of sucrose by the chemical method.[1]

Glucose and Fructose

These monosaccharides were converted into oximes before silylation and were determined by gas chromatography using a capillary column coated with OV17 as described by Nurok and Reardon.[6]

Kestose

This trisaccharide was determined as a TMS derivative by gas chromatography using a two phase system and conditions described by Nurok.[6,7]

Results and discussion

The results of sucrose analysis in molasses by the three methods as well as the pol determinations are reproduced in Table 1. Reducing sugars, glucose, fructose and kestose contents of these samples are given in Table 2.

Although the reproducibilities of isotope dilution and gas chromatographic methods were very similar (Mean Deviation of 51 pairs of duplicates 0,09% and 0,08% respectively), sucrose determined by the former method was on the average 0,5% higher. The difference in the results obtained by the two methods was shown by statistical analysis to be significant at the 99% level. The reason for this discrepancy was found by gas chromatographic analysis of the combined "pure" sucrose residues on which radioactive count was made. In the trisaccharide region (Fig. 1) kestose (about 0,4%) and small amounts of other unidentified compounds were detected, indicating that the steps used in the isotope dilution method for purification of sucrose are not sufficient to completely eliminate oligosaccharides present in molasses. As any impurity remaining in sucrose inflates the results of the isotope dilution method, the sucrose values obtained by this method were obviously too high. The results obtained by gas chromatography are therefore considered to be more accurate and were accepted in this work as true sucrose values.

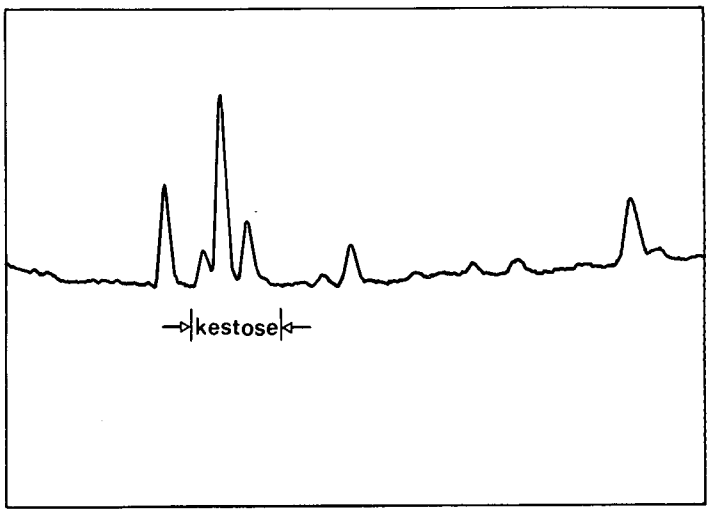

FIGURE 1 GC analysis in trisaccharide region of "pure" sucrose residues on which radioactive count was performed in I.D. method. Conditions as in Ref. 7.

Perusal of Table 1 confirms the previous findings[1, 11, 14] that the chemical method overestimates sucrose in molasses. The differences between the results of this method and sucrose as determined by gas chromatography are listed in Table 3. However, as kestose which is present in molasses also produces reducing sugars upon hydrolysis and will be recorded in the chemical method as sucrose, the results have to be corrected for kestose content of respective samples and these are given in Table 2. The correction factor is 1,02. Although the difference from true sucrose becomes considerably smaller after this correction, as is apparent from Table 3, it is not completely eliminated. This is possibly due to the presence in molasses of other substances which produce reducing sugars on hydrolysis. The mean and standard deviation of the differences for each mill before and after correction are given in Table 4.

TABLE 3

Difference between the chemical method (in bold corrected for kestose) and gas chromatographic method of sucrose determination.

	DL	JB	ML	TS	UF	UK
May	3,66	1,93	2,22	2,47	—	—
	2,76	**1,35**	**1,57**	**1,65**		
June	1,97	1,72	0,25	2,52	0,41	1,15
	1,04	**1,15**	**-0,32**	**1,73**	**-0,26**	**0,70**
July	2,81	1,37	1,33	2,05	0,98	1,36
	1,84	**0,83**	**0,70**	**1,29**	**0,25**	**0,76**
August	2,29	1,57	0,47	1,87	1,37	1,31
	1,22	**1,04**	**-0,20**	**0,99**	**0,62**	**0,72**
September	1,63	1,10	1,68	1,16	0,71	0,93
	0,29	**0,49**	**0,99**	**0,25**	**0,10**	**0,31**
October	1,79	2,44	1,79	2,39	1,51	1,63
	0,30	**1,52**	**0,94**	**1,07**	**0,50**	**0,85**
November	1,80	2,03	1,59	1,74	1,57	1,06
	0,11	**1,02**	**0,71**	**0,41**	**0,57**	**0,33**
December	1,93	1,14	-0,09	1,47	1,92	0,98
	0,33	**0,20**	**-1,00**	**0,24**	**0,87**	**0,19**
January	1,82	2,11	1,07	1,75	0,93	
	0,20	**1,25**	**0,37**	**0,42**	**0,00**	

TABLE 4

Mean difference of various methods from GC results and standard deviations.

Difference		DL	JB	ML	TS	UF	UK
Chem. — GC	Mean	2,19	1,71	1,15	1,94	1,18	1,20
	St. Dev.	0,66	0,46	0,78	0,47	0,50	0,25
Corrected chem. — GC	Mean	0,90	0,99	0,42	0,89	0,33	0,55
	St. Dev.	0,91	0,42	0,79	0,59	0,38	0,26
GC — Pol	Mean	2,17	4,69	2,87	1,08	1,37	0,98
	St. Dev.	1,06	1,13	0,87	0,99	0,59	0,58

The concentration of kestose in molasses, as can be seen from Table 2, does not vary much from an average value of 0,9%. In view of this a simple correction by subtracting 1,0 from the results of the chemical method will give acceptable sucrose figures for routine purposes. It is therefore suggested that this procedure be adopted by mill laboratories, where specialised equipment required for kestose analysis is lacking.

The difference between pol of molasses when 10 g of lead is used for clarification and sucrose by GC are listed in Table 5 and the means of these differences and standard deviations in Table 4. As expected pol values are lower than sucrose but the magnitude of the difference and its fluctuations are surprisingly high and illustrate the great influence which impurities present in molasses have on pol reading.

TABLE 5

Difference between sucrose by gas chromatography and pol when 10g of lead is used for clarification.

	DL	JB	ML	TS	UF	UK
May	1,50	3,56	2,35	1,57	—	—
June	3,23	5,53	3,80	1,34	1,30	1,30
July	1,49	4,91	4,03	1,35	2,23	1,31
August	3,30	4,49	3,89	2,01	1,98	1,74
September	2,84	5,89	3,02	2,69	1,26	1,32
October	2,98	6,12	2,71	0,56	1,57	0,42
November	2,75	5,27	2,11	-0,37	1,04	0,15
December	0,47	3,28	2,27	-0,10	0,30	0,60
January	1,01	3,14	1,64	0,65	1,30	—

That optically active substances affect polarisation of sugar solutions is well known[8, 9]. Glucose and fructose, in view of their high concentration in final molasses, are of special interest in the context of this investigation. Fructose in particular, being strongly levorotatory, will profoundly influence pol readings. It is therefore of interest to note (Table 2) that fructose and not glucose is more abundant in molasses and that the amounts of these two monosaccharides as well as their ratio vary over a wide range. Consequently, the contribution of these substances to pol of molasses should differ from one sample to another. No correlation could be found however between either the amounts of reducing sugars, fructose, glucose or the F/G ratio and the difference between sucrose and pol as determined in this work.

The influence of lead used for clarification of molasses on various reducing sugars has also been studied in the past but the conclusions reached are somewhat contradictory. That lead suppresses levorotation of fructose and under certain conditions can even make fructose dextrarotatory seems to be well established, but to what extent, if at all, reducing sugars

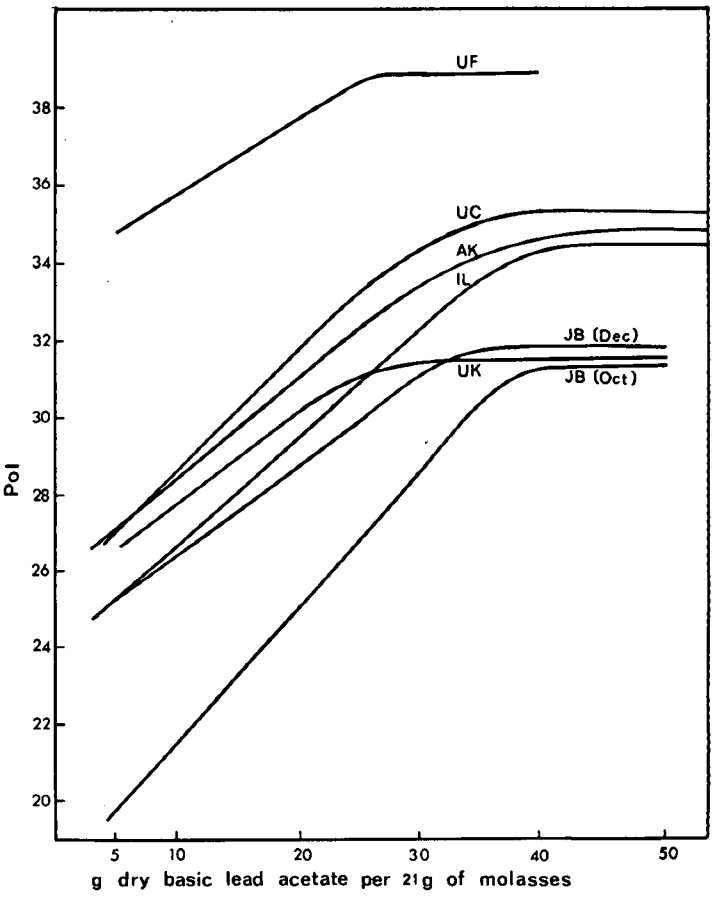

FIGURE 2 Influence of lead used in clarification on pol of final molasses

are precipitated and removed from solution is not quite clear[8, 9]. There is also no indication of exactly what amount of lead should be used for clarification purposes, "minimum quantity required for good clarification" being usually specified.

The effect of lead on pol in molasses is illustrated in Fig. 2 in which the pol readings obtained with molasses from several factories are plotted against the amount of lead used for clarification. An almost linear increase in polarisation is obtained with the increase of dry basic lead acetate until a plateau is reached. In Fig. 2 this can represent as much as 12 pol units. However, both the slope of the curves and the amount of lead required to reach the plateau vary from one sample to another. It is further apparent that the "minimum amount required" fluctuates in these examples between about 3 and 6 g of lead which in terms of pol represents nearly one unit.

The preliminary results with diluted molasses indicate that the quantity of fructose removed during clarification increases with the amount of lead used while glucose remains largely unaffected as shown in Table 6. When dilute acetic acid is added to filtered molasses solution in order to restore levorotation of fructose[8, 10] the obtained pol readings are dependent on the amount of lead originally used (Table 7).

TABLE 6

Removal of fructose and glucose during clarification.

Lead used in clarification (g)	Found in solution %	
	Fructose	Glucose
0	1,14	0,34
7	1,12	0,30
10	1,09	0,37
20	0,85	0,34
30	0,68	0,41
50	0,36	0,18

TABLE 7

Polarisation of molasses after addition of acetic acid.

ml of 20% acetic acid added	Horne's dry lead used for clarification (g)				
	5	6	7	8	10
0	25,55	25,80	26,05	26,80	27,75
2	24,45	24,80	25,20	25,30	25,55
4	24,35	24,70	24,95	25,30	25,55
6	24,35	24,70	24,95	25,20	25,55
8	—	24,70	24,95	24,90	25,55

Conclusions

It is apparent from the data presented that the GC method of sucrose determination in final molasses should be the method of choice. However, as mill laboratories are at present not equipped to carry out this analysis, the chemical method corrected by a subtraction of one from the obtained sucrose values, will give acceptable results for routine purposes. The resulting error will influence a Factory Balance by about 0,2% on the average.

While pol determination on factory products of high purity may be a reasonable substitute for sucrose analysis,[12] the results of this investigation support the view expressed by Clayton[13] that "the pol . . . of final molasses is practically meaningless". The amount and the composition of optically active impurities vary to such an extent in final molasses and the interaction of lead with these substances is so complex that there is no way in which sucrose content can be computed from pol readings.

It is further clear that if reproducible pol readings are to be achieved the quantity of lead in the clarification stage must be specified. Ten grams of dry basic lead acetate as used in this work are proposed for this purpose. Under these arbitrary conditions an error in a Factory Balance of between 0,3% and 1,5% can be expected.

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