

THE DETERMINATION OF SUCROSE IN FINAL MOLASSES

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Abstract

Three methods for the determination of sucrose in molasses recommended by I.C.U.M.S.A. are compared with two methods currently in use in South Africa, and the uncorrected results obtained by these methods are compared with the results obtained by the isotope-dilution method. The reproducibility and inter-analyst error of a simple acid inversion chemical method are assessed. Comments are made on the theoretical corrections for oligosaccharides in both chemical and polarimetric methods.

Introduction

During the 1970/71 season three samples of final molasses were circulated to all South African sugar factories for analysis, in order to assess the comparative value of the analytical data published weekly by the S.M.R.I. The results returned by the mills showed considerable scatter, particularly those for sucrose, pol and reducing sugars. Equally disturbing was the wide variety of methods which were found to be in use. Statistical analysis of the results confirmed that agreement within one laboratory was a great deal better than agreement between laboratories. This situation is not uncommon, and agrees with that found in Mauritius by Randabel⁸.

As a direct result of this survey a study was made of the methods for sucrose determination recommended by I.C.U.M.S.A.⁶ The methods included in this study were:

- (i) The isotope-dilution method (Sibley *et al*¹⁰).
- (ii) The polarimetric method proposed by Dutton⁶.
- (iii) The acid-inversion method proposed by Sugar Research Institute, Mackay⁶.
- (iv) The Canadian National Committee method⁵.
- (v) The Jackson and Gillis method IV (Laboratory Manual⁷), at present the most widely used method in South African mills.
- (vi) The S.M.R.I. acid-inversion method.

The Sugar Research Institute method, which we shall refer to as the Mackay method to avoid confusion with the S.M.R.I. method, appeared to be relatively quick and straightforward, at the same time yielding results comparable to those obtained by isotope-dilution. This method is therefore being studied further, with a view to recommending it for use in routine factory analysis.

Analytical Methods

1. Isotope-Dilution Method.

The method used was basically that described by Sibley, Eis and McGinnis¹⁰. Molasses containing ¹⁴C labelled sucrose was diluted and clarified with basic lead acetate in the usual way. After de-leading the filtrate the sucrose was precipitated as barium saccha-

rate. The sucrose was recovered from the washed precipitate by carbonation, and passed through a de-ionising column. The clear solution was evaporated to a syrup, from which sucrose was crystallized with absolute ethanol. After further purifying by recrystallization the activity of the pure sucrose was measured in a Beckmann β -mate II scintillation counter.

2. The Polarimetric Method proposed by Dutton.

This method followed the Clerget principle of determining the polarisation before and after inversion. The inversion was by invertase, at a controlled pH and temperature. The divisor, determined under experimental conditions, was found to be 1.31. Corrections for raffinose and kestose were included in the published method, but have been omitted in this work for reasons given in the discussion.

3. The Mackay Acid-Inversion Method.

As the name suggests, an acid-inversion stage was part of this method. Reducing sugars were determined by the Lane and Eynon method before and after inversion. Potassium oxalate was used as a sequesterant for calcium. No corrections were made for reducing substances other than sucrose, glucose and fructose.

4. The Canadian National Committee Method.

Reducing sugars were determined before and after inversion by the Lane and Eynon method but in this case E.D.T.A. was the sequesterant, and inversion was achieved with invertase.

5. The Jackson and Gillis Method IV.

The polarisation was measured before and after inversion by hydrochloric acid of known strength. To compensate for the influence of chloride ions on the optical rotation of the invert solution an appropriate volume of sodium chloride solution was added to the uninverted molasses solution before it was polarised.

6. The S.M.R.I. Method.

As this method has not been published as such it will be given in full.

26.00 g of molasses were weighed and dissolved in distilled water. The solution was transferred to a 200 ml volumetric flask, and made up to the mark. From this solution two 20 ml aliquots were pipetted into separate 200 ml standard flasks. One aliquot was set aside for total reducing sugar determination, and the remaining aliquot was made up to the mark for reducing sugar determination.

(a) *Reducing Sugars.* Determination was carried out by titrating the above solution against 10 ml Fehling solution according to the method of Lane and Eynon.

(b) *Total Reducing Sugars.* To the aliquot set aside above were added approximately 60 ml of

distilled water, and the flask and contents were heated in a water-bath to exactly 65°C. The flask was then removed from the water-bath and 10 ml HCl (S.G. 1,1029) were added immediately.

The contents were mixed by rotation and the flask was left to stand at room temperature for at least 30 minutes. After cooling the solution was made to the mark, and this solution was titrated against 25 ml Fehling's solution according to the Lane and Eynon method.

Results

Various aspects of Dutton's method and the Mackay method were investigated before carrying out comparative analyses.

1. Dutton's Method.

(a) Basic lead acetate solution.

It was reported⁶ that a solution containing 45% basic lead on total lead and with an S.G. of 1.25 caused sucrose to precipitate. The use of basic lead acetate with a basicity of 36% was recommended by Dutton, who gave a detailed description of the preparation of this solution. Analysis of the solution prepared in this way in our laboratory gave a basic lead content of 9,33 g/100 ml as PbO, with a calculated basicity of 38,8% on total lead. This solution was used throughout these experiments.

(b) The Influence of Inversion on the Rotation of Sucrose.

The change in rotation per original 1° rotation for sucrose was determined, and was initially found to be 1,317 under our conditions of inversion, using refined sugar. As this figure was not in agreement with the value of 1,31 reported by Dutton⁶ the effect of sucrose concentration was investigated. It was found that in the case of unbuffered solutions the scatter of results on sucrose concentrations ranging from 4,5 to 6,0% was quite wide. However, when the solutions were buffered with anhydrous disodium hydrogen phosphate the change in rotation for the same range in concentration was 1,311 per 1° original rotation. These results are shown in Table I.

TABLE I

Sucrose Concentration	Change in rotation per original 1°	
	Unbuffered	Buffered
6,0%	1,297	1,310
5,5%	1,300	1,311
5,0%	1,298	1,311
4,5%	1,333	1,312

(c) The Corrections for Raffinose and Kestose.

In Dutton's method the % sucrose is calculated as follows:

$$\% \text{ sucrose} = \frac{2P_1 - 4P_2 - A (\% \text{ raffinose}) - B (\% \text{ kestose})}{C}$$

where P₁ = polarisation before inversion.

P₂ = polarisation after inversion

$$A = \text{change in rotation per original } 1^\circ \text{ of rotation for raffinose} \times \frac{104,5}{66,5}$$

$$B = \text{change in rotation per original } 1^\circ \text{ of rotation for kestose} \times \frac{25,5}{66,5}$$

$$C = \text{change in rotation per original } 1^\circ \text{ of rotation for sucrose.}$$

Values for A, B and C quoted by Dutton were 0,75, 1,13 and 1,31 respectively.

Using the official method of Albon and Gross³ for the determination of raffinose and kestose, we were unable to obtain a good separation of sugars from our molasses samples. We consequently resorted to the thin-layer separation according to Schaffler and Jukes⁹, which gave results which could be estimated as three bands, viz. a neo-kestose band, a 1-kestose band, in which were included whatever raffinose and 6-kestose were present, and a third band containing higher oligosaccharides. However, for reasons discussed later we have not applied any corrections to our results, but have calculated the sucrose from

$$\% \text{ sucrose} = \frac{2P_1 - 4P_2}{C}$$

2. The Mackay Method.

(a) The importance of removing calcium from solutions to be titrated against Fehlings solution was investigated by determining the reducing sugars and sucrose of a molasses sample using varying amounts of potassium oxalate. Additional calcium was added to the sample to raise the calcium level to 5%, and the reducing sugars and sucrose were again determined with and without oxalate addition. The results are shown in Table II.

TABLE II

Conditions for Calcium Removal	No. of Analyses	Ave. Reducing Sugars	Ave. Total Invert	Ave. Sucrose
No Potassium Oxalate	3	14,14	53,41	37,31
1g Potassium Oxalate	8	14,49	53,77	37,33
2g Potassium Oxalate	3	14,40	53,75	37,38
<i>Calcium increased to 5%</i>				
No Potassium Oxalate	6	13,87	51,91	36,14
1g Potassium Oxalate	1	14,74	53,24	36,58

(b) One of the biggest variables under factory conditions of molasses analysis is the inversion pro-

cedure. The effect of this variable was studied as follows:

- (i) Inversion at 60°C was allowed to proceed for 5 minutes, the recommended time of 10 minutes, and for 15 minutes.
- (ii) Inversion for 10 minutes was carried out at 54°C, the recommended temperature of 60°C, and at 65°C.
- (iii) As the South African mill laboratories have been accustomed to doing inversions by the Walker method¹, in which the acid is added at exactly 65°C and the solution is then left to stand at room temperature for at least half an hour, that method was included in the comparison.

Results are shown on Table III.

TABLE III

Conditions of Inversion	No. of Analyses	Ave. Total Invert
5 mins at 60°C	2	53,87
10 mins at 60°C	8	53,78
15 mins at 60°C	2	53,86
10 mins at 54°C	4	53,11
10 mins at 60°C	8	53,78
10 mins at 65°C	4	53,72
Walker's method	4	53,65

(c) *Recovery Experiments.* The recovery of sucrose when known amounts of sucrose and invert were added to molasses was determined by both the Mackay method and Dutton's method. The results are shown in Table IV.

TABLE IV

Conditions	% Recovery of Sucrose	
	Mackay Method	Dutton's Method
Molasses + 2% invert	100,1	100,05
Molasses + 5% sucrose	100,5	100,5
Molasses + 2% invert + 5% sucrose	101,4	99,9
Molasses + 2% 60:40 glucose/fructose	—	100,3
Molasses + 2% 60:40 glucose/fructose + 5% sucrose	—	100,3

Comparative Analyses by all Methods under Investigation

Six samples of final molasses from various sources were analysed according to the six methods listed in the introduction. With the exception of the isotope-dilution method each sample was analysed five times by each method by an experienced analyst. In each case the analyst most familiar with a particular method was chosen. With regard to the isotope-dilution method, we had no prior experience with this method and were not able to assess our results statistically over any length of time. However, the fact that the results obtained by this method bore a more or less fixed relationship to the results obtained by more traditional methods bestowed a considerable degree of credibility on the isotope-dilution results.

Table V summarizes the results, which are also shown in figure 1.

Comparison between different Analysts using the Mackay Method

In order to assess the influence of different analysts on the Mackay method, five samples of final molasses were analysed in duplicate by five analysts, all using basically the same apparatus and reagents. The results of these analyses are shown in Table VI.

Discussion

The intention, when this investigation was started, was to find a method for sucrose which was both accurate and relatively simple, analytically. In South Africa we have a large number of factories publishing analytical results for comparative purposes. In order to get meaningful comparisons the methods must be specified in considerable detail, and must be such that they can be performed by relatively untrained staff. The determination of sucrose in final molasses is at present done at most mills by the Jackson and Gillis Method IV.

It is felt that under the variable conditions obtained in the factory laboratories polarimetric methods may be more liable to errors than chemical methods. The clarification of molasses solutions using varying amounts of lead acetate is a very real source of error in polarimetric methods. Others are the variations in temperature, the variable amounts of evaporation under different humidity conditions, polarimeter errors, both instrumental and reading, and the effects of optically active impurities. On the other hand the Lane and Eynon titration is not stoichiometric, and

TABLE V

Sample	Ave. Sucrose %					
	Isotope Dilution	Dutton's Method	Mackay Method	Canadian Nat. Com. Method	Jackson and Gillis	S.M.R.I. Method
A	36,90	36,50	37,25	37,63	36,40	37,44
B	36,84	37,42	37,65	38,94	37,25	38,37
C	38,85	39,74	40,08	40,94	40,75	40,23
D	34,38	34,93	35,54	36,52	34,13	36,28
E	33,04	33,82	34,20	36,20	33,90	35,27
F	36,14	36,22	37,33	—	—	—
Average Standard Error	—	0,06	0,27	0,22	0,59	0,27

FIGURE 1

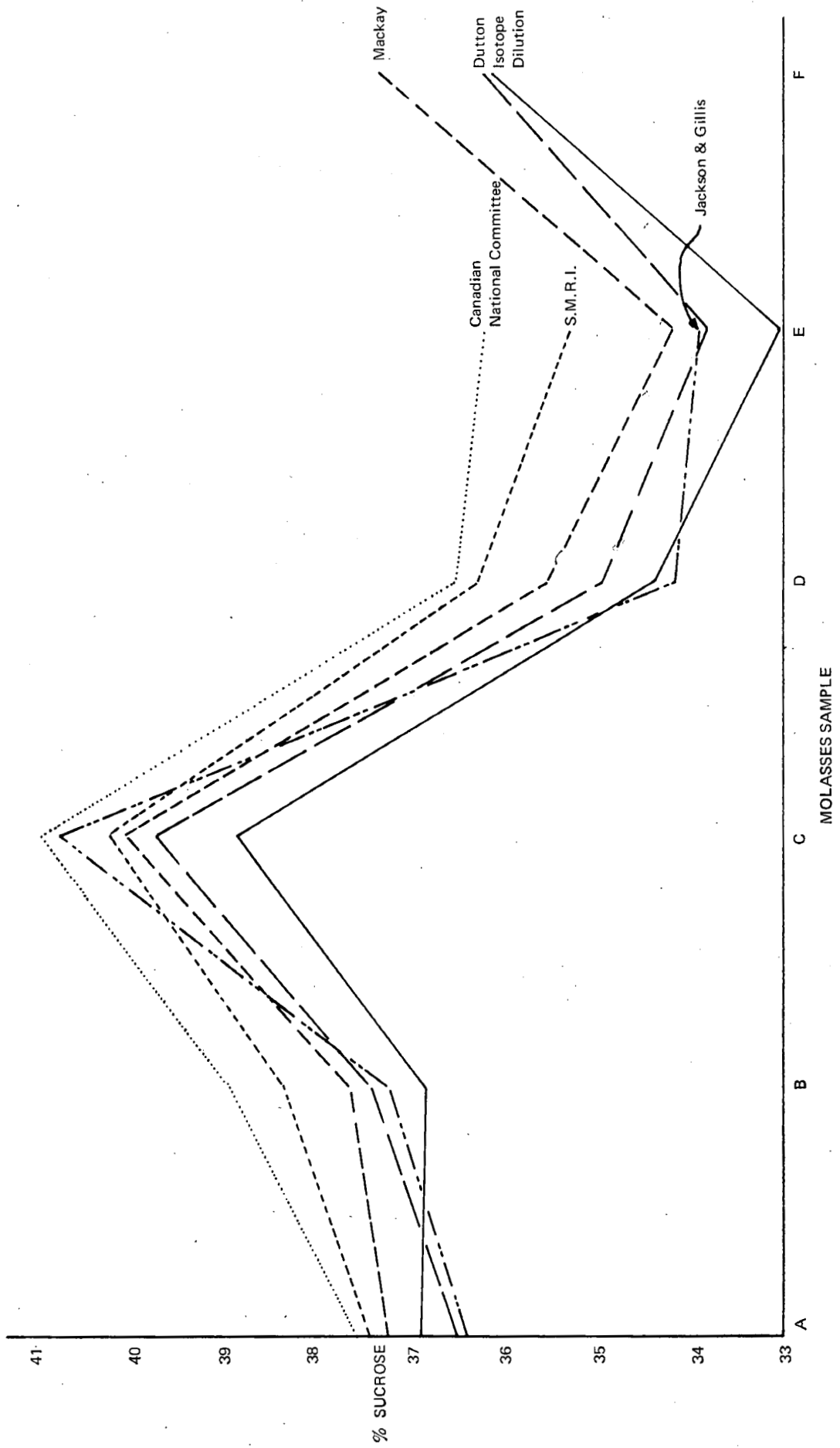


TABLE VI

Sample	Analyst					Mean
	1	2	3	4	5	
A	39,02	37,27	36,94	36,96	36,32	37,30
B	38,18	38,40	36,75	37,43	36,89	37,53
C	40,09	40,31	39,27	38,52	39,82	39,60
D	35,66	35,45	35,26	35,35	34,64	35,27
E	34,79	33,98	34,28	34,89	33,66	34,32
Std. Error (S.E.)	0,602	0,692	0,660	0,632	0,863	0,691
Coeff. of Var. = $\frac{\text{S.E.} \times 100}{\text{Mean}}$	1,637	1,882	1,794	1,718	2,346	1,875

requires rigid adherence to procedural details in order to be reproducible.

The results shown in Table V indicate that the polarimetric method according to Dutton is highly reproducible, when performed under ideal conditions. However at this stage we were trying to move away from polarimetric methods, for the reasons given above, and we did not submit this method to any inter-analyst or inter-laboratory tests.

The three methods determining total reducing sugars by titration showed a similar degree of reproducibility. Figure 1, which shows the relationship between the various methods and the isotope-dilution method indicated that of the three the Mackay method gave the result closest to the true value for sucrose. This method was therefore chosen for a more detailed investigation.

The importance of removing calcium from solutions to be titrated against Fehling's solution is well known⁴. Our results, in Table II, show that low results were obtained for reducing sugars when calcium was not sequestered prior to titrating. The 1 g of potassium oxalate recommended in the Mackay method was found, by atomic absorption spectroscopy, to reduce the calcium present in solution from 5% on molasses to 5 ppm. An excess of oxalate was not found to be significant.

The period of inversion, within the limits shown in Table III, had a very small influence on the total invert content determined. The temperature was more critical, particularly when it was less than 60°C. In order to have a method of inversion which did not require the refinement of a constant temperature water bath, and which was familiar to local laboratory assistants, we compared Walker's inversion method with that recommended in the Mackay procedure. The results agreed to within reasonable limits of analytical error, and the former method was adopted in all subsequent determinations.

Five samples of molasses were analysed by five analysts by the Mackay method, each analyst doing the determination in duplicate. An analysis of variance was made to assess the difference between analysts as opposed to the random experimental error. This is shown in Table VII.

The difference between analysts is significant at the 5% level, which is a considerable improvement over the Jackson and Gillis method IV. Randabel⁸, in a similar type of assessment in Mauritius with the

TABLE VII

Source of Error	Degrees of Freedom	Sum of Squares	Mean Square	F. Level
Samples	4	171,321	42,83	4,279*
Analysts	4	10,457	2,61	
Analyst & Samples	16	9,799	0,61	
Total ₁	24	191,577	7,98	
Within analysts	25	13,740	0,55	
Total ₂	49	205,317	4,19	

Jackson and Gillis method IV, found a 0,1% significance level between two analysts working in the same laboratory. To this extent we have almost achieved one of our objectives, that of getting comparable results. It remains to be seen whether these results can be repeated between factory analysts.

The average standard error for all analysts was 0,69, with a coefficient of variability of 1,875%. These results compare favourably with those published by Dowling², for refinery final molasses. In our preliminary survey of factory laboratories last year the average standard error using the Jackson and Gillis method was 0,86%.

The corrections for raffinose and kestose have not been applied to any of the results quoted in this paper. The average South African mill laboratory is not equipped to analyse for oligosaccharides in molasses. Moreover the quantitative estimation of kestose and raffinose by the method of Albon and Gross is made somewhat more difficult in cane molasses by the relatively high concentrations of neo- and 1-kestose. Thin-layer chromatography does appear to give a good separation of these sugars, and using this method the molasses samples used in this survey were found to have kestose levels of from 2,0 to 2,8%. When the corrections proposed by I.C.U.M.S.A.⁶ for polarimetric and chemical methods are applied to the results obtained with Dutton's method and the Mackay method respectively they yield figures which are considerably below those found by isotope-dilution. As mentioned earlier, our experience with the isotope-dilution method is not very great, and we should therefore view our results with caution. However, the corrections involved are very substantial, and therefore until we have further experience with

both the isotope-dilution method and the quantitative estimation of interfering oligosaccharides we prefer to omit the corrections entirely.

Conclusions

The determination of sucrose in final molasses can be carried out quickly and reproducibly by the chemical estimation of reducing sugars before and after acid inversion. Analytical procedures must be specified in considerable detail, and must be adhered to absolutely rigidly in order to get comparative results. Polarimetric methods can be extremely reproducible and accurate, but in general are more time-consuming and involve a greater number of analytical steps.

The absolute accuracy of both chemical and polarimetric methods rely on the estimation of interfering sugars such as kestose and raffinose. At present it is not practicable to make these corrections, and we should appreciate that our results for sucrose are probably higher than the true values.

The isotope-dilution method is free from the type of interference caused by other sugars mentioned above, but it is not a method suitable for routine use in factory laboratories. Its usefulness lies in the fact that it provides a result against which we can compare the results obtained by routine methods.

An advantage of the chemical methods of analysis is that they provide a figure for reducing sugars as well as for sucrose. If, in future, a method such as the Mackay method is adopted as an official I.C.U.M.S.A. procedure for sucrose it would be useful if the reducing sugars were determined by official methods. At present this means using the constant-volume modification of the Lane and Eynon titration, but if any changes are made under the subject of reducing sugars these should be taken into account when proposing methods for sucrose.

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Discussion

Mr. Bruijn: With reference to the isotope dilution method it must be mentioned that the figures are not reliable and err on one side. There is evidence that the sucrose samples we had for counting were not absolutely pure so the counts were too high. Therefore, the solid line in the graph in Figure 1 for the isotope dilution method should drop about one per cent.

Mr. MacGillivray: The other lines on the graph are uncorrected for oligosaccharides. Oligosaccharide figures were available and had the correction been applied most of the figures for the other methods would have been below those of the isotope dilution method.

Mr. Schaffler: It is mentioned that the kestose content of the samples ranged between 2 and 2,8%. On the samples I checked the kestoses were 1,69, 1,75, 2,1, and 2,2%.

The difference between the S.M.R.I. and isotope dilution methods were 0,54, 1,53, 1,38, 1,90 and 2,23. Would not a pol method be better than a chemical method owing to a lower correction factor?

Mr. MacGillivray: I think these differences must have arisen through my taking the total oligosaccharide figure, not just the kestose figure. I will recalculate the results using the figures you have just quoted. The corrections for raffinose and kestose on a polarimetric method would be 0,75% and 1,13% respectively whereas on a chemical method the raffinose correction is 1,07%.

Our tendency is to move away from the polarimetric method because of other errors in that type of method.

Mr. d'Hotman de Villiers: For inversion, why is hydrochloric acid used instead of invertase?

Mr. MacGillivray: Invertase is not all that specific. It has an effect on oligosaccharides, which are the main interfering sugars.

Also, as many factory laboratories do not have refrigerators we were perturbed about the stability of invertase if kept for some time in the factory.

Mr. Jennings: A water bath is also necessary if invertase is used and these also are not always available in factory laboratories.

Mr. Carter: It is a good thing that an attempt has been made to standardise the analysis of molasses because the factories are all interested in the molasses purity figures published by the S.M.R.I.

The S.M.R.I., having found differences in measuring molasses purities, should keep a close check on other analytical methods being used in factories.

Mr. MacGillivray: S.M.R.I. staff members are now visiting factories and checking methods and will keep a check on comparative values in future.

Miss Morel du Boil: In the correction for raffinose and kestose, for polarimetric method should not the values for A and B, 0,75 and 1,13, be divided by 1,31?

Mr. MacGillivray: They should be in terms of the final results but their relative importance is just as great because the sucrose and reducing sugar pols are divided by this amount.

Mr. Alexander: It is a good thing that the S.M.R.I. is investigating some of the commonly accepted methods of determination.

I still am not sure what method to use and none will apparently give the correct answer.

Under the circumstances I think we should stop recording the second decimal point.

Mr. Radford: Has the S.M.R.I. method been used for high test molasses and how did it compare with other methods?

Mr. MacGillivray: It has been used but the corrections for HTM are much greater than for final molasses because of the oligosaccharides present. It has not been compared with other methods.

Mr. Dawes: When ME started making high test molasses I carried out a survey on different methods being used for determining sucrose and the one giving the best results was very similar to the S.M.R.I. method.

Dr. Matic: We are carrying out a second survey on the use of the S.M.R.I. method and if it appears to be an improvement we will probably propose it to the Chemical Control Committee as a possible method to be adopted by factories.