ESTIMATION OF SUGARS LOSS IN STORED HIGH-GRADE MOLASSES

I. A. SMITH and J. S. CAZALET

Tongaat-Hulett Sugar Limited

Abstract

A and B molasses was held in the laboratory at temperatures from 25°C to 55°C, and at brix levels between 70 and 80. Composition of samples withdrawn was monitored over periods up to 48 weeks. At high temperatures most of the sucrose was hydrolysed to fructose and glucose. Degradation of these monosaccharides also took place but at a lower rate than the sucrose inversion. Initially more glucose was lost but later on degradation of fructose was the main feature. Low temperature and low brix conditions resulted in microbial conversion of some sugars to ethanol. This study and practical considerations indicate that temperature of high-grade molasses stored for later fermentation should be in the range 35°C to 45°C.

Introduction

The diversion of molasses and some crystal sugar to production of fuel ethanol now takes place in a number of countries. For practical convenience this can be done by omitting the later boiling(s) from normal sugar factory operations and sending A or B molasses to ethanol production.

Another factor is the seasonal nature of sugar factory operations. Cane milling typically takes place for only 6 to 8 months of the year. Ethanol plants can however be operated year-round and this improves utilisation of capital equipment. Bulk storage of molasses for use outside the sugar factory operating period then becomes necessary.

One result of this situation is that molasses of higher value is now being stored in larger quantities, relative to the norms for exhaust molasses. Loss of sugars is known to occur in exhaust molasses stored at elevated temperatures. The purpose of the present investigation was to assess the effect of different storage conditions on loss of sugars in higher purity molasses.

A potential danger in storage of high purity molasses is crystallisation of sucrose in heat exchangers and tanks. Dilution can overcome this but in turn introduces the possibility of microbial consumption of sugars. To gain some insight into these effects, diluted molasses was included in the investigation.

Experimental Procedure

A and B molasses obtained from factories in South Africa and Zimbabwe was placed in 4 litre plastic containers. Brix was adjusted downwards in some cases by mixing in water. The containers were held in laboratory incubators at 25°C, 35°C, 45°C and 55°C.

At intervals of 4 weeks on average, samples were withdrawn from each container. Analyses carried out on these samples included:

- Refractometer brix; pH; Lane and Eynon total sugars; and GC sucrose, fructose and glucose; all by the methods detailed in the Laboratory Manual for South African Sugar Factories.[1]
- Unfermentable reducing substances by diluting a sample and fermenting with excess yeast for 48 hours, followed by the Luff Schoorl reducing substances determination in the Laboratory manual.
- Ethanol by gas chromatography at 155°C on a Chromosorb 102 column.

Results and Discussion

A large body of data was built up over the 18 months of the investigation. The results are not presented in detail but are drawn on as necessary to illustrate different aspects of estimation procedures and of reaction mechanisms. Data for high temperatures is frequently referred to only because the larger changes show a clearer picture; we do not suggest that excessive loss of sugars in storage is the norm.

Estimation of fermentable sugars.

The traditional method for determination of total sugars is the Lane and Eynon copper reduction procedure. Specific gas chromatographic methods have been developed more recently. Figure 1 shows estimates of total sugars, expressed as invert sugar, for A molasses stored at 55°C. The Lane and Eynon method clearly overestimates and the difference increases with time.

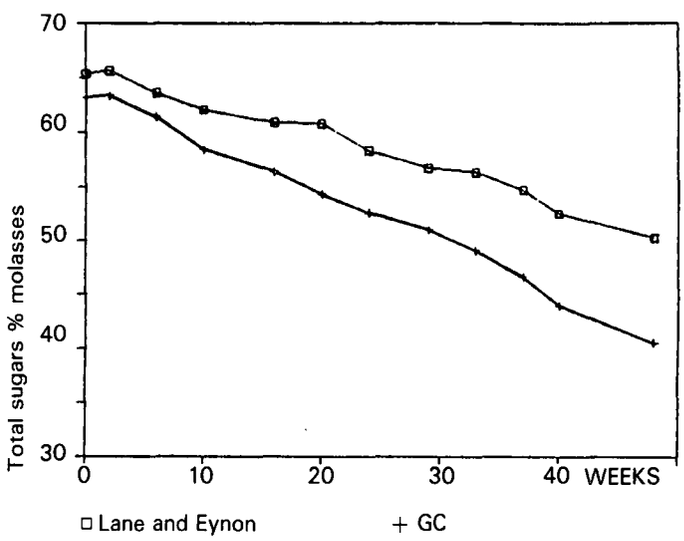

FIGURE 1 Total sugars (as invert) by GC and by Lane & Eynon: A molasses at 55°C

Under high temperature conditions Maillard type reactions between monosaccharides and amino acids are known to occur. Reaction products include substances which have reducing power but are not fermentable. This component was determined after fermenting the simple hexose sugars with excess yeast. Figure 2 shows that unfermentables in A molasses start at a low level but increase progressively in storage at 55°C.

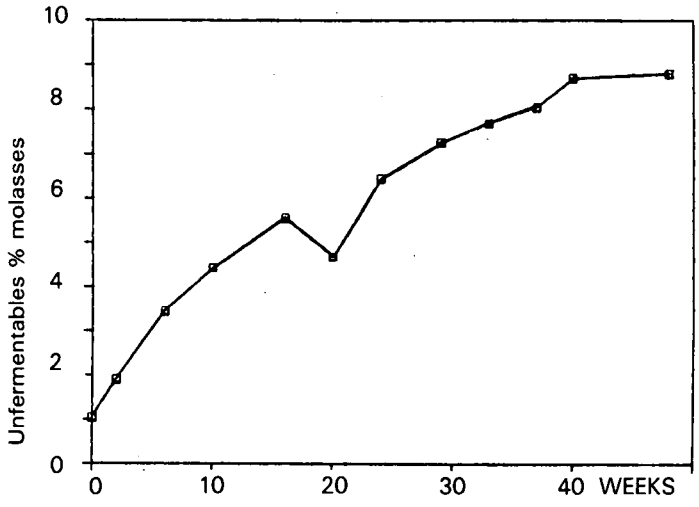

FIGURE 2 Unfermentable reducing substances (UFRS): A molasses at 55°C

Unfermentables can be subtracted from Lane and Eynon total sugars to give an alternative estimate of fermentables. Figure 3 shows that this agrees well with the direct measurement via GC.

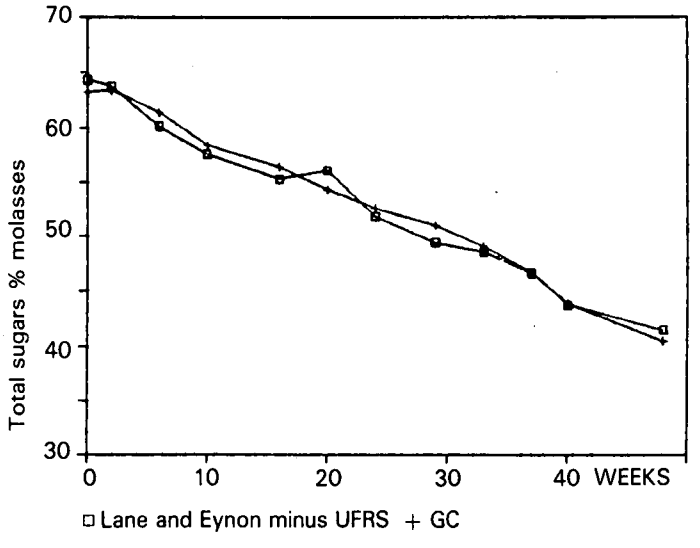

FIGURE 3 Total sugars by GC and by Lane & Eynon corrected for UFRS

Figures for yield of ethanol on Lane and Eynon sugars are depressed when unfermentables content is high, as in exhaust molasses or in higher purity material which has undergone degradation in storage. Our experience however is that for laboratory fermentations, ethanol yields expressed on true fermentables are effectively constant. All sugars data quoted below are derived from GC analysis and can be regarded as fully fermentable.

Acid inversion of sucrose

At high storage temperatures monosaccharides undergo thermal degradation to products which include organic acids. These drop the pH and increase the rate of acid hydrolysis of sucrose. The inversion products are of course fructose and glucose which can in turn create more acid and lower the pH still further.

Table 1 gives the time taken to reach various pH levels for A molasses stored at 55°C. Sucrose inversion rates estimated from Stadler's tables[2] are also shown.

Table 1

Measured pH values and calculated sucrose inversion rates for A molasses stored at 55°C

Weeks Storage	pH	% Sucrose inverted per week
0	6,0	0,3
3	5,5	1
10	5,0	3
25	4,5	10
45	4,0	35

Inversion rate trebles for each 0,5 unit drop in pH. On the other hand, the time taken for each such change increases. Both effects are a feature of the logarithmic pH scale.

The change in sugars content of A molasses stored for 48 weeks at 55°C is shown in Table 2.

Table 2

Change in individual and total sugars % A molasses at 55°C

Weeks storage	GC Suc	GC Fruc	GC Gluc	Total (as invert)
0	52,3	4,6	3,6	63,2
2	52,4	4,6	3,7	63,4
6	48,0	6,3	4,6	61,4
10	41,4	8,4	6,5	58,5
16	31,2	12,6	11,0	56,4
20	23,0	15,7	14,5	54,4
24	15,8	18,1	17,9	52,7
29	9,9	19,5	21,2	51,1
33	5,4	20,3	23,1	49,2
37	2,3	20,3	24,0	46,7
40	1,2	18,9	23,9	44,0
48	0,2	16,8	23,6	40,5

Net effect of an increasing inversion rate and a falling level of remaining sucrose is a fairly linear reduction in the sucrose content. The inversion products fructose and glucose increase sharply. Total sugars (expressed as invert) reduce but only by about one-third as opposed to the almost total disappearance of sucrose.

Effect of brix on acid inversion.

B molasses adjusted to 70 brix and to 80 brix was held at 45°C. The changes in total monosaccharide with time are shown in Figure 4 as an indicator of sucrose hydrolysis.

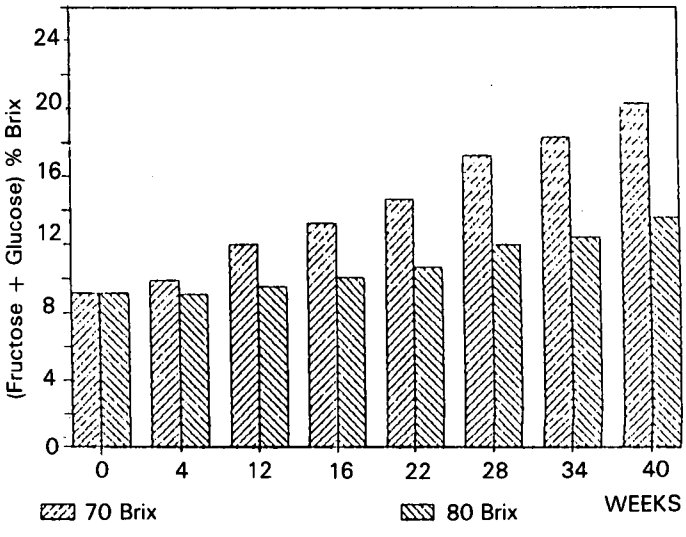

FIGURE 4 Invert formation: B molasses at 45°C

Rate of inversion is some 60% higher at 70 brix than at 80 brix. This is in broad agreement with the inversion/concentration relationship derived by Vukov.[3] The loss of total sugars is however identical for the two brix levels, at 9% over 40 weeks (Figure 5).

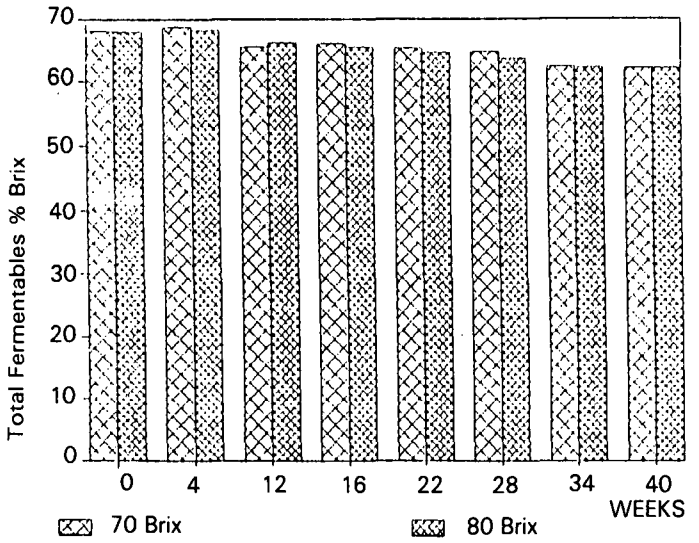

FIGURE 5 Total fermentables: B molasses at 45°C

In terms of fermentables therefore, dilution (within reason) appears to have no effect at higher storage temperatures.

High temperature monosaccharide loss

Sucrose on inversion yields fructose and glucose. Quantities of monosaccharide equivalent to sucrose lost from A molasses at 55°C were calculated, assuming uptake of one molecule water per molecule sucrose and formation of equal quantities of fructose and glucose. Adding these values to the amount originally present gives the quantity of monosaccharide that would be expected if sucrose inversion were the only change taking place. The resulting data for fructose is plotted together with observed values in Figure 6.

A net loss of monosaccharide is obviously taking place. To provide an indication of reaction rates, the net loss data for each interval between sampling and analysis has been expressed as units monosaccharide % molasses per week (Table 3).

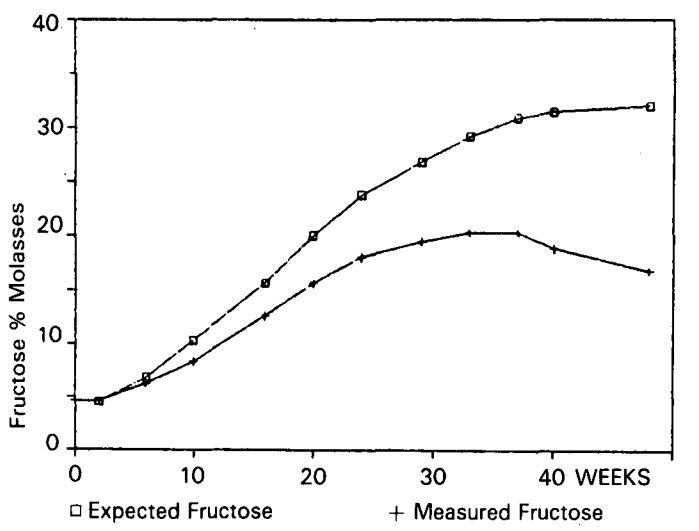

FIGURE 6 Expected and actual fructose: A molasses at 55°C

Table 3

Net change in monosaccharide % molasses per week for A molasses at 55°C

Storage period (Weeks)	Net change per week in:	
	Fructose % molasses	Glucose % molasses
0 to 2	0	+0,1
2 to 6	−0,2	−0,4
6 to 10	−0,4	−0,4
10 to 16	−0,2	−0,1
16 to 20	−0,3	−0,2
20 to 24	−0,4	−0,1
24 to 29	−0,3	0
29 to 33	−0,4	−0,1
33 to 37	−0,4	−0,2
37 to 40	−0,7	−0,2
40 to 48	−0,3	−0,1

The simultaneous formation of fructose and glucose, at generally higher rates than their disappearance, means that the net loss estimates in Table 3 may not be very accurate. They do however show certain broad trends.

Glucose loss is high from 2 to 10 weeks but then drops off. In the back ends of South African factories, more glucose than fructose is lost. This has been ascribed by Newell[4] and others to Maillard type reactions. Reasons why the glucose loss reduces are not clear but could be associated with depletion of other reactants. The amino acids which react with glucose will be present in lower concentrations in the A molasses stored here than in exhaust molasses.

Loss of fructose increases more slowly but after 20 weeks averages 3 to 4 times the glucose loss rate. This ratio is in line with reported data for thermal degradation, but the absolute rate values are not. Monosaccharides are in fact quite stable at low pH values and the reaction rate constants given by Sapronov[5] explain only a fraction of the observed disappearance.

Effect of temperature on fermentables loss

The loss of total fermentable sugars in A molasses stored for 20 weeks at three different temperature levels is shown in Table 4.

Table 4

Loss in total fermentables (as invert) in A molasses stored for 20 weeks

Storage temperature, °C	% Loss of total fermentables
35	~1,5
45	3
55	15

Loss at 55°C is significant but at 35°C is too low to quantify with any confidence. The level of 35°C also tends to represent a practical limit for the climatic conditions in cane production areas. Any lower temperatures would require chilling facilities in heat exchange circuits.

Low temperature situation

As noted above loss of sugars through chemical reaction can be pronounced at high temperatures but is small at 35°C. At still lower temperatures losses from this cause may be ignored for practical purposes, but can potentially be replaced by a completely different loss mechanism. High purity molasses stored at low temperatures requires to be diluted to avoid crystallisation in storage. This brings with it a danger of microbial activity.

Molasses was diluted to varying degrees and stored at low temperature. Table 5 shows change in sugars content for B molasses diluted to 70 Brix and held at 25°C.

Table 5

Change in individual and total sugars % B molasses at 70 Brix and 25°C

Weeks storage	GC Suc	GC Fruc	GC Gluc	Total (as invert)
0	39,5	3,9	2,5	48,0
4	40,1	4,1	2,5	48,7
12	40,2	0,9	1,9	45,2
16	40,4	0,5	2,1	45,1
22	38,8	1,1	2,6	44,6
28	36,8	1,8	3,6	44,2
34	34,9	2,3	4,1	43,1
40	33,9	2,7	4,7	43,1

pH remained in the range 5,4 to 5,7 and under these conditions acid inversion is negligible. What did occur was disappearance of most of the fructose present, and some glucose, between weeks 4 and 12. After that the sucrose content reduced while fructose and glucose increased progressively. 10% of the original total fermentables was lost over the full test period (40 weeks in this case).

These samples were analysed for ethanol. None was found up to 4 weeks, but after 12 weeks 2 ml ethanol per 100g molasses was present. The ethanol level then rose slowly to 3,4 ml/100g at 40 weeks.

Laboratory fermentations typically yield 0,6 ml ethanol per gram of monosaccharides originally present. This figure was used to estimate the mass of sugars consumed in forming the ethanol found here. The equivalent sugars are plotted together with the sugars actually present in Figure 7.

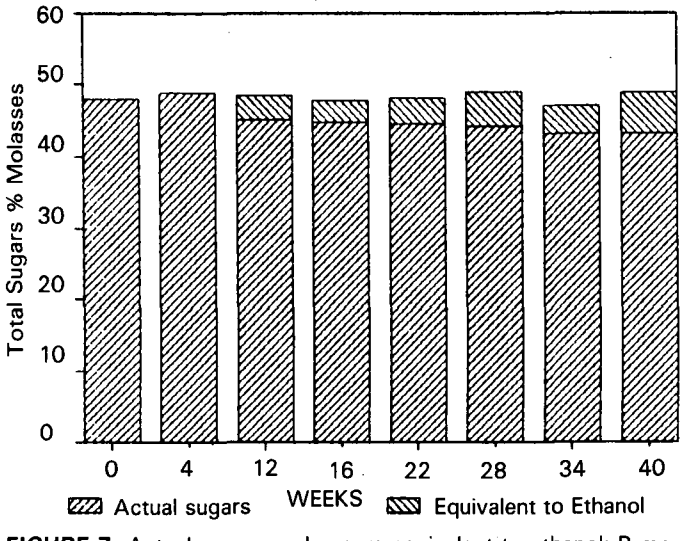

FIGURE 7 Actual sugars and sugars equivalent to ethanol: B molasses at 70 brix and 25°C

It is clear from Figure 7 that the total of sugars determined and sugars equivalent to ethanol produced remains essentially constant.

The interpretation of these results in that micro-organisms fermented most of the available monosaccharide to ethanol between 4 and 12 weeks. After that sucrose was inverted to fructose and glucose, presumably by invertase produced during the bout of microbial activity. Some consumption of total sugars continued but at a lower rate than initially.

There are one or two unusual aspects to this picture. Fructose was preferentially consumed, whereas in our experience it is generally glucose that is taken up first. Also, the reasons why sugars consumption slowed again after 12 weeks are obscure. A factor here may be the low level of nutrients, particularly nitrogen, due to a higher purity in relation to exhaust molasses.

A 5% loss of total fermentables over 20 weeks is indicated for this situation of diluted molasses stored at 25°C. Storage of A and B molasses samples at 80 brix and 25°C also resulted in loss of fermentables but at lower rates, averaging 2% over 20 weeks.

The low temperature findings do not necessarily reflect what would happen in bulk storage. The laboratory test procedure included a thorough mixing of the molasses at each sampling period. This would have dispersed any active organisms throughout the remaining material and also provided a degree of aeration. Neither of these effects would normally apply in bulk storage and the loss of fermentables could be significantly less than suggested by the laboratory findings.

Conclusions

Extended storage of high-grade molasses at 55°C resulted in marked pH drops and resultant inversion of almost all the sucrose originally present. There were significant net losses of total sugars.

At low temperatures smaller amounts of sugars were converted microbiologically to ethanol. Actual loss in bulk storage may not be the same due to differences in handling the laboratory-stored molasses. Also, biological systems are more prone to variation than are chemical reactions.

The optimum storage temperature appears to lie between 35 and 45°C. Below this range problems of sucrose crystallisation or microbial activity, and of high cost of cooling, may arise. At higher temperatures the rate of sugars loss due to degradation reactions increases sharply.

As a final comment, Lane and Eynon analyses do not measure fermentables because of the formation in processing and storage of substances which have reducing power but cannot be utilised microbiologically.

Acknowledgements

The extensive analytical work reviewed here was carried out by the analysts of the Sugar Technology Department laboratory, Tongaat-Hulett Sugar Limited. Thanks are also due to Mr. K. J. Schäffler for advice on kinetic aspects.

REFERENCES

1. *Laboratory Manual for SA Sugar Factories* (1985). SA Sugar Technologists Association.
2. Honing, P (1953). *Principles of sugar technology*, Vol. 1, p 448. Elsevier.
3. Vukov, K (1965). Kinetic aspects of sucrose hydrolysis. *Int Sug J 67* (798), 172-175.
4. Newell, GM (1979). A preliminary investigation into factors affecting gas formation in massecuite and molasses. *Proc S Afr Sug Technol Ass 53*, 62-65.
5. Sapronov, AR and Koltschewa, RA (1975). Die kinetik der saccharosezertgung und die von pH und temperatur abhangige hexose. *Journal Commission Internationale Technique de Sucrerie*, 195-205.