

QUANTITATIVE DETERMINATION OF SUGARS IN FACTORY PRODUCTS BY GAS CHROMATOGRAPHY USING OPEN TUBULAR COLUMNS

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Abstract

Glucose, fructose, sucrose and kestose in a variety of factory products were determined as trimethylsilyl derivatives by gas chromatography on open tubular columns. The advantages of using these columns are discussed.

Introduction

This paper deals with some preliminary results on the quantitative analysis of mono-, di- and trisaccharides by gas chromatography on open tubular columns. The advantage of using gas chromatography for the analysis of these compounds is that it is a rapid method that can in principle be fully automated. In contrast to the polarimetric and chemical methods of sucrose determination, impurities present in a mixture do not in general interfere with results obtained.

Many papers have been published (see e.g. ref. 2 and references therein) on the separation of various volatile derivatives of sugars by gas chromatography. Trimethylsilyl (TMS) ethers have been the most widely used derivatives due to the ease of their preparation. There are several reports of the quantitative analysis of TMS derivatives of sugars by gas chromatography (see references 2, 3, 4, 6, 10, 11, and references therein) including some with application to the sugar industry (see references 6, 9, 11, 14 and references therein).

The work described here differs from other published reports in that wall coated open tubular columns have been used instead of packed columns. Open tubular columns have been widely used to analyse a large variety of sample types including sugars^{7, 13} but there appear to be no reports of quantitative analysis of sugars on these columns. An open tubular column has approximately the same efficiency per unit length as a packed column, but has a very much lower resistance to flow with the result that a long column with a very high efficiency may be prepared. Open tubular columns have been used in this laboratory⁷ to separate the TMS derivatives of the three kestose isomers where a very high chromatographic efficiency is needed. The efficiency is illustrated by Figs. 1 and 2 which show the chromatograms of the TMS derivatives of the kestose isomers and melezitose on a 2 m packed column and on a 190 m open tubular column. The high efficiency of open tubular columns results in lower retention times for the same

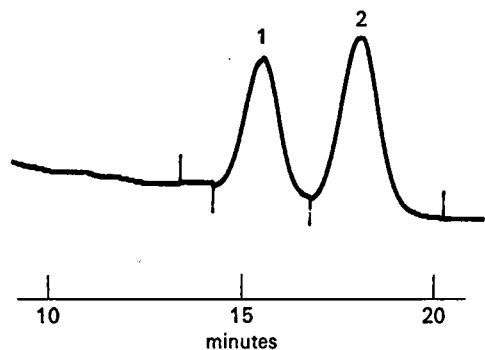


FIGURE 1 TMS derivatives of kestose and melezitose separated at 250 °C on a 2 m column packed with 5% SE 30 on Gas-chrom Q. 1, kestose; 2, melezitose.

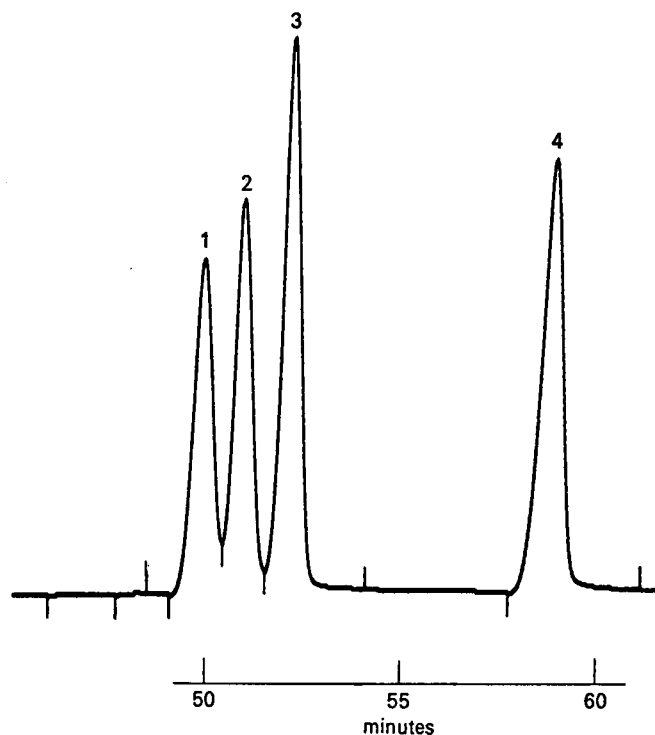


FIGURE 2 TMS derivatives of a synthetic mixture of the kestose isomers and melezitose separated at 260 °C on a 190 m open tubular column coated with OV 17. 1, 1-kestose; 2, 6-kestose; 3, neo-kestose; 4, melezitose.

separation and in very much sharper peaks. This latter quality is of importance, as the integrated boundaries of the peaks are more sharply defined, which should allow greater precision of integration than in the case of broader peaks obtained with packed columns. The reagent used for silylating the sugars is trimethylsilylimidazole (TMSI). Mahoney⁶ has used this reagent for the analysis of dry solutions of sucrose, and this laboratory⁸ has used the reagent for the quantitative analysis of a dilute aqueous solution of the kestoses. The reagent has also been recommended for the analysis of reducing sugar oximes¹.

Experimental

Sample preparation.

(i) Reducing Sugars.

(i)a *In molasses:*

Five grams molasses and 250 mg arabinose (BDH Biochemicals) were dissolved in 2.5 cm³ distilled water. Between 10 and 12 mg of this solution was treated with 1 cm³ of a 2.5% solution of hydroxylamine hydrochloride in dry pyridine, heated for 30 minutes at 73 °C, cooled, treated with 1 cm³ of pure TMSI and then allowed to stand for a minimum of 30 minutes before use. The sample size for chromatography was 2 μl.

(ii) Sucrose.

(ii)a *In molasses:*

One gram of molasses and 330 mg trehalose dihydrate

(BDH Biochemicals) were dissolved in 1 cm³ distilled water. Between 10 and 12 mg of this solution was weighed into a Pierce reacti-vial fitted with a teflon faced disc. This was then treated with 0,5 cm³ of a reagent consisting of 4 parts TMSI (Ohio Valley Speciality Chemical Inc.) and 1 part dry pyridine (BDH Analar, stored over potassium hydroxide pellets) and allowed to stand a minimum of one hour before use. The sample size for chromatography was 1 µl.

(ii)b *In juice:*

As for molasses except that the solution for silylation was prepared by dissolving 100 mg trehalose dihydrate in 1 g juice. The sample size for chromatography was 1 µl.

(ii)c *In VHP sugar:*

As for molasses except that the solution for silylation was prepared by dissolving 1 g of sugar and 1,1 g of trehalose dihydrate in 2 cm³ distilled water. The sample size for chromatography was 1 µl.

(iii) Kestoses.

(iii)a *In molasses:*

Five grams of molasses and 20 mg melezitose dihydrate (Fluka) were dissolved in 2,5 cm³ of distilled water. Between 10 and 12 mg of this solution was weighed into a reacti-vial fitted with a teflon faced disc and silylated with the same reagent used for sucrose. The vial was allowed to stand a minimum of 1 hour. Sufficient dry imidazole was then added to saturate the solution and 0,1 cm³ of hexamethyldisiloxane was added to form a small top phase. The vial was shaken to extract the TMS derivatives into the top phase and was then centrifuged to separate the two phases. The sample size for chromatography was 2 µl.

(iii)b *In juice:*

As for molasses except that the solution for silylation was prepared by dissolving 20 mg melezitose dihydrate in 25 g juice. The sample size for chromatography was 10 µl.

(iii)c *In VHP sugar:*

As for molasses except that the solution for silylation was prepared by dissolving 8 g sugar and 10 mg melezitose dihydrate in 10 ml distilled water. The sample size for chromatography was 5 µl.

Chromatography

Two columns were used for this work.

Column 1:

A 190 m × 0,5 mm stainless steel open tubular column (Handy and Harman Tube Co.) was coated by the plug method with OV17 (Applied Science Laboratories Inc.) using benzyltriphenylphosphonium chloride (Aldrich Chemical Co.) as a wetting agent. The column was used in a Beckman G.C. 55 gas chromatograph fitted with an inlet splitter and flame ionization detector.

Column 2:

Similar to column 1 but 40 m long. The column was used in a Perkin Elmer model 3920 gas chromatograph fitted with an inlet splitter and a flame ionization detector.

The conditions of analysis are listed in Table 1. Chromatographic peak areas were determined using a Hewlett Packard model 3380 electronic integrator. Samples containing known masses of sugar and internal standard were run each day to establish a response factor between the sugar and the internal standard.

TABLE 1

Sample Type	Column No.	Inlet Pressure kPa	Inlet Temp. °C	Column Temp. °C	Carrier Gas
Reducing sugars	1	186	200	175	H ₂
Sucrose	1	186	300	260	H ₂
Kestoses	2	103	300	250	H ₂
Kestoses	1	186	300	260	H ₂

Results and discussion

Reducing sugars.

The mixture of fructose and glucose in molasses consists of a number of isomers, which when silylated and chromatographed, yield several peaks some of which overlap. Some authors (10, 11 and references therein) have overcome this problem by allowing the mixture of isomers to equilibrate. One peak only for fructose and one peak only for glucose is measured. The mutarotation equilibrium must be established under carefully controlled conditions; the equilibrium composition of reducing sugars is dependent on the solvent used and may only be reached after several hours¹⁰. In principle a more satisfactory method is to treat fructose and glucose chemically so that the TMS derivative of each is presented as a single peak. Sweeley, Bentley, Makita and Wells¹² reported that this may be achieved by preparing oximes of the reducing sugars before silylation. In the work reported here oximes were also used and were prepared by a modification¹ of the technique reported by Sweeley and co-authors. A chromatogram of the TMS derivatives of the reducing sugar oximes on the 190 m column is shown in Fig. 3. Arabinose is included as an internal standard for quantitative analysis. It is seen that glucose and fructose are well separated and that each is not a single peak but is in fact a doublet due to the cis and trans forms of the oximes. The peaks obtained with a sample of molasses are small as shown in Fig. 3 and difficulty was occasionally experienced in obtaining an integrated area for the second peak of the glucose doublet in the case of samples containing a low percentage of glucose.

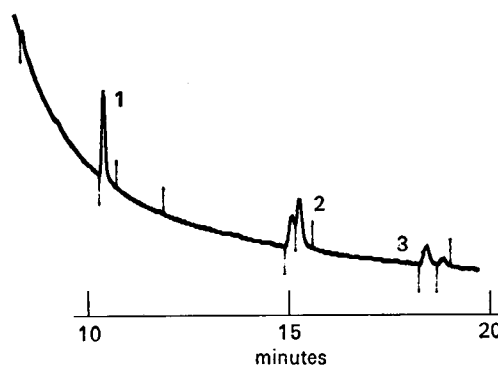


FIGURE 3 TMS derivatives of reducing sugar oximes separated at 175 °C on a 190 m open tubular column coated with OV 17. 1, arabinose; 2, fructose; 3, glucose.

The determination of reducing sugars in 51 samples of molasses discussed in an accompanying paper⁵ was performed using this column. Each determination was performed in duplicate. For these results the standard deviation for glucose is 0,31 and for fructose is 0,37. These are a measure of the precision of the chromatographic method and not of the method of sample preparation, as a single molasses derivative was used for duplicate determinations. The poor results are partially explained by the small peaks obtained and indications

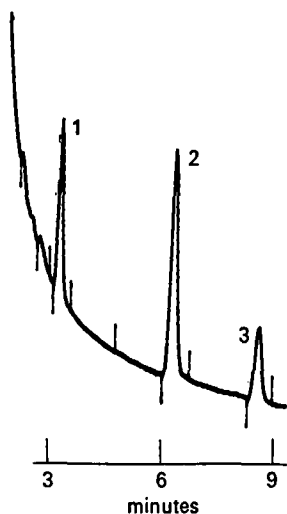


FIGURE 4 TMS derivatives of reducing sugar oximes separated at 140 °C on a 40 m open tubular column coated with OV 17. 1, arabinose; 2, fructose; 3, glucose.

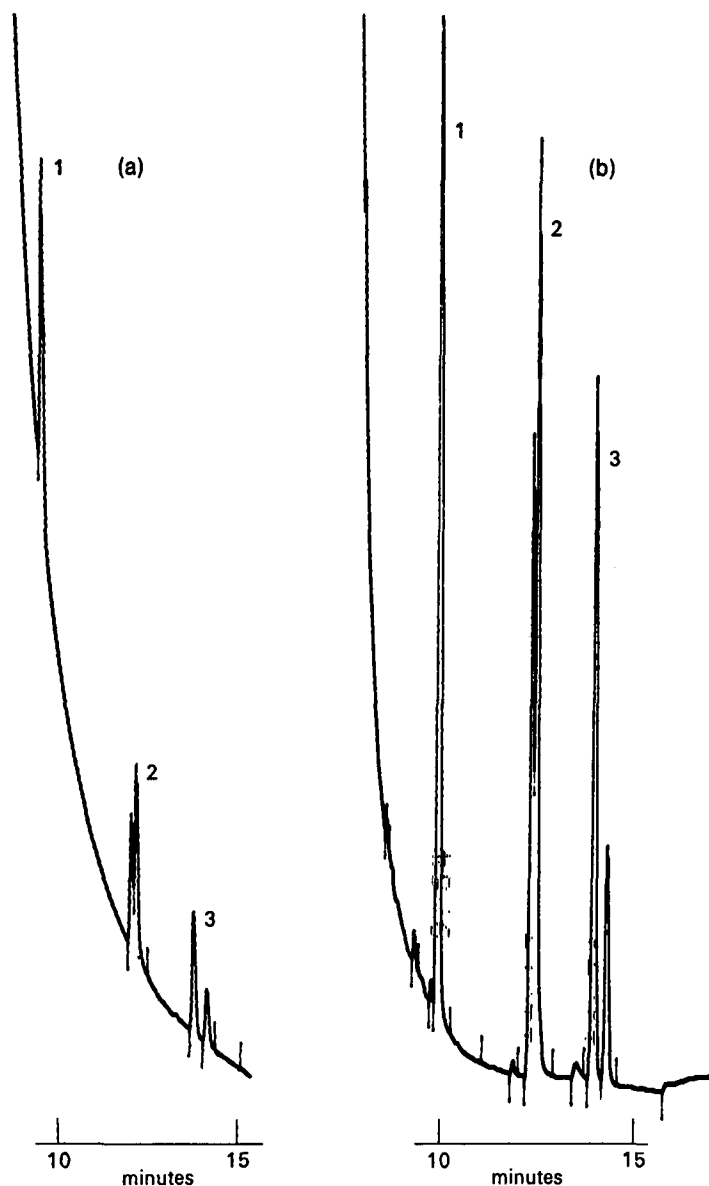


FIGURE 5 TMS derivatives of reducing sugar oximes separated at 200 °C on a 190 m open tubular column coated with OV 17. 5a single phase system; 5b double phase system. 1, arabinose; 2, fructose; 3, glucose.

are that more precise results would be obtained using a shorter column which would give sharp single peaks instead of doublets as is shown in Fig. 4. A shorter column would also afford a faster analysis time which it is estimated could be lowered to about 2 minutes. The 190 m column, instead of the 40 m column, was used for the analysis of the molasses samples discussed above as temporary technical difficulties precluded the use of the shorter column.

A two phase sample preparation step, whereby the derivatives are concentrated in a small top phase, has been developed in this laboratory⁸. Fig. 5a and 5b show the same sample of molasses analyzed using the single and the two phase systems. With the latter system larger peaks are obtained and the solvent exhibits significantly less tailing which is of importance when using an electronic integrator. The quantitation using this method has not been fully investigated but preliminary indications are that in the case of reducing sugars it is not as accurate as the single phase system. However it is of value for determining reducing sugars by gas chromatography in very dilute solutions where the peaks obtained using the single phase system would be too small for satisfactory determination. The two phase system was used for the analysis, discussed in an accompanying paper⁵, of reducing sugars present at concentrations varying from 0,3% to 1% in a dilute solution of molasses clarified by lead acetate.

The temperature of the injection block has a marked effect on the fructose to glucose ratio which increases by about 25% when the temperature is raised from 180 °C to 300 °C. The injection block was set at 200 °C for the analyses of reducing sugars referred to in this paper.

Sucrose.

Sucrose may be quantitatively determined on the 190 m open tubular column as a TMS derivative using trehalose as an internal standard. A typical analysis on this column is shown in Fig. 6. The peaks are sharp and the separation is more than adequate. The precision is good and appears to be better than that of other reported gas chromatographic methods of sucrose analysis using TMS derivatives^{2, 3, 6}. The standard deviation for 10 consecutive replicate injections of the same sample (Malelane final molasses containing 31,19% sucrose) is 0,06. The use of an electronic integrator has undoubtedly contributed to this precise value. There are very small peaks between sucrose and trehalose but no attempt was made to determine if these were caused by impurities in the molasses or by incomplete derivitization of the sucrose and trehalose. The analysis took 15 minutes but it should be possible to reduce this time to approximately 1 minute by using a shorter column.

The determination of sucrose in 51 samples of molasses, discussed in an accompanying paper⁵, was performed using this column. Each sample of molasses was analysed twice chromatographically, using a separate sample preparation for each analysis. In a few cases the duplication was poor and this was assumed to be due to the presence of insoluble matter such as bagasse in the molasses. In all such cases the analysis of a third sample was found to give good agreement with one of the first two analyses. The standard deviation for the above analyses is 0,11. This is a measure of the precision of the whole method including gas chromatography and sample preparation.

Table 2 illustrates the agreement in sucrose value between gas chromatography and pol for samples of Amatikulu mixed juice and direct analysis of cane extract. With the exception of two samples the agreement between pol and gas chromatography is within 2% relative.

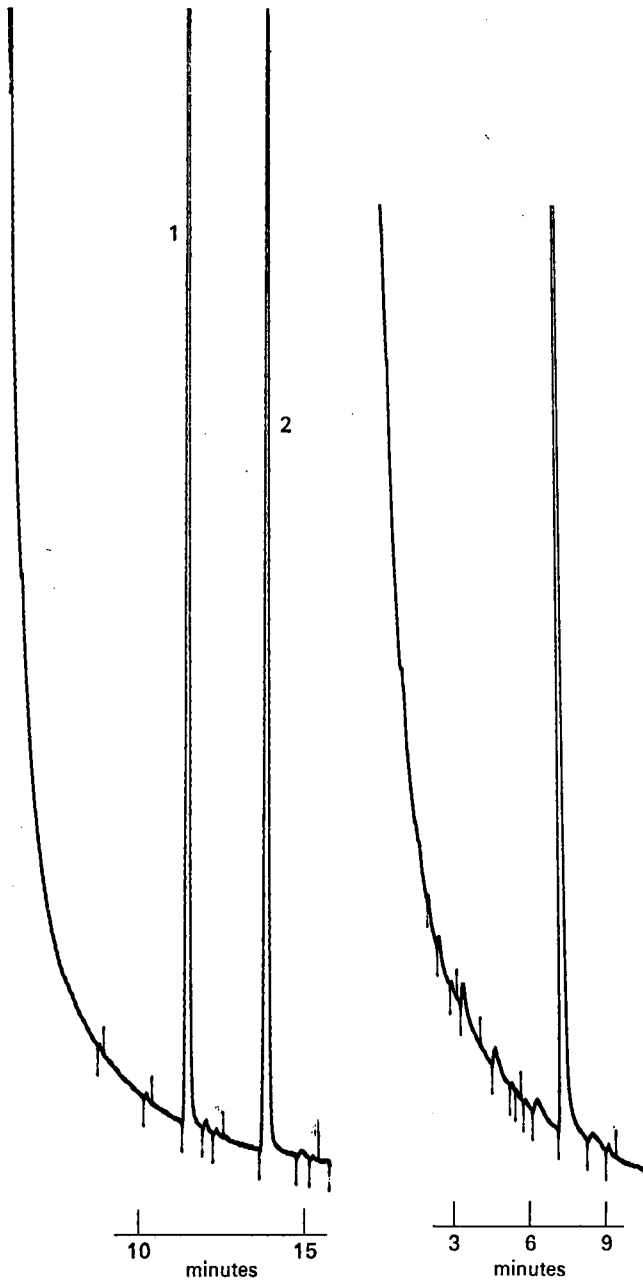


FIGURE 6 TMS derivatives of sucrose and trehalose separated at 260 °C on a 190 m open tubular column coated with OV 17. 1, sucrose; 2, trehalose.

FIGURE 7 TMS derivative of sucrose in last expressed juice. Two phase system.

validity. Nevertheless it is of interest to note that the mean gas chromatographic sucrose is within 0,11 % of the pol value for VHP sugar.

TABLE 3

Sample Number	Description of sample	Percentage Sucrose		
		Run 1	Run 2	Mean
1	Mixed Juice	11,96	11,52	11,74
2	Syrup	41,21	41,26	41,24
3	A molasses	54,00	53,35	53,68
4	B molasses	34,54	34,89	34,72
5	C molasses	29,20	29,26	29,23
6	VHP sugar of 99,34 pol	99,34	99,11	99,23

Sucrose may also be analysed in very dilute solution by using the two phase method referred to earlier. This is illustrated in Fig. 7 which shows a large sucrose peak in a sample of last expressed juice of 0,5 brix. No internal standard was included as the quantitation of this method for sucrose has not been investigated.

The Kestoses.

The kestoses may be determined as TMS derivatives on either the 40 m or the 190 m open tubular column. The shorter column is used for the rapid analysis of total kestose illustrated in Fig. 8 and the longer column is used where it is necessary to know the amount of individual isomers present (see Fig. 2). The analysis time is under 10 minutes on the 40 m column and could most probably be reduced to about 6 minutes by using a shorter column. A still faster analysis may be possible by using a different stationary phase or by using a column of smaller internal diameter. The analysis time on the 190 m column is 1 hour. Raffinose has the same retention time as 1-kestose on both columns with the result that kestose values include any raffinose that may be present.

TABLE 2

Sample Number	Description of sample	Gas chromatographic* sucrose	Pol
1	Mixed juice	8,65	8,61
2	Mixed juice	7,77	7,77
3	Mixed juice	7,17	6,99
4	Mixed juice	7,05	6,98
5	Direct analysis of cane extract	4,24	4,20
6	Direct analysis of cane extract	2,64	2,73
7	Direct analysis of cane extract	3,73	3,80
8	Direct analysis of cane extract	4,39	4,44

* Gas chromatography was performed on single samples analysed once only.

Table 3 illustrates the sucrose analysis in syrup, molasses and in VHP sugar. These results are for single samples chromatographed twice and do not have any statistical

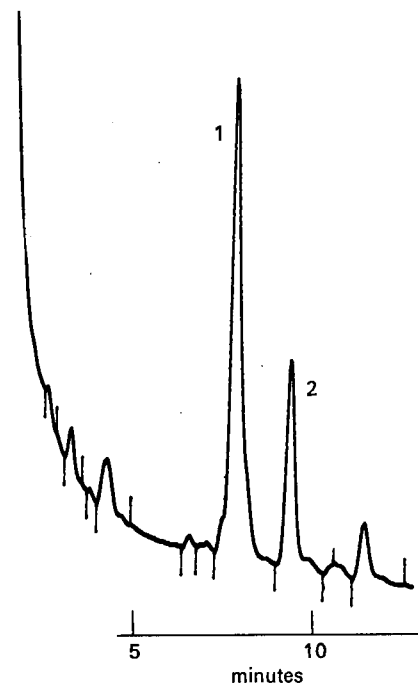


FIGURE 8 TMS derivatives of kestose and melezitose separated at 250 °C on a 40 m open tubular column coated with OV 17. 1, kestose; 2, melezitose.

Melezitose may be used as an internal standard⁸ but as the pure kestose isomers were not available in sufficient quantities to determine their detector response relative to that of melezitose, relative rather than absolute results are obtained. These are likely to be within approximately 10% of the true value³.

The two phase system whereby the kestoses are concentrated in a small top phase was used for the work described here. A measure of the sensitivity of the method is that it has been used to detect traces of kestoses in Analar Sucrose. Preliminary results⁸ have indicated that the relative error using this method is about 7%.

The analysis of kestoses in final molasses described in an accompanying paper⁵ were performed by the two phase method and thus may have relative errors as high as about 15%. However for a sample containing 1% of kestose the absolute error should be less than 0,2%.

TABLE 4

Sample Number	Description of sample	1-kestose %	6-kestose %	Neo-kestose %	Total kestose %
1	Mixed Juice . . .				0,023
2*	Syrup				0,129*
3	A molasses				0,235
4	B molasses				0,705
5	Final molasses . .				0,875
6	June composite final molasses ex Umzimkulu . . .				0,44
7	Nov. composite final molasses ex Darnall				1,66
8	Final molasses from fire damaged cane ex Illovo	0,53	0,17	0,12	0,82
9	Affinited C sugar corresponding to sample No. 8 . . .	0,048	0,037	0,035	0,12
10	Final Blackstrap molasses from a Canadian refinery	0,89	0,60	0,43	1,92
11	Affinited sugar corresponding to sample No. 10	0,08	0,12	0,06	0,26
12	Molasses from crystallised refinery return syrup†	1,20	0,71	0,58	2,49
13	Affinited sugar corresponding to sample No. 12	0,072	0,082	0,042	0,20

* Sample 2 is from a different factory to that of samples 1, 3, 4 and 5 which were all sampled on the same day.

† This refers to a sample of refinery return syrup that crystallized on standing in the laboratory.

Table 4 lists the amount of kestoses found in various factory products. Samples 1, 3, 4 and 5 show levels of kestose in mixed juice and molasses all sampled on the same day. As would be expected the kestose becomes more concentrated as it passes through the factory. Samples 6 and 7 are included as they have the highest and lowest kestose values found in a survey of 51 samples of final molasses⁵. Samples 8 and 9; 10 and 11; 12 and 13 are samples of molasses together with the corresponding sugars crystallized from them. The ratio of 6-kestose to total kestose is about 50% higher in all three of the crystalline sugars than it is in the corresponding molasses. It has been found that 1-kestose is the major isomer in all samples of final molasses that have been analysed in this laboratory.

Conclusions

The analysis of the TMS derivatives of reducing sugars, sucrose and kestoses on open tubular columns as described in this paper takes a total of 45 minutes. This time could be reduced to approximately 12 minutes if shorter columns were used and possibly to as little as 5 minutes if temperature programming were used. Such a quick analysis would not lead to a significantly lower time for the determination of these compounds in a factory product as sample preparation takes a minimum of 10 minutes actual working time. However a quick chromatographic run would allow more precise quantitative results to be obtained as a greater number of replicate analyses could be conveniently performed on a given derivatized sample.

The chromatographic precision for the sucrose determination is good ($\sigma = 0,06$) whereas that for glucose ($\sigma = 0,31$) and fructose ($\sigma = 0,37$) is lower but is still satisfactory for the determination of reducing sugars in final molasses. These are preliminary results that were determined without optimizing the various chromatographic parameters. In the case of sucrose the standard deviation compares very favourably with those reported for packed columns.

The peak separation on the open tubular columns is far superior to that reported for comparable runs on packed columns. This allows for greater confidence in the analysis, as the chance of impurity peaks contributing to measured peak areas is diminished. Thus in the sucrose analysis the small peaks reported between sucrose and trehalose may be included with the major peaks on a packed column.

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