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A Comparison of Sample Preparation Procedures for High Performance Liquid Chromatographic Determination of Sucrose in Molasses

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ABSTRACT

Sucrose was determined in a series of molasses samples by two high performance liquid chromatographic (HPLC) methods which differed only in sample preparation, a cleanup procedure being included in one method. Small, but significant, method differences and method × sample interactions were observed. Further work is required to establish the causes of the observed differences and the most suitable cleanup procedure.

INTRODUCTION

The determination of sugars, in particular sucrose, in molasses with the greatest possible degree of accuracy and precision is of considerable importance to the sugar industry worldwide. Chromatographic methods have been extensively used, in particular gas–liquid chromatography (Schäffler & Morel du Boil, 1981, 1984). The technique has been carefully evaluated and has been shown to give accurate and precise results. High performance liquid chromatography (HPLC) (Wong-Chong & Martin, 1979; Damon & Pettitt, 1980; Charles, 1981; Abeydeera, 1983) has been investigated as a possible alternative as analytical procedures are generally more rapid and simple than those of GLC. Chorn & Hugo (1984) obtained higher values for sucrose in molasses, by an HPLC method utilising a cation exchange resin column and refractive index

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detection, than those obtained by the reference GLC method. The use of a guard column containing a mixture of cation (H^+) and anion (OH^-) exchange resins (Fitt, 1978) removed salts and greatly improved method to method agreement.

In the HPLC methods currently being evaluated by the International Commission for Uniform Methods for Sugar Analysis (ICUMSA), no sample cleanup is applied prior to injection, other than filtration of diluted samples.

Sample cleanup may improve accuracy and increase column life by removing interfering components but it may also introduce errors, such as losses of sugars. A preliminary investigation of these effects is described in this paper.

MATERIALS AND METHODS

Molasses samples

Twelve samples, consisting of three pairs each of cane and beet molasses, were analysed as part of a collaborative study (ICUMSA: Subject 8 (sucrose)). Samples were warmed until fairly fluid and stirred thoroughly before taking sub-samples.

Calibration standards

A prime standard (1·0% w/v) and two secondary standards (0·5% and 1·5% w/v sucrose (AnalaR, BDH)) were used. Calibrations (peak area versus concentration) were linear throughout this range.

Sample preparation

The two HPLC methods differed only in the procedure used for sample preparation. In both cases, samples were diluted so that the final sucrose concentration was intermediate between those of the two secondary standards. The first method (Method 1) incorporated clarification with neutral lead acetate solution (sufficient to complete precipitation), followed by deionisation with mixed bed (H^+/HCO_3^-) resin and filtration (0·6 μm membrane filter). This procedure was shown to be generally

applicable for the estimation of sugars in food and plant material (Wight & van Niekerk, 1983) but possible minor effects of sample cleanup on sucrose quantification were not studied. No cleanup other than filtration was applied prior to chromatography in the second method (Method 2).

Treatment of the samples with neutral lead acetate facilitated filtration and thus the additional stages required for sample cleanup did not greatly increase the time required for analysis.

Design of experiment

Two independent sets of triplicate determinations were made on each sample by Method 1, using different sub-samples for each individual determination. The two sets of determinations were made independent by running them on different days using different calibration standards. A single set of duplicate determinations was made on each sample by Method 2. The determinations by Method 2 for a given sample were done on the same day as the first series by Method 1. For reasons of column life and analyst's time required, determinations by Method 2 were kept to the minimum necessary for useful comparison with Method 1.

Chromatography

A cation exchange column in the calcium form (Bio-Rad HPX-87C) was used for analysis. The column temperature was 65°C and deionised distilled water was used as the mobile phase at a flow rate of 0·6 ml/min. A guard column consisting of two microguard cartridges (cation H^+ + anion OH^-, Aminex HPX-85H and Aminex A-25 from Bio-Rad) was connected to the main column.

The chromatographic system consisted of a Varian model 5000 pump with a column heating attachment, a loop injector (loop size 11 μl), a Waters model R401 differential refractometer and a Hewlett–Packard model 3390A integrator.

Quantification

A least squares regression analysis of the data (peak area versus concentration) for replicates of the three standards was used to calculate the sucrose content of the samples.

Statistical methods

A general linear model with dummy variables was used to estimate the various main and interaction effects of interest (*viz.* method effects, sample effects and sample × method interaction). The significance of the observed constant method differences, as well as sample × method interaction, was assessed by means of an analysis of variance procedure based on the above model (Graybill, 1976). The pooled error variances pertaining to the two methods were compared by means of an *F*-statistic.

RESULTS AND DISCUSSION

The results obtained for sucrose content are summarised in Table 1. The figures in this table were calculated on the basis of an unconstrained least squares regression analysis of the data obtained from replicate injections of the standards (of the form $Y = A + BX$ where Y = peak area and X = concentration) and were used as a basis for comparison of the two sample preparation methods.

The corresponding values, calculated on the basis of a constrained calibration ($Y = BX$), were also used for this purpose.

Satisfactory replicates were obtained for all samples by Method 1 and for all samples except B9 by Method 2. Inclusion of data for this sample led to a much higher estimate of error variance for Method 2 than for

TABLE 1
Molasses Samples (ICUMSA, Subject 8)
(Sucrose content (g/100 g) calculated from unconstrained plot: $Y = A + BX$)
(Method 1—With sample cleanup. Method 2—Without sample cleanup)

Sample No.	*Method No.*		*Sample No.*	*Method No.*	
	1 *Sucrose*	*2* *Sucrose*		*1* *Sucrose*	*2* *Sucrose*
C1	31·99	31·67	B9[a]	41·29	41·09
	31·77	31·80		41·65	38·71
	32·02			41·69	
	32·35			41·22	
	32·55			41·80	
	32·74			41·56	

TABLE 1—*contd.*

Sample No.	*Method No.*		*Sample No.*	*Method No.*	
	1 *Sucrose*	*2* *Sucrose*		*1* *Sucrose*	*2* *Sucrose*
C2	36·02	35·88	B10	41·53	40·41
	35·85	36·00		41·54	41·15
	35·70			41·69	
	35·97			41·39	
	35·32			41·46	
	36·01			41·11	
C3	28·41	28·39	B11	48·39	48·08
	28·41	28·86		48·58	47·82
	28·40			47·95	
	28·67			47·85	
	28·31			47·77	
	28·56			48·47	
C4	27·45	27·58	B12	49·43	49·16
	27·88	27·71		48·76	49·08
	28·11			49·27	
	28·00			49·27	
	27·99			50·24	
	28·05			50·28	
C5	25·74	24·89	B13	47·26	47·33
	25·61	24·93		47·28	47·16
	25·61			47·49	
	25·97			47·83	
	26·03			47·89	
	25·75			47·47	
C6	29·40	27·93	B14	49·30	48·47
	29·41	28·94		48·89	48·48
	29·60			48·82	
	29·53			48·87	
	29·53			49·39	
	29·66			48·83	

Two sets of triplicate determinations on each sample were made by Method 1 and a single set of duplicate determinations was made on each sample by Method 2.

[a] Results for sample B9 were not used for comparison of Methods 1 and 2 because of one anomalously low value for sucrose obtained using Method 2.

TABLE 2
Table of Mean Concentrations Obtained Under the Two Methods
(Unconstrained calibration: $Y = A + BX$)

Sample No.	*Method 1* ($n = 6$)[a]	*Method 2* ($n = 2$)[a]	*Differences*	
			'Absolute'	*'Per cent of method 1'*
C1	32·24	31·74	0·50	1·55
C2	35·81	35·94	−0·13	−0·36
C3	28·46	28·63	−0·17	−0·60
C4	27·91	27·65	0·26	0·93
C5	25·79	24·91	0·88	3·41
C6	29·52	28·44	1·08[b]	3·66
B10	41·45	40·78	0·67	1·62
B11	48·17	47·95	0·22	0·46
B12	49·54	49·12	0·42	0·85
B13	47·54	47·25	0·29	0·61
B14	49·02	48·48	0·54	1·10
Average	37·77	37·35	0·42	1·11

[a] n refers to number of replicates per sample.
[b] Mean difference between these sample means differs significantly from overall mean difference (0·42) between methods.

Method 1, but the variances for the two methods could not be shown to differ significantly if the data for B9 were excluded. The remainder of the analysis was, therefore, limited to eleven samples, excluding B9.

On the basis of the unconstrained calibration results for these eleven samples, the analysis of variance procedure revealed significant constant method differences ($p < 0{\cdot}001$), as well as significant method × sample interaction effects ($p = 0{\cdot}003$). The actual differences recorded were, however, relatively small (Table 2). The pooled error variance estimate for the two methods amounted to 0·0686 with 55 degrees of freedom. This figure corresponds to a coefficient of variation of 0·7% relative to the grand mean of 37·7 g/100 g. The conclusions were essentially the same in the case of the constrained calibration.

It is surprising that higher sucrose values were generally obtained by the method incorporating sample cleanup. If any losses of sucrose do occur during cleanup, these are offset by a concentration effect. The latter may occur as a result of size exclusion effects during deionisation.

The observed method × sample interactions may be due to removal

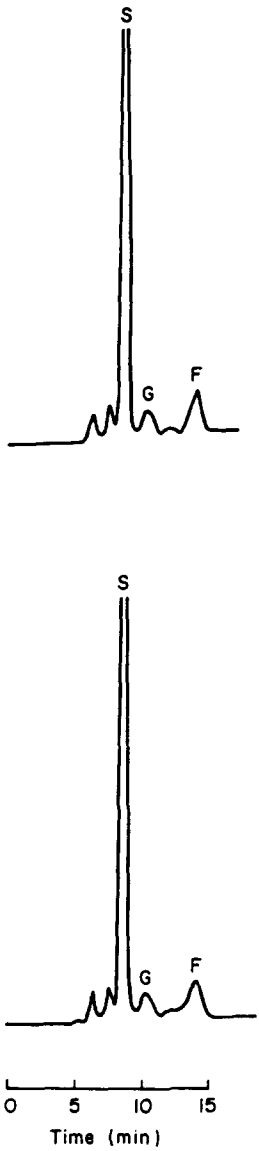

Fig. 1. Chromatograms of a cane molasses sample. With sample cleanup (upper chromatogram) and without sample cleanup (lower chromatogram). Methods and chromatographic conditions as described in the text. S = sucrose, G = glucose, F = fructose.

by sample cleanup of unidentified minor components which coelute with sucrose or indirectly influence sucrose peak area in some samples. The effect of cleanup on chromatograms is illustrated in Fig. 1. This particular sample (No. C2) differed from most of the others as sample cleanup resulted in a slightly lower sucrose value. The two chromatograms are similar but sample cleanup resulted in improved separation of a minor monosaccharide component eluting between glucose and fructose (probably mannose). Similar chromatograms were obtained for sample C3 where sample cleanup also resulted in a slightly lower sucrose value.

These results indicate that the use of a suitable cleanup procedure may improve the overall accuracy of the method. An investigation of the causes of the observed method differences, and also of alternative cleanup procedures and recoveries of added standards, would be desirable in order to establish the most appropriate procedure for sample cleanup.

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