ALTERNATE METHODS OF SUCROSE ANALYSIS

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INTRODUCTION

Chromatography has come to stay as the best method for sucrose analysis. Although different chromatographic procedures give accurate and reproducible results, these methods lack the quickness that factory situations demand. Thus polarimetry although not very accurate still is the basic tool of analysis for sucrose in most factory laboratories.

The factory chemist ideally would like to determine the carbohydrates of interest in a couple of minutes, if not in seconds. Therefore a method to identify each component simultaneously may give the desired quick results. In the recent past there has been a flurry of activity in the field of enzymatic analysis[1] as enzymes are capable of identifying each carbohydrate component specifically.

Although there is a wide array of modified enzymatic analyses, all involve immobilized enzymes and belong two main types. Their approaches are different from one another. In one the sample is analyzed in a flow system and appropriately called flow injection analysis (FIA).[2,3] In here the enzyme is adsorbed on to a column of amino-cellulofine, and the injected sample flows simultaneously through each of the columns containing an immobilized enzyme.

The second method involves a "static" system. Here the sample is introduced in to a reactor containing immobilized enzyme membranes. There are three approaches to this method. In the first approach[4] the reactor contains one enzyme electrode for glucose and another for sucrose and glucose. Therefore subtraction of one value from the other gives results for both sucrose and glucose. In the second approach[5] a single reactor containing a glucose electrode and immobilized invertase enzyme measures the variation of current with time. The current in the initial phase is considered to come from glucose whose reaction at the glucose electrode takes place immediately while sucrose takes time to react with invertase and then mutarotates to glucose. Here the change of current after the initial phase is considered to be for sucrose. A simple modification of this approach is to measure the glucose concentration first and subsequently introduce an immobilized invertase enzyme to the reactor.[6] Another similar approach proposed[7] in the sugar industry is the measurement of glucose using a glucose analyzer before and after adding invertase to the sample thus determining both the glucose and the sucrose content. The third approach is the glucose elimination methodology which is achieved by removing glucose from the system by glucose oxidase and a catalase bienzyme before sucrose analysis.

In a recent article some Japanese workers[2] have described simultaneous analysis of glucose, fructose, and sucrose by the flow injection analysis (FIA) method, where immobilized enzymes are used for each carbohydrate.

Here parallel configuration of enzyme immobilized reactors with a multichannel amperometric detector and a glucose eliminating reactor is used. In addition ascorbic acid, an interfering substance is removed from the sample by the use of an ascorbate-eliminating reactor. This system can determine glucose, sucrose, and fructose simultaneously.

The general reaction scheme for glucose, sucrose, and fructose in the presence of immobilized membranes is given in Scheme I.

SCHEME I

REACTION PATHWAYS OF GLUCOSE, SUCROSE AND FRUCTOSE

1. Glucose

α-D-Glucose + H_2O + O_2 $\xrightarrow{\text{Glucose Oxidase}}$ D-glucono-δ-lactone+H_2O_2

2. Sucrose

Sucrose + H_2O + $\xrightarrow{\text{Invertase}}$ D-Fructose + α-D-glucose

α-D-glucose $\xrightarrow{\text{Mutarotase}}$ β-D-glucose

β-D-glucose + H_2O + O_2 $\xrightarrow{\text{Glucose Oxidase}}$ D-glucono-δ-lactone + H_2O_2

3. Fructose

D-Fructose $\xrightarrow{\text{Fructose 5-dehydrogenase}}$ 5-Keto-D-fructose

Hexacyanoferrate(III)----->hexacyanoferrate(II)

In both reactions 1 and 2, the production of hydrogen peroxide or consumption of oxygen can be used as a measure of the reaction. In all three cases hydrogen peroxide and reduced hexacyanoferrate (II) are detected amperometrically.[2] Other methods of detections are also available.[9]

This paper discusses a preliminary study we carried out on the YSI 2700 a dual channel sucrose and glucose analyzer and how the values determined for sucrose compare with that of Ion Chromatography. In a separate experiment sucrose values obtained from five different factory samples were compared with High Pressure Liquid Chromatography, Ion Chromatography, and polarimetry. Similar comparisons without the enzymatic analysis for sucrose have been carried out in the past.[10]

EXPERIMENTAL

The YSI 2700 is a commercial enzymatic analyzer, capable of determining sucrose and glucose in addition to other compounds. This apparatus was obtained from Yellow Springs Instruments, Ohio. The sucrose and glucose membranes were obtained from the same source. The instrument was calibrated using sucrose (5.00 g/L) and glucose (2.50 g/L) solutions and the variables (sample size etc.) in the instrument were set to default values unless otherwise stated. When molasses, brei or

pellet samples were run, 10.00 g/L of sucrose and 2.50 g/L of glucose were used as calibration standards. The concentration of the samples analyzed were maintained around 10.00 g/L for sucrose. Two sucrose standard solutions containing 12.00 and 8.00 g/L of sucrose were read before and after each calibration. An error of +2% was allowed for the standards and if these limits were exceeded a recalibration was done. No filtration or other purifications were carried out on any of the samples.

The Ion Chromatograph used is a Dionex 2020i with a pulsed amperometric detector (PAD). The analysis was carried out using lactose as an internal standard.

The High Pressure Liquid Chromatograph used is a Waters Sugar Analyzer fitted with a Bio-Rad Aminex HPX-87N column. An external standard method was used in the analysis of samples.

The Polarimeter was an automated Crystal Tek Model.

RESULTS AND DISCUSSION

In order to assess the enzymatic analyzer's (YSI-2700) reproducibility, linearity and accuracy 3 g, 5 g, 10 g, 25 g standard sucrose solutions were read. The following results (Table 1) are for five readings obtained on each standard sucrose sample. The instrument was calibrated with sucrose (5.00 g/L) and glucose (2.50 g/L) solutions. The sample size was 25 µL.
The same experiment was carried out with a sample size of 15 µL. The results are as given in Table 2.

The results obtained for pure sucrose solutions (Tables 1 and 2) indicate that the values obtained on the YSI apparatus was sufficiently accurate and reproducible (RSD = 0.07%-0.98%) 3 g - 10 g samples. When a higher concentration (25 g/L) with 25 µL sample size was used, it deviated from linearity (11%) more than the specified amount (YSI literature, 5%) and in addition the reproducibility (RSD 1.4%-1.7%) was poor. When the sample size was reduced to 15 µL, both linearity and reproducibility improved indicating that at high concentrations the membrane enzyme kinetics determine the reaction rate.[4]

In a separate experiment six molasses samples were compared and analyzed by 1. An enzymatic method using YSI-2700, 2. Ion Chromatography, 3. High Pressure Liquid Chromatography, and 4. Polarimetry. The results from the six different factory samples are given in Table 3 and indicate that IC, HPLC and Pol values are in general agreement while the YSI values tend to be low.

In addition a number of brei and pellet samples were read on the YSI and were compared with IC values. Table 4 gives a comparison of YSI and IC values of factory pellet samples obtained from four consecutive weekly composites.

Table 5 gives a comparison of sucrose values obtained by YSI and IC for 22 randomly selected brei samples.

CONCLUSIONS

The preliminary results obtained indicate enzymatic methods in general and the use of YSI-2700 in particular are suitable and may be used as a fast method for sucrose analysis in beet end samples. A sample run takes only approximately 80 seconds, and there is no sample preparation although appropriate dilutions have to be made.

Pure sucrose solutions around the calibration standards give reasonably accurate results (Relative

deviation of mean from true value <1.6%, Tables 1 and 2). Data obtained on pellets though promising is inconclusive. For weeks #1, 2, and 4 there is better agreement between IC and YSI values, than for week #3. Week 3 results may indicate problems with the method or only "random" sorts of problems with calibration and sample preparation. These results look promising, but there is a clear need for further work. Data obtained on twenty four brei samples show that there is very good agreement between on IC and YSI values. Only two samples show greater than 3% bias. In the case of molasses consistent low values were obtained on YSI, when compared to the other methods. This result is not unexpected due to the presence of materials in molasses which are known to inhibit the enzyme.

A remaining concern is the instrument's occasional irregular behavior. This may be due to the quality of the membranes used at different times. It also could be due to fouling of the probe which we have observed at times. We have come to understand the instrument better with time and we are getting more reproducible and accurate results now than before. However we constantly keep a check on the YSI readings by random comparisons with the IC.

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TABLE 1

YSI ANALYSIS OF PURE SUCROSE SOLUTIONS

	3 g/L	5 g/L	10 g/L	25 g/L
	2.99	4.97	9.89	23.0
	3.00	4.92	9.91	22.5
	3.02	4.94	9.91	22.3
	3.05	4.97	9.90	22.1
	3.05	4.92	9.90	21.9
Mean	3.02	4.94	9.90	22.36
SD	.025	.022	.0075	.37
RSD, %	.83	.45	.075	1.68
Relative deviation of mean from true value, %	.6	1.2	1.0	10.56

TABLE 2

ANALYSIS OF PURE SUCROSE SOLUTIONS

	3 g/L	5 g/L	10 g/L	25 g/L
	2.95	5.00	9.96	23.9
	2.99	5.02	9.78	23.5
	2.94	4.89	9.81	23.0
	3.00	4.92	9.81	23.2
	2.97	4.95	9.76	23.1
Mean	2.97	4.96	9.82	23.26
SD	.023	.048	.070	.326
RSD, %	.77	.98	.718	1.4
Relative deviation of mean from true value, %	1.0	.88	1.76	6.96

TABLE 3

COMPARISON OF MOLASSES SAMPLES FROM SIX DIFFERENT FACTORIES

	IC%	HPLC %	YSI %	POL %
Sample 1	51.55	52.88	50.23	51.98
Sample 2	46.95	47.73	44.24	46.42
Sample 3	48.80	49.83	47.19	48.58
Sample 4	48.38	48.72	46.07	47.90
Sample 5	49.43	48.25	47.34	48.88
Sample 6	45.81	45.28	43.78	

TABLE 4

COMPARISON OF PELLET OF FIVE DIFFERENT FACTORY SAMPLES FOR FOUR WEEKLY COMPOSITES

	YSI %	IC %	Bias (YSI - IC)	YSI %	IC %	Bias (YSI - IC)
	Week 1			Week 2		
1.	7.47	7.70	-0.23	6.64	6.23	0.41
2.	6.03	6.36	-0.33	4.88	4.75	0.13
3.	9.03	9.35	-0.33	10.20	9.92	0.28
4.	8.63	8.90	-0.27	8.15	8.08	0.07
5.	10.85	10.20	0.65	12.45	11.80	0.65
6.	7.80	8.10	-0.3	7.92	7.75	0.17
	Week 3			Week 4		
1.	6.98	6.47	0.51	6.85	6.84	0.01
2.	4.80	4.37	0.43	5.45	5.53	-0.08
3.	9.10	8.75	0.35	9.35	9.05	0.03
4.	7.40	6.70	0.30	7.52	7.26	0.26
5.	10.2	10.25	-0.05	8.92	8.71	0.21
6.	7.92	7.42	0.5	7.97	7.73	0.24

TABLE 5

COMPARISON OF SOME DATA OBTAINED FROM YSI AND IC OF RANDOMLY SELECTED BREI SAMPLES

	YSI g/L	IC g/L	Bias (YSI - IC)		YSI g/L	IC g/L	Bias (YSI - IC)
1.	9.00	8.88	0.12	12.	9.70	9.73	-0.03
2.	9.80	9.85	-0.05	13.	9.41	9.71	-0.30
3.	9.93	9.91	0.02	14.	10.0	10.13	-0.13
4.	10.1	10.07	0.03	15.	9.97	10.07	-0.10
5.	9.56	9.49	0.07	16.	9.61	9.74	-0.13
6.	9.63	9.81	-0.18	17.	10.7	10.71	-0.01
7.	9.73	9.66	0.07	18.	9.75	9.84	-0.09
8.	9.69	9.63	0.06	19.	9.61	9.77	-0.16
9.	10.8	10.70	0.1	20.	9.78	9.94	-0.16
10.	9.94	9.84	0.10	21.	9.19	9.36	-0.17
11.	10.2	10.40	-0.20	22.	10.0	9.43	0.57