Estimation of total sugar and reducing sugar in molasses using near infrared diffused reflectance spectroscopy

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Introduction

Final cane molasses usually contains 30–40% sugar by weight, which is around 10% of the total sugar (non-reducing sugar and reducing sugar) content in cane. The control of this loss is a challenging problem in sugar industries. Also, the ratio of reducing sugars (RS) (glucose and fructose) to non- reducing sugar (sucrose), RS/sucrose, is an important factor which influences the exhaustibility of molasses. Hence, estimation of total sugar and reducing sugar content in molasses is a very important task in sugar refineries. The polarimetric method is commonly employed in sugar industries to determine sugar content in molasses. Although the technique is adequate for sugarcane juice samples under ideal conditions, it has serious limitations in the case of molasses with high a concentration of reducing sugar. Chemical analysis for the estimation of total sugar and reducing sugar in cane molasses is the standard conventional method. However, the complexity of analysis, time required for analysing each sample and the use of hazardous chemicals have made this technique inefficient for process control in any sugar industry. Therefore, a more accurate method is required to separately quantify total sugar and reducing sugar content. Hence, the development of a rapid, inexpensive, physical and also accurate method for sugar determination in molasses will be highly useful.

The near infrared (NIR) spectroscopic technique has emerged over the past several years as an alternative analytical tool for sugar refineries.[1–7] Several types of sugarcane materials with different types of physical or chemical properties have been studied using NIR reflectance as well as transmittance spectroscopy. The work by Berding *et al*.[2] and Clarke *et al*.[8] had pointed out the use of NIR as a realistic method for analysis of sugarcane juice, bagasse and molasses. However, very few attempts to determine the chemical composition of molasses by NIR have been made. In a case study on cane molasses R.A. Pax[9] applied multiple linear regression for quantification of sucrose in molasses using double polarisation data as reference. Large errors of prediction have, however, been reported. It will be of immense value to further explore the potential of near infrared diffused reflectance spectroscopy as a rapid and automated analytical technique for the determination of total sugar and reducing sugar content in molasses.

Materials and methods

The molasses samples were collected during and after the sugarcane season from sugar industry. Care was taken to obtain samples with a wide range of concentration of total sugar and reducing sugar. Each sample was analysed by the standard polarisation method using a digital automatic

saccharimeter after clarifying the molasses solution with lead acetate to get the pol value. The samples were also analysed using conventional chemical analysis to find out total sugar and reducing sugar present in the sample. The pol values and chemical values of total sugar and reducing sugar obtained were used as reference to develop the calibration model. An indigenous ELICO NIR system specially designed for the sugar industry was used for taking spectral measurements. The samples were scanned in the region of 1100–2500 nm in diffused reflectance mode. A ceramic block was used as reference.

Chemical analysis

All the samples were analysed chemically for total sugar and reducing sugar content using the standard titration against Fehling's solution. The Fehling's solutions A and B were purchased from the market and used as it was. (1 mL of Fehling's solution equivalent to 0.005 g of invert sugar). Neutral lead acetate solution was prepared by dissolving 100 g lead acetate in 1 L of water. Sodium phosphate–potassium oxalate solution was prepared by dissolving 70 g of di-sodium hydrogen phosphate dodecahydrate and 30 g of potassium oxalate in 1 L water.

Sample preparation

12.5 g of molasses sample was weighed and transferred to a 250 mL volumetric flask with the help of water. 25 mL of lead accetate solution was added to it, volume was made up and the contents were shaken and filtered. 10 mL of sodium phosphate–potassium oxalate mixture was added to 100 mL of this filtrate in a 500 mL volumetric flask. Once again after making up the volume, the contents were shaken and filtered. The solution so obtained was used for determination of total sugar and reducing sugar content. All the samples were titarted against 10 mL of Fehling's solution and the total and reducing sugar in all the samples were calculated as follows.
1 mL of Fehling's solution = 0.005 g invert sugar based on the equation

$$C_{12}H_{22}O_{11} + H_2O = 2\ C_6H_{12}O_6 \tag{1}$$

342 g of sucrose gives 360 g of invert sugar or 0.00475 g of sucrose gives 0.005 g of invert sugar = 1mL of Fehling's solution. The Fehling factor (invert sugar equivalent to 10 mL of Fehling's solution) = .05

Total sugars (TS) in the molasses samples were calculated according to the formula given as

$$TS\% = \frac{\text{Fehling factor} \times 250 \times 500 \times 100 \times 100}{12.5 \times 100 \times 50 \times \text{Titer volume (mL)}} \tag{2}$$

Reducing sugars (RS) in molasses samples were calculated according to the formula given as:

$$RS\% = \frac{\text{Fehling factor} \times 250 \times 500 \times 100}{12.5 \times 100 \times \text{Titer volume (mL)}} \tag{3}$$

Results and discussion

A commercial program provided with the NIR spectrophotometer, based on GRAMS 386 for multivariate spectral data analysis was used to process the data and to develop the model for pol, total sugar and reducing sugar content in molasses samples. Data were analysed in three different sets. In first set, pol values of the samples were taken as the reference to develop the model. In second the set, reference values were obtained by chemical analysis of the samples. The calibration model was obtained for two components; total sugar and reducing sugar. The wavelength used for calibration was the full spectrum 1100–2500 nm. In the third set, the reference values were again obtained using

Table 1. NIR calibration statistics.

Reference method	Range (nm)	No of samples	Factors	*R*	*RMSD*	No of outliers
Pol	1100–2500	40	5	0.8316	5.0195	
Chemical Analysis	1100–2500	40	6 (TS)	0.9395(TS)	2.6455(TS)	4
	—	—	6(RS)	0.8063(RS)	0.6118(RS)	4
Chemical Analysis	2100–2500	40	6(TS)	0.9556(TS)	1.4833(TS)	2
	—	—	6(RS)	0.9288(RS)	0.3142(RS)	2

R is the correlation coefficient: *RSMD* is the root mean square difference

chemical analysis for total sugar and reducing sugar but the wavelength used for calibration was 2100–2500 nm.

Prior to calibration, spectral data were mean centered. Partial least square (PLS) regression was employed for the calibration. The PLS used both the spectral response and respective reference data for the examined samples to determine PLS factors on the data set. The optimum number of PLS factors used in a model was determined by a cross-validation procedure. One sample was temporarily removed from the calibration set to be used for validation. With the rest of the samples, a PLS model was developed and applied to predict total sugar and reducing sugar content in molasses samples. The results were compared to their respective reference values. This procedure was repeated until the prediction for all the samples were obtained. The optimum number of PLS factors in each set was defined as the one that corresponds to the lowest standard error of cross-validation. The values of the correlation coefficient (*R*) and root mean square difference (*RMSD*) were calculated for each set.

The calibration statistics for all the three cases are summarised in Table 1. Using the pol values as reference, a low correlation coefficient value of 0.8316 was obtained and even the standard error index, root mean square difference was quite high, 5.0195. Poor correlation in calibration based on pol values was not unexpected because polarimetry is not a correct technique for measuring sugar content in molasses which have a substantial concentration of reducing sugars. Hence calibration developed using polarisation values was unsatisfactory and could not be considered suitable for evaluation of sugars in molasses. In the second set, an optimal correlation was obtained with the correlation coefficient value of 0.9395 for total sugar and 0.8063 for reducing sugar when full spectrum was used in developing the calibration model. Although the calibration is acceptable, the error index is a little higher, 2.6455 for total sugar and 0.6118 for reducing sugar. A robust calibration could be developed by selecting the region 2100–2500 nm. The choice was based on the fact that all the relevant combination bands of sucrose, glucose and fructose are located in this region. The total sugar and reducing sugar values obtained by chemical analysis were again used as the reference. High values of correlation coefficients, 0.9556 for TS and 0.9288 for RS with relatively low *RMSD* values, 1.4833 and 0.3142, respectively, could be achieved.

The developed calibration models using the chemical method as a reference were used for prediction of total and reducing sugars in unknown samples of molasses. A prediction set consisting of eight samples was created and values of total sugar and reducing sugar were determined. Table 2 gives the concentration of total and reducing sugars in unknown molasses samples predicted using the full spectral range (1100–2500 nm) calibration model. Similarly, the sugar content in these molasses samples was also determined using range 2100–2500 nm calibration curve. The values are indicated in Table 3. As can be seen, the values obtained in the latter case are more close to the actual values.

Table 2. Value of total sugar and reducing sugar in molasses samples in prediction set (1100–2500).

Chemical	NIR	Bias	Chemical	NIR	Bias
TS(%)			RS(%)		
35.32	36.17	–0.85	9.90	8.92	0.98
33.69	32.66	1.03	9.94	9.72	0.22
39.91	40.41	–0.50	8.19	8.35	–0.16
41.13	41.98	0.77	8.35	7.73	0.62
41.85	41.48	0.37	8.14	8.40	–0.26
43.98	42.81	1.17	8.16	8.59	–0.43
36.26	32.57	3.69	9.96	10.20	–0.24
47.18	46.02	1.16	8.26	8.61	–0.35

Table 3. Value of total sugar and reducing sugar in molasses samples in prediction set (2100–2500).

Chemical	NIR	Bias	Chemical	NIR	Bias
TS(%)			RS(%)		
35.32	33.93	1.39	9.90	9.58	0.32
35.85	36.17	–0.32	8.17	8.00	0.17
39.91	39.33	0.58	8.19	8.42	–0.23
41.13	40.71	0.42	8.35	8.52	–0.17
41.85	42.15	–0.30	8.14	8.03	0.11
46.67	45.73	0.94	8.22	8.02	0.20
47.18	47.54	–0.36	8.26	8.54	–0.28
55.96	56.66	–0.70	8.18	8.32	–0.14

Conclusion

The present study shows that near infrared diffused reflectance spectroscopy has considerable potential in rapid estimation of the total sugar and reducing sugar in molasses samples. The approach can be developed into a completely automated on-line technique in a sugar refinery where no sample preparation is required.

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