

RAPID ANALYSIS OF CANE JUICE BY NEAR INFRA-RED REFLECTANCE

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Abstract

An investigation was carried out to test the suitability of near infra-red reflectance (NIR) for rapidly estimating cane quality components. A large number of mixed cane juice samples of known pol, brix, sucrose, fructose, and glucose content were used to calibrate a Technicon Model 450 Infra-Alyzer. Regression analyses indicated that brix, pol, and sucrose values obtained were closely correlated with those obtained by the conventional methods of analysis based on the refractometer, saccharimeter, and gas chromatograph ($r = 0,90$). Glucose and fructose values, however, were not well-correlated with those obtained by gas chromatography. The NIR method was also compared with the whole stalk analysis procedure used for determining pol, brix, fibre and moisture in cane, and preliminary results are reported as well as results using shredded cane samples. Indications are that a reasonably accurate estimate of cane juice quality can be rapidly achieved by this new technique.

Introduction

The use of near infra-red reflectance spectroscopy (NIRS) for the analysis of constituents in food and other related products has increased considerably during the past 5 years (Clark²). This technique is now well-established for the determination of protein, oil, carbohydrate and moisture contents of raw materials used in the baking industry (Wetzel¹). Interest in this technique stems from the fact that it is rapid, and it can be used to determine up to 9 constituents simultaneously.

A search for new applications for near infra-red reflectance (NIR) has been in progress at the SASA Experiment Station for some time and so far it has been used for the routine determination of nitrogen (N) in cane leaf samples (Meyer⁵), and more recently for the rapid simultaneous estimation of organic matter, texture and N-mineralization potential of soils (Meyer *et al.*⁶). The possible application of this technique to the determination of the sucrose content of cane juice led to an experiment in which a series of prepared sugar solutions, ranging from 5 to 20% sucrose, were used to calibrate a Technicon 300 Infra-Alyzer. Although the results obtained were promising, the filters supplied with the instrument did not cover the correct spectral range entirely. A larger instrument which covers a wider spectral range and has an effective liquid sampling device, has been used more recently. An investigation was conducted to assess the NIR technique for the simultaneous determination of brix, pol and sucrose contents as well as the presence of reducing sugars in cane juice. The direct determination of sugars, dry matter, and fibre components of shredded cane samples was also investigated.

Experimental procedure

Equipment

The instrument used was a Technicon Infra-Alyzer Model 450 (Figure 1) interfaced with an IBM personal computer.

Absorption bands in the near infra-red region (1 000 to 2 500 nm) are used to determine the desired constituents. In the Technicon instrument the primary light beam is passed through a series of 18 filters, covering the range 1 445 to 2 348 nm, and the narrow bands of filtered light are directed onto the sample. The scattered reflected rays of each wavelength are then concentrated onto a gold-plated integrating sphere. The amount of reflected light is inversely proportional to the amount of light energy absorbed at a particular wavelength which in turn is proportional to the concentration of the constituents which are optically active at that wavelength.

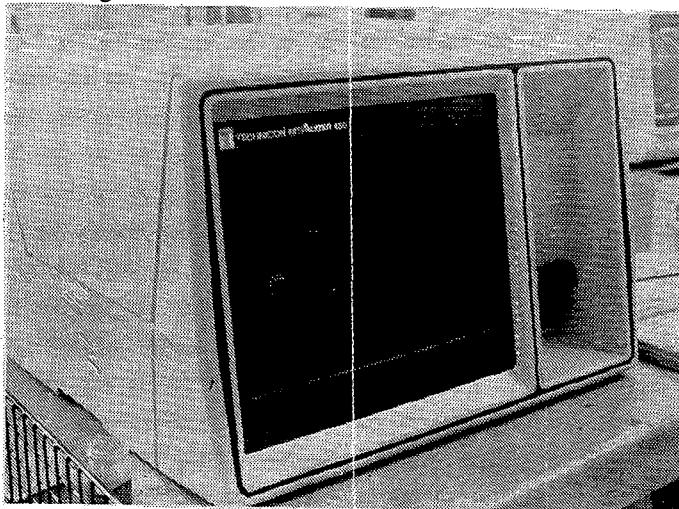


FIGURE 1 The Technicon near infra-red spectrophotometer.

The usefulness of NIRS in determining the concentrations of various constituents is due to the rotational and vibrational energies associated with hydrogen bonds. The vibrational motions of hydrogen bonded to other atoms (C, O, N and S) and the carbon-oxygen double bond stretching vibrational motion can be measured indirectly through the amount of near infra-red radiation absorbed. Reflectance varies depending on the number and types of bonds present. The wavelengths of functional groups that can be detected by NIR have been listed by Kaye.³

Procedure

Calibration and testing of the instrument proceeded in 3 stages: initial calibrations were carried out with standard sugar solutions of known sucrose content (1 to 20%), and with a series of cane juice samples of known brix and pol contents supplied by the millroom of the SASA Experiment Station. The cane juice samples included both diluted extracts prepared according to the 'full test' method (equivalent to the Sugar Industry Central Board (SICB) DAC procedure) and first expressed juice samples based on the Java ratio method (Anon¹). Most of the diluted extracts were prepared from different parts of stalk samples collected from various field trials. In this way a wide range of brix and pol values were obtained (Table 1).

Table 1

Nature and composition of samples and a comparison of calibration performance

Nature of sample	Source (no)	Constituent (% cane)	Range			Calibration performance		
			Low	High	Mean	Correlation coefficient	Standard deviation	CV%
Standard sugar solutions	SASA millroom (46)	Pol	1,00	20,00	11,00	0,99	0,19	1,7
2:1 diluted extracts		Brix Pol	1,71 0,69	5,77 5,38	4,49 4,07	0,93 0,91	0,29 0,28	6,0 6,9
Shredded cane samples	SASA millroom (35)	Brix	5,00	16,70	13,70	0,88	0,47	3,4
		Pol	1,00	15,40	11,68	0,86	0,55	4,7
		Purity	19,70	96,20	82,03	0,89	3,90	4,7
		Dry matter	12,60	30,00	25,11	0,92	0,86	3,4
		Fibre	7,60	14,10	11,42	0,87	0,69	6,0
Mixed cane juice	SICB laboratory, Mount Edgecombe (90)	Brix	7,93	13,95	10,95	0,97	0,35	3,2
		Pol	5,96	12,22	8,96	0,98	0,32	3,6
		Sucrose	6,14	12,37	11,14	0,97	0,31	3,4
		Glucose	0,16	0,76	0,36	0,51	0,09	25,6
		Fructose	0,19	0,59	0,34	0,54	0,07	21,0

In the second phase of the investigation almost 100 mixed cane juice samples that had been analysed by the SICB laboratory at Mount Edgecombe were used to calibrate the instrument.

Apart from brix and pol values, sucrose, fructose, and glucose contents had been determined by gas chromatography (GC).

In the third stage sucrose, dry matter, and fibre contents in shredded cane samples were estimated. Separate calibrations were carried out for fresh and dried shredded cane samples.

Calibration

All samples were run through the Infra-Alyzer and the reflection values for each filter, together with the millroom or SICB analyses were stored on computer discs. A minimum of 30 samples is required for calibration and the various constituent values should ideally follow a normal distribution pattern. Once the recording of the reflection values is complete, a cycle of regression is started on the computer in order to develop a set of weighting factors or coefficients called 'F' values which relate sample measurement to sample composition. Each set usually represents the best combination of 5 wavelengths which give the lowest standard error of estimate in a multiple regression analysis. Equations were produced for the prediction of each constituent, having the following general form:

constituent value.

$$= F_0 + F_1 \log \frac{1}{R_1} + F_2 \log \frac{1}{R_2} \dots F_{20} \log \frac{1}{R_{20}}$$

where $R_1, R_2 \dots R_{20}$ are the measured reflectance values of the sample at selected wavelengths

$F_0, F_1 \dots F_{20}$ are the regression coefficients

The computed F values of the best set of filters for each of the various constituents were then entered into the memory bank of the Infra-Alyzer. Calibrations were evaluated by using the equations to predict the brix, pol, sucrose, glucose, and fructose contents in separate batches of juice samples.

The NIR technique is non-destructive and once the instrument is programmed it is relatively simple to operate. In practice, about 5 drops of juice are placed on a circular stainless steel sample-holder (40 mm diameter) and this is covered by a microscope slide. Results are displayed digitally on a screen or a printer within 30 s of activating the sample-holder. In the case of shredded cane another device is used.

Results

Calibration

Comparative statistical information obtained for the calibrations derived from the different types of juice samples and the shredded cane samples is given in Table 1. Significant correlations were obtained between data from the NIR analyses and the various constituents as determined by conventional methods. In terms of the correlation coefficients and standard deviations, NIR appeared to be more reliable for determining pol and brix in cane juice than for shredded cane samples. However, in terms of the coefficient of variation (CV), the brix and pol % cane were not very different from the CV% values obtained for brix and pol measurements in mixed cane juice and full test analysis samples.

The relationship between the sucrose contents determined by NIR and those obtained by GC is shown graphically in Figure 2. The differences between the two methods are plotted in Figure 3, showing a slightly skew distribution. For about 70% of the samples examined, differences between GC and NIR were smaller than 0,30 units of sucrose (Table 2), while for pol and brix % the differences were generally less than 0,35 and 0,40 units respectively. For glucose and fructose the agreement between NIR and CG methods was unsatisfactory and no further comparisons were made. It seems that NIR is not sufficiently sensitive to determine concentrations of fructose and glucose below 0,5%.

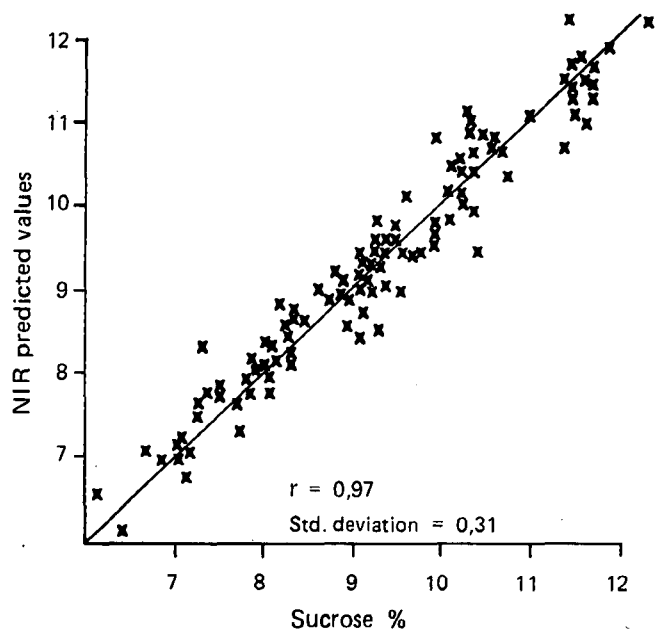


FIGURE 2 Relationship between GC and NIR methods for determining sucrose.

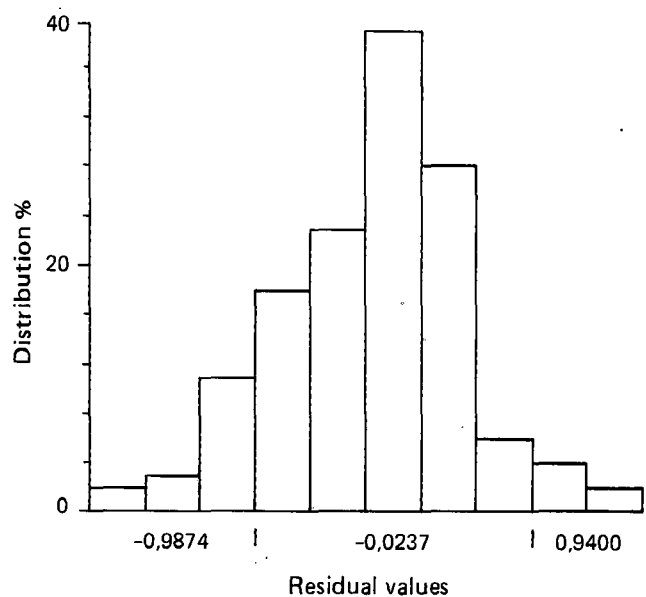


FIGURE 3 Distribution of deviations between the GC and NIR methods.

Table 2

Agreement between near infra-red and conventional methods of cane juice analysis

Measurement	Conventional method	Limit of deviation	% samples correlating
Brix	Refractometer	±0,20	44
		±0,30	60
		±0,40	70
		±0,50	80
Pol	Saccharimeter	±0,20	45
		±0,30	65
		±0,40	75
		±0,50	87
Sucrose	Gas chromatograph	±0,20	47
		±0,30	68
		±0,40	79
		±0,50	89

For shredded cane samples the correlations obtained when calibrating the instrument for brix and pol % cane were not as satisfactory as those for the juice calibration, but the results indicated that in 70% of the samples examined, differences between results obtained by NIR and by the conventional methods were smaller than 0,55 units of brix % cane and 0,60 units of pol % cane. Dry matter % cane values obtained by NIR were generally well correlated ($r = 0,86$) with the computed values in the 12 to 30% range (Figure 4) and in about 70% of the samples, differences were smaller than 0,70 units. Though fibre values estimated by NIR were less well correlated with values obtained by conventional analysis, the relationship obtained with the use of 4 filters was significant (Figure 5).

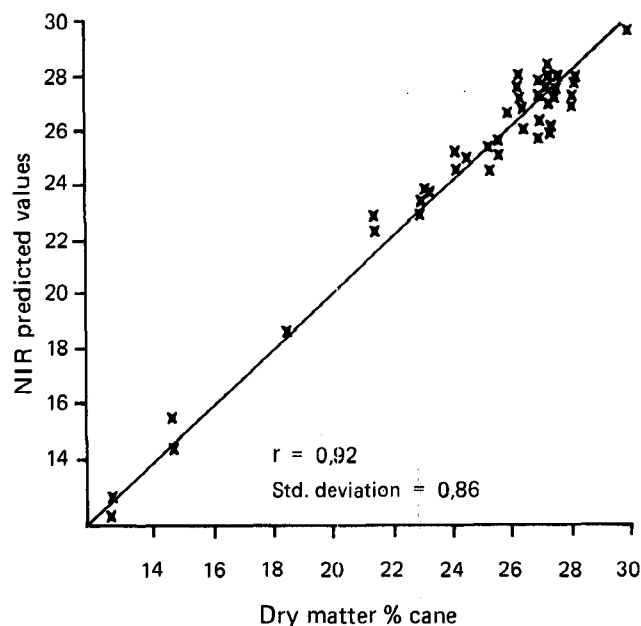


FIGURE 4 Comparison between NIR and conventional methods for determining dry matter % cane.

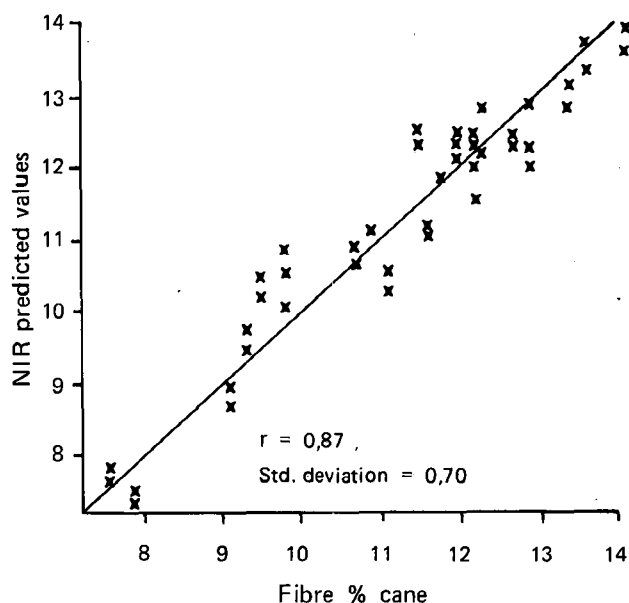


FIGURE 5 Comparison between NIR and conventional methods for determining fibre % cane.

Validation

A number of independent samples not included in the calibration exercise were used to validate the reliability of the equations to be used in the Infra-Alyzer programme. A comparison between conventional and NIR results for brix and pol % values in filtered juice obtained from partitioned cane stalks is given in Table 3. Results from the independent mixed juice samples submitted by the SICB laboratory are shown in Table 4. A wide range of brix and pol % values was covered, and in general the correlations between the results obtained by NIR and conventional methods were similar to those established in the calibration exercise.

The consistency of the NIR method was also investigated by analysing a number of prepared samples 5 times. Coefficients of variation ranged from 1 to 3% for juice samples and from 3 to 8% for shredded cane samples.

For the shredded cane samples agreement between the various constituents based on NIR and the conventional method was not very satisfactory and further calibration is required with a wider range of samples.

Discussion and Conclusions

The preliminary results obtained in this investigation indicated that it is possible to measure simultaneously the brix, pol, and sucrose contents of either first expressed or diluted cane juice. Similar results were recently reported for the

analysis of brix and pol % cane juice and molasses by Mantovani.⁴ Although the technique is currently not considered to be sufficiently accurate to replace any of the standard methods of analysis, it does have potential for rapidly evaluating cane quality. The usefulness of the technique for cane juice analysis could be improved by developing separate calibrations for the low, medium, and high ranges of brix and pol values. The use of a 'flow-through' cell might also produce more reliable results.

While results of the shredded cane analysis were not as satisfactory as those for juice analysis, it is possible that substantial improvements to the accuracy of the NIR technique could be made.

Results from similar work conducted in Louisiana (Sverzut *et al.*⁷) showed no statistical differences between the infra-red and conventional methods for determining pol, fibre, sucrose, and moisture contents in shredded cane. A reason for their improved results may be the use of a more sophisticated infra-red analyzer which is able to scan between 1 100 to 2 500 nm at every 2 nm. Sample uniformity is also an important factor and in this regard a more finely shredded cane sample would contribute to sample homogeneity. Once moisture has been determined in a sample by NIR, rapid drying in a microwave oven (Sverzut and Verma⁸) followed by grinding and pressing into a disc should improve the sensitivity of the technique for determining brix, sugar and fibre contents.

Table 3

Comparison between conventional and infra-red analysis of diluted (2 : 1) cane juice from partitioned cane stalks

Variety	Segment (from top)	Brix % juice			Pol % juice		
		Millroom	Infra-red	Difference	Millroom	Infra-red	Difference
NCo376	1	2,57	2,37	-0,20	0,95	0,51	-0,44
	2	3,60	3,58	-0,02	2,32	1,87	-0,45
	3	4,08	4,29	+0,21	3,10	2,87	-0,23
	4	4,30	4,59	+0,29	3,35	3,27	-0,08
	5	4,32	4,69	+0,37	3,40	3,30	-0,10
	6	4,39	4,87	+0,48	3,50	3,44	-0,06
	7	4,37	4,63	+0,26	3,50	3,37	-0,13
	8	4,61	5,15	+0,54	3,87	3,77	-0,10
N12	1	2,94	2,90	-0,04	1,62	1,15	-0,47
	2	4,14	3,89	-0,25	3,18	2,86	-0,32
	3	4,42	4,30	-0,12	3,47	2,95	-0,52
	4	4,62	4,67	+0,05	3,73	3,43	-0,30
	5	4,63	5,20	+0,57	3,73	3,86	+0,13
	6	4,76	4,88	+0,12	3,96	3,45	-0,51
	7	4,82	4,87	+0,05	4,03	3,77	-0,26
	8	5,05	5,62	+0,57	4,35	4,30	-0,05
Overall mean		4,22	4,40	+0,18	3,25	3,01	-0,24

Table 4

Near infra-red analysis of mixed juice samples from several mills

Mill	Brix % juice			Pol % juice			Sucrose % juice			
	SICB	NIR	Diff	SICB	NIR	Diff	SICB	NIR	Diff	
Glendale	12,48	12,34	-0,14	10,80	10,71	-0,09	10,89	10,98	+0,09	
Sezela 1	10,59	10,39	-0,20	9,12	9,05	-0,07	9,18	9,31	+0,13	
Sezela 2	10,66	10,14	-0,52	9,20	8,64	-0,56	9,28	8,86	-0,42	
Mt Edgcombe	12,74	12,79	+0,05	10,94	10,95	+0,01	11,05	11,31	+0,26	
Entumeni	10,75	10,74	-0,01	9,04	9,51	+0,47	9,14	9,58	+0,44	
Gledhow 1	11,15	10,88	-0,27	9,52	9,19	-0,32	9,57	9,40	-0,17	
Gledhow 2	11,44	11,58	+0,14	9,77	10,05	+0,28	9,85	10,23	+0,38	
Overall mean		11,40	11,26	-0,14	9,77	9,73	-0,04	9,85	9,95	-0,10

The greatest advantage of NIR over standard methods which make use of the saccharimeter is that only simple filtering of the juice is required instead of clarification by lead acetate. The results are immediately available (less than 30 s for each sample) and therefore the laboratory could process samples more rapidly.

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REFERENCES

1. Anon (1975-76). Sugarcane analysis. *Ann Rep SA Sug Ass Exp Stn*, Mt Edgecombe: 32-33.
2. Clark, DH (1985). History of near infra-red reflectance spectroscopy analysis of agricultural products. *ARS Agric Handb* 643, US Government Printing Office, Washington DC.
3. Kaye, W (1954). Near infra-red spectroscopy. Spectral identification and analytical application. *Spectrochim Acta* 6: 257-287.
4. Mantovani, G (1986). Report of the 18th session of ICUMSA, subject 13, dry substances in sugar products other than sugars.
5. Meyer, JH (1983). Rapid determination of nitrogen in cane leaves. *Proc S Afr Sug Technol Ass* 57: 109-112.
6. Meyer, JH, Wood, RA and Leibbrandt, NB (1986). Recent advances in determining the N requirement of sugarcane in the South African sugar industry. *Proc S Afr Sug Technol Ass* 60: 205-211.
7. Sverzut, CB, Verma, LR and French, AD (1987). Sugarcane analysis using near infra-red spectroscopy. *Trans Am Soc Ag Engrs* 30: 255-258.
8. Sverzut, CB and Verma, LR (1986). Microwave analysis of shredded sugarcane. *Trans Am Soc Ag Engrs* 2(2): 215-219.
9. Wetzel, DL (1983). Near infra-red reflectance analysis. *Anal Chem* 55: 1165A-1176A.