

NEAR INFRA RED ANALYSIS OF SHREDDED CANE: A POTENTIAL REPLACEMENT FOR THE DIRECT ANALYSIS OF CANE

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Abstract

Sugar industries world wide are continuing to show increasing interest in the development of near infra red (NIR) spectroscopy as a possible replacement for conventional laboratory analyses. In South Africa, the direct analysis of cane (DAC) is used to allocate payments to individual growers. DAC analysis is time-consuming, labour intensive and expensive. In contrast, previous NIR studies have shown that analysis of shredded cane can be carried out efficiently in 5 to 10 minutes. Earlier NIR estimates were not as accurate as the standard laboratory methods. However the introduction of scanning instruments and software improvements has enabled the re-evaluation of over 500 SMRI and SASEX shredded cane samples. Factors examined include sample deterioration prior to scanning, the use of different sample cells, the effect of sample packing using different operators, the accuracy of primary laboratory data, required for calibration, and the use of different NIR instruments. Calibration predictabilities, developed at one mill in 1994, were evaluated using data from four other mills over two different seasons (1992 and 1995).

Keywords: Analysis, NIR, DAC, shredded cane

Introduction

Direct analysis of individual cane (DAC) consignments is time consuming and expensive. A weighed aliquot of prepared cane (shredded) is added to water in a cold digester and extracted for 20 minutes. Suspended material is removed by filtration prior to using a refractometer for brix analysis. For pol analysis, a clarification/filtration step, using toxic lead acetate is essential, and this is then followed by reading the clear supernatant in a polarimeter. Moisture in cane is determined by oven drying a subsample for 60 minutes at 105°C.

The possibility of using NIR on shredded cane has been proposed as an alternative to DAC for determining cane quality. This procedure eliminates sample preparation as shredded cane can be scanned directly by the NIR instrument. Provided the instrument has been correctly calibrated, estimates of brix, pol and moisture can be obtained within minutes of the laboratory receiving the sample.

In 1987, the first calibrations for shredded cane in South Africa were developed at SASEX (Meyer and Wood, 1988). Unfortunately the small size of the sample cell and the restricted number of wavelengths available for the filter instrument limited the accuracy of this initial work. In a 1991 study, Berding *et al.* (1991) found that inadequate sample preparation, mixing and sample presentation were the major sources of error during NIR estimates. Visual inspection of the coarse-sample cell after packing ensured uniform sample presentation and the absence of voids. In 1993, a comparison of the calibration performance of a NIRSystems 6500 (scanning) and a Bran and Luebbe 450 (filter) instruments showed

that the scanning instrument was marginally better than the filter instrument for measuring pol, brix and moisture (Schaffler *et al.*, 1993). Berding and Brotherton (1994) used both early and late season cane for calibration and prediction work. They found that NIR errors were greater than those for laboratory analyses. However, these laboratory results were obtained under ideal conditions (clean cane, low sample throughput, research station laboratory). Compared to commercial cane testing, Berdings' predictions may be regarded as being more than adequate. The problems of cane deterioration prior to sample shredding, filling of the NIR sample cell and scanning were highlighted by Berding (1995). He also examined the lack of spectral references for high moisture standards, the problem of transferring calibrations between instruments and the need for maintaining calibration after serious instrument breakdown. Brotherton and Berding (1995) found that calibrations for components in mill-prepared cane were less satisfactory than calibrations for clean cane. Clarke *et al.* (1994) used frozen cane samples for NIR work. Simple step-wise regression rather than the partial least squares (PLS) technique was used to develop calibrations for analytes in shredded cane. Laboratory data were obtained from mill laboratories and sugarcane testing stations. Clarke *et al.* (1995) found that cane taken directly from mill carriers was too coarse for acceptable NIR calibrations. Additional preparation through a Jeffco cutter-grinder produced NIR results that compared favourably with laboratory procedures.

Comparisons of prediction data from various published papers are shown in Table 1.

Table 1
Statistical results from a number of published predictions for analytes* in shredded cane

Description	Analyte	Bias	Slope	r	SEP**
Berding <i>et al.</i> , (1991)	Brix	0,06		0,91	0,53
	Moisture			0,92	0,57
Berding and Brotherton, (1994)	Brix	0,03	1,01	0,98	0,21
	Moisture	0,02	0,99	0,99	0,28
Berding (1995)	Brix	-0,30	0,94	0,97	0,42
	Moisture	0,46	0,93	0,95	0,62
Clarke <i>et al.</i> , (1994)	Brix			0,96	0,54
	Moisture			0,82	1,65
Clarke <i>et al.</i> , (1995)	Brix			0,93	0,62
	Moisture				

* Pol results were not included as certain publications expressed pol as an instrument reading (°Z) rather than pol % cane

** SEP = Standard error of prediction

Recent work at the SMRI (Schaffler 1996a) has established that:

- Conversion of the original spectra to their second derivatives did not improve NIR predictions, based on PLS calibrations. This observation is useful as the use of the original data rather than treated data saves time and disk space.
- There was no advantage in including the visible and lower NIR region (400–1098 nm) for brix, pol and moisture predictions. This is important as visible-NIR spectrophotometers are significantly more expensive than units restricted to the 1100–2500 nm range.

NIR is a secondary technique, and quantitation of subsequent unknown samples is based on the assumption that these calibrations are robust and representative. When multivariate calibration (eg PLS) rather than spectral procedures are used to develop calibrations then validation is even more important. This paper examines several factors that could affect the accuracy of NIR estimates. These include:

- (a) The use of either fresh samples of shredded cane or samples that were frozen and later thawed for analysis.
- (b) The use of sample cells made by different manufacturers.
- (c) The effect of different operators packing the shredded cane into the sampling cells.
- (d) The effect of using a NIRSystems 5000 instrument for calibration and using spectral data from a NIRSystems 6500 for a subsequent validation.
- (e) The effect of using calibration data derived from one mill in 1994 to predict analytes in cane from other mills in 1992 and 1995.
- (f) The effect of using calibration data derived from commercial cane (burnt and dirty) to predict analytes in clean cane obtained from variety trials.

Experimental Procedure

Shredded cane preparation

SMRI Shredded cane was always obtained from one of the Cane Testing Service (CTS) laboratories situated at each sugar mill. A CTS sample shredder (Anon, 1985), especially designed to reduce the particle size of the cane from the sub-sampler, ensured that the prepared cane was suitable for NIR scanning.

SASEX Composite cane samples were taken from plant breeding trials. After all the leaves were removed, the samples were shredded in a Jeffco cutter-grinder.

Brix, pol and moisture analyses used for NIR studies

SMRI All primary laboratory results were obtained from CTS laboratories. The procedures used by these laboratories are outlined in the SA Laboratory Manual for SA Sugar Factories (Anon, 1985).

SASEX After thorough mixing the shredded sample was split in two. Moisture was determined gravimetrically by drying overnight in an oven at 85°C. Dissolved solids were extracted in water for three minutes using a high speed Ultraturrax blender followed by separation of insoluble fibre using vacuum filtration.

Sampling cells

The normal coarse-sample cell (NIRSystems part number NR 7080) was used for most of the reported work. A second cell, produced by Dr Berding (Bureau of Sugar Experiment Stations, PO Box 122, Gordonvale, 4865, Australia) was used during the 1995 study to compare the effect of different sample cells on NIR predictions.

Scanning

A NIR Systems 6500 spectrophotometer, on approval from Premier Technologies, was installed in the CTS Laboratory at Maidstone on 1 July 1992. Duplicate sub-samples of shredded cane (59), used for routine DAC analysis, were packed into the NIR's coarse-sample cell and scanned from 400–2500 nm, in the reflectance mode, using default parameters. Laboratory data for the scanned samples were taken from the CTS computer. A NIRSystems 5000 spectrophotometer was subsequently purchased and installed in the Cane Testing Service laboratory at Mount Edgecombe on 5 May 1994. Routine shredded cane samples (116), used for DAC analysis, were packed into the NIR's coarse-sample cell. Duplicate sub-samples were scanned from 1100–2500 nm, in the reflectance mode, using default parameters. Again laboratory data for the scanned samples were taken from the CTS computer. In 1995, shredded cane samples from Umzimkulu (19), Umfolosi (20), Darnall (21) and Maidstone (20) mills were collected and heat-sealed into polythene sachets (20 x 20 cm). The samples were immediately frozen, transported to the Institute where they were kept deep frozen and thawed prior to analysis. Once samples had reached room temperature, the sachets were opened and the contents were mixed well prior to sample presentation.

Results and discussion

Laboratory analyses

NIR is a secondary technique entirely dependent on laboratory analyses for calibration. The primary laboratory data for the various sub-sets of shredded cane used for this study are included in Tables 2 and 3.

Over the past 3 to 4 years the local sugar industry has suffered from one of the worst droughts in living memory. This is reflected in the low purities for commercial cane arriving at sugar mills. An example of this is seen in Table 2, which reflects average purities of 82, 79 and 79 for 1992, 1994 and 1995 respectively. Cane from SASEX on the other hand produced purities of between 84 and 88.

Reliability of primary laboratory data

In the South African sugar industry, cane is analysed in parallel at every factory by *two* methods:

- the direct analysis of individual cane consignments for brix, pol and fibre content.
- the mill balance which entails the determination of brix, pol and fibre from the analysis of mixed juice and final bagasse and, where applicable, diffuser press water mud.

These methods are *independent* of one another. At the end of each week, the two measures of total pol are compared in the following manner:

$$\text{Pol factor} = \frac{\text{Tons pol in cane by mill balance}}{\text{Tons pol in cane by direct analysis}}$$

Table 2
SMRI laboratory details of shredded cane samples used for the NIR study

File	Date of origin	No. of samples	Cane type	Analyte	Mean	Range	SD
ALL92	July 1992	59 Mean purity = 82	FCBD* Maidstone mill	Brix	16,9	14,1– 21,1	1,2
				Pol	13,8	10,4– 17,7	1,6
				Moisture	68,6	62,4– 74,2	2,2
ALL94	May 1994	116 Mean purity = 79	FCBD* Mount Edgecombe Mill	Brix	14,5	11,1– 17,7	1,3
				Pol	11,5	7,7– 14,5	1,4
				Moisture	71,1	66,8– 75,2	1,7
SCDG	May 1995	80 Mean purity = 79	FCBD* Umzimkulu Umfolozi Darnall, Maidstone	Brix	13,1	10,3– 16,0	1,5
				Pol	10,4	6,2– 14,1	1,9
				Moisture	71,0	65,4– 76,6	2,2

*FCBD = Factory cane, burnt, dirty

Table 3
SASEX laboratory details of shredded cane samples used for the NIR study

File	Date of origin	Number of samples	Cane type	Analyte	Mean	Range	SD
SHRED CNS	June 1992	68 Mean purity = 87	CCVT**	Brix	15,7	12,0–17,9	1,3
				Pol	13,7	9,3– 16,0	1,5
				Moisture	71,6	67,7– 76,2	na
SRD COMP	Jul 1992	183 Mean purity = 84	CCVT**	Brix	15,6	11,1– 17,9	1,2
				Pol	13,1	4,0– 13,2	2,1
				Moisture	71,2	71,1– 76,2	na
SHRLG SAM	Aug 1993	40 Mean purity = 86	CCVT**	Brix	15,8	13,5– 17,2	0,8
				Pol	13,6	11,1– 15,0	0,9
				Moisture	69,6	66,3– 72,3	na
SHR CANEC	Sep 1993	150 Mean purity = 88	CCVT**	Brix	16,2	13,1– 19,3	1,2
				Pol	14,2	10,8– 17,8	1,3
				Moisture	70,6	66,3– 74,4	na

**CCVT = Clean Cane, Variety Trials
na = not available

This factor and a similar brix factor are useful monitors for maintaining effective technical control in raw sugar mills. These factors have a useful spin-off for NIR studies in that the reliability of DAC results are continually monitored by comparison with the mill balance. Few other NIR applications have the luxury of this sort of quality assurance.

Calibration data set

The samples scanned in May 1994, with the NIRSystems 5000 instrument, were chosen as the calibration data set. A single scan of duplicate sub-samples produced 232 spectra for the 116 samples. NSAS software (version 3.25) was used for calibration development. Earlier work at the Institute (Schaffler, 1993) had shown that PLS calibrations, using all

700 spectral data points, was superior to multi-linear regression where only two or three wavelengths were used. The use of chemometric software rather than spectral selection is also less time-consuming and less subjective. No averaging of the spectra was done. If the PLS software indicated outliers, the samples were removed and the calibration was repeated. The number of outliers removed was extremely small (Table 4). It must be remembered that the primary laboratory data used for calibration development was obtained from routine CTS laboratories working under high throughput conditions, so that occasional mistakes are bound to occur. The summary statistics for the calibration of brix, pol and moisture are reported in Table 4 and shown graphically in Figure 1. Figures have been included as correlation data can very often be misleading (Anon, 1988; Fearn, 1996).

Table 4

Summary statistics for the PLS calibration of brix, pol and moisture from 116 shredded cane samples (ME Mill) using data scanned by NIR in May 1994

Analyte	No of outliers	No of factors	MSECV**	R	SEC ⁺
Brix	3 (2,5%)*	11	0,027	0,979	0,29
Pol	4 (3,4%)*	14	0,036	0,982	0,30
Moisture	2 (1,7%)*	9	0,093	0,945	0,57

* = outliers expressed as percentage of total number of samples
 ** MSECV = mean square error of cross-validation
 + SEC = Standard error of calibration

Table 5

Prediction statistics for analytes in the 1992 shredded cane samples (Maidstone mill) using calibrations developed in 1994 (Mount Edgecombe mill)

Analyte	Bias	Slope	R	SEP
Brix	0,1	1,110	0,96	0,33
Pol	-0,9	0,999	0,94	0,52
Moisture	-0,4	0,940	0,91	0,90

The correlation coefficients for all three analytes were excellent (R ranged from 0,95 to 0,98). Standard errors for calibration were also very good, yielding relative errors of less than 5% for all three constituents.

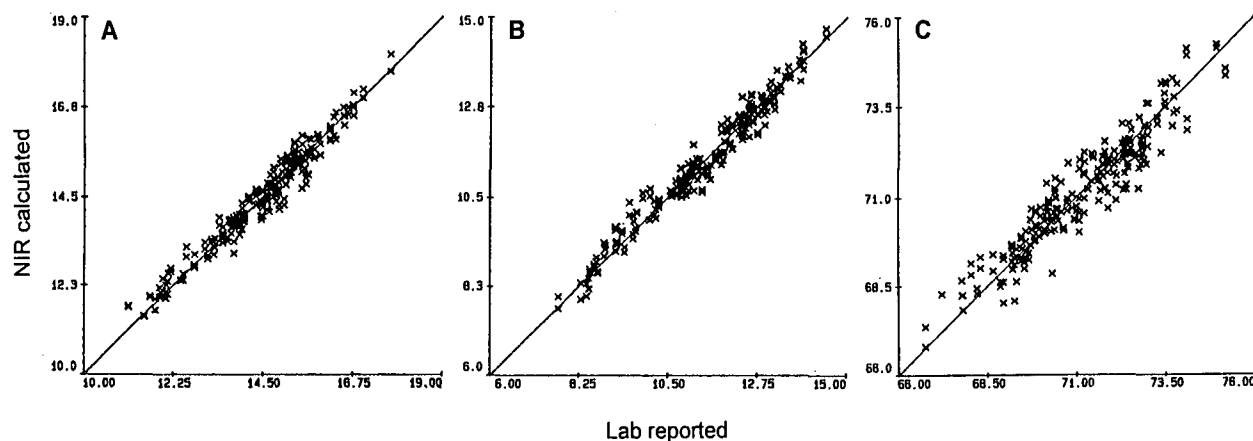


FIGURE 1: Scatter plots from the PLS calibration of brix (A), pol (B) and moisture (C) in 116 shredded cane samples from ME, 1994.

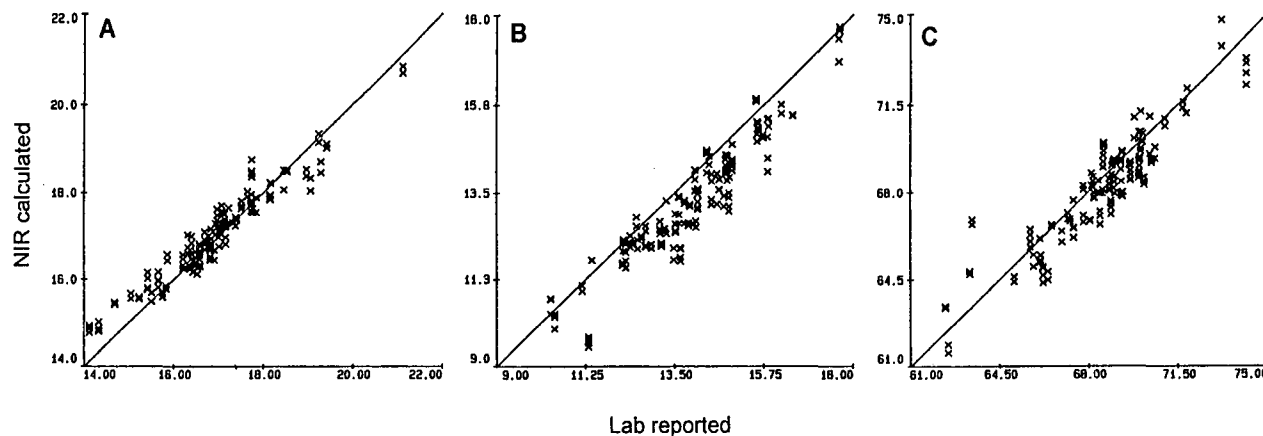


FIGURE 2: Scatter plots for predictions of the 1992 data (59 samples from Maidstone mill) using the 1994 calibrations for brix (A), pol (B) and moisture (C). Calibrations were obtained from Mount Edgecombe mill.

Evaluation of the 1994 calibrations using shredded cane samples scanned in 1992

The calibrations developed for the 1994 data set, with the NIRS 5000 instrument, were used to predict the three analytes in a series of shredded cane samples taken from MS mill in 1992, where samples were scanned with a NIRS 6500 instrument. Comparisons between NIR predictions and the laboratory data are included in Table 5 and shown graphically in Figure 2.

Results for brix were particularly pleasing (virtually no bias, the slope was near unity, high correlation and low standard error of performance). Although the statistics for pol and moisture were not as impressive, they were still most satisfactory. The findings were specially pleasing as:

- Calibrations and predictions were carried out on different NIR instruments. Berding (1995) has expressed reservations on the transferability of calibrations between instruments. The results in Table 5 tend to suggest that the relative errors of prediction across the two NIR instruments are not greater than 5 to 10%.
- Pol is an extremely precise analysis. Routine repeatability tests for pol in juice can produce repeatabilities of 0,02 to 0,05. This is significantly better than the standard errors reported in Tables 4 and 5. It must be remembered, however, that these NIR results are based on the shredded material and not on the juice extracted from the cane. Brokensha (1995) recently reported that the present CTS sampling method will ensure that two-thirds of a cane consignment will produce pol % cane data that is within 0,4 unit of the correct result (units are sucrose % cane). The remaining third of the data will show fluctuations greater than 0,4 unit of sucrose. The inaccuracies stem mainly from sampling precision and the problems associated with trying to gain proper representation of a 20 ton mass of heterogeneous cane from a 2 kg sample. The results in Table 5 compare favourably with those reported by CTS. The statistical results of five published predictions of brix and moisture in shredded cane were shown in Table 1. The results of the SMRI study in Table 5 correspond most favourably with the published findings.

Predicting brix, pol and moisture in 1995 shredded cane samples using the 1994 calibrations

Freshly shredded cane samples (Maidstone 1992) were used in the prediction study discussed in the preceding section. This was not possible in 1995 and samples from four mills were collected, frozen and despatched to the SMRI. Thawing and scanning produced indifferent results. This was attributed to analyst inexperience. The samples had fortunately been refrozen. These were once again thawed, mixed well and duplicate samples were re-scanned by a second analyst. The calibrations developed from the 1994 sub-set were used to predict the three analytes. The results of this exercise are shown in Table 6. Once again scatter plots of the predictions are shown in Figure 3.

Table 6
Prediction statistics for analytes in the 1995 shredded cane samples, from 4 mills, using calibrations developed in 1994

Analyte	Bias	Slope	R	SEP
Brix	-0,08	1,012	0,91	0,96
Pol	-1,71	0,83	0,92	0,73
Moisture	0,56	1,07	0,83	1,15

Although the results of brix prediction (bias = -0,08, slope = 1,01, R = 0,91 and SEP = 0,96) were not as striking as those obtained for the 1992 predictions, the results were surprisingly good when one considers that:

- The NIR validations are entirely dependent on the original laboratory data which were produced by four different laboratories. Increased scatter should therefore be expected.
- The samples were collected, frozen, thawed, refrozen and finally thawed before scanning. Shredded cane is exceptionally unstable (Berding 1995) and ideally only fresh samples (delay of less than 30 minutes prior to scanning) should be used for comparisons. The brix predictions are still reasonably good despite sample deterioration as there is no loss of brix in the early stages of deterioration.

The pol predictions support the assumption that the samples had deteriorated. The scatter plot in Figure 3B indicates

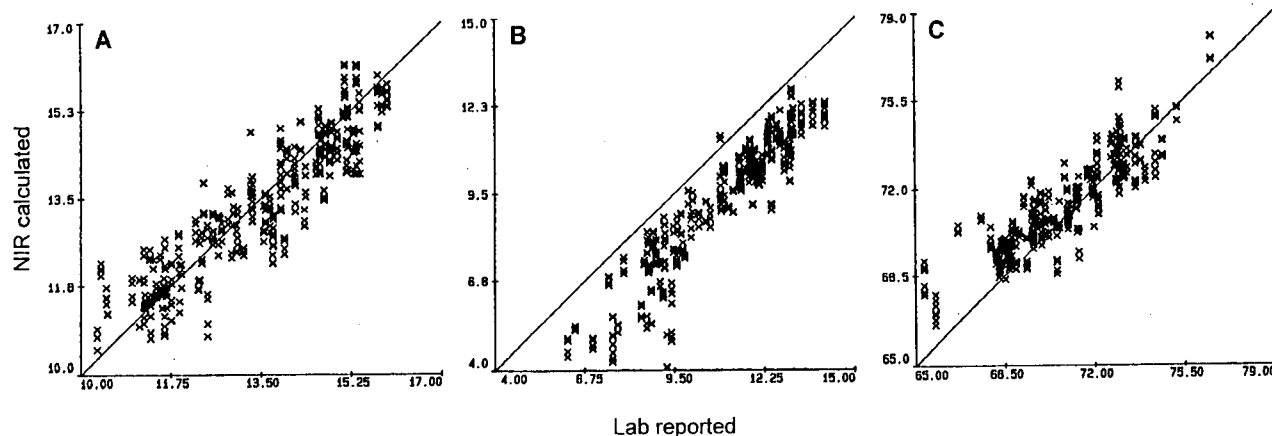


Figure 3 Scatter plots for predictions of the 1995 data (80 samples from four mills) using the 1994 calibrations for brix (A), pol (B) and moisture (C). Calibrations were obtained from Mount Edgecombe mill.

that NIR estimates were too low, which would occur if the samples had deteriorated. Laboratory staff confirmed that the samples in question had an 'off-odour'. Moisture predictions were also not as good as those obtained for the 1992 sub-set. Future work will address the problem of freezing shredded cane for subsequent analyses. Ideally only fresh cane should be used. However this approach, especially during calibration development and testing, can create logistical problems, including:

- Moving an expensive instrument from mill to mill and leaving the instrument unattended for prolonged periods.
- Sending an analyst to mill laboratories throughout the milling season. It has been demonstrated that operator skill is crucial to keep NIR errors to a minimum. Other tasks (instrument diagnostics, scanning the reference as well as spectral data backup) need special training. Mill or CTS staff have other priorities and cannot be expected to give NIR a high priority. Direct comparisons between spectral data from fresh and frozen cane that has been preserved and thawed *only once* needs to be done.

Predicting brix, pol and moisture in clean cane samples from SASEX using the 1994 SMRI calibrations

Although the calibrations developed at the SMRI were based on commercial cane (ie burnt and dirty), the concept of global equations for different types of cane is still intriguing. The SASEX samples were all from clonal evaluation trials. All cane was stripped clean of leaf material before processing through a Jeffco cutter-grinder. Four sets of SASEX NIR spectral/constituent data (see Table 3) were used to test the SMRI calibration (1994). The prediction results are shown graphically in Figure 4.

It is obvious from the results that all four sub-sets were significantly biased. NIR estimates were almost one unit higher than the laboratory results. The bias could be due to:

- the SMRI calibration for brix being inaccurate due to spectral differences between clean and dirty industrial cane.
- the laboratory methods, used at the two laboratories, being different.

All CTS laboratories use a cold digester/extractor to ex-

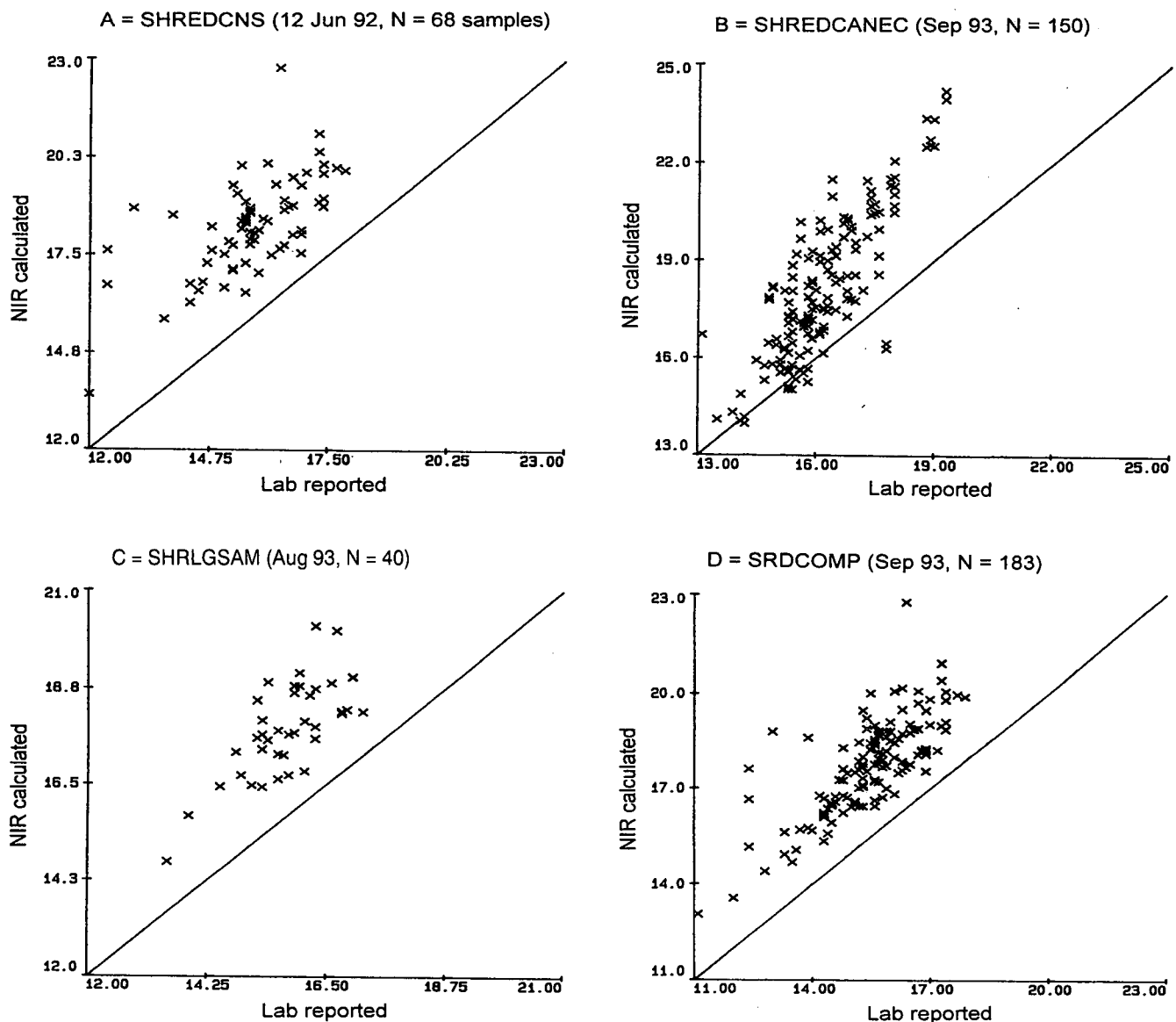


FIGURE 3: Scatter plots for predictions of brix in SASEX shredded cane for four different sub-sets using the SMRI 1994 calibration for brix (calibrations from Mount Edgecombe mill samples).

tract dissolved solids from cane. Extraction times are exactly 20 minutes. The mill room at SASEX does not use cold extractors and dissolved solids are extracted in water for three minutes using a hand-held Ultraturrax. A collaborative cane deterioration trial between SASEX and SMRI was carried out in 1993 and a comprehensive comparison of the two extraction procedures was made, the comparisons are summarised in Figure 5.

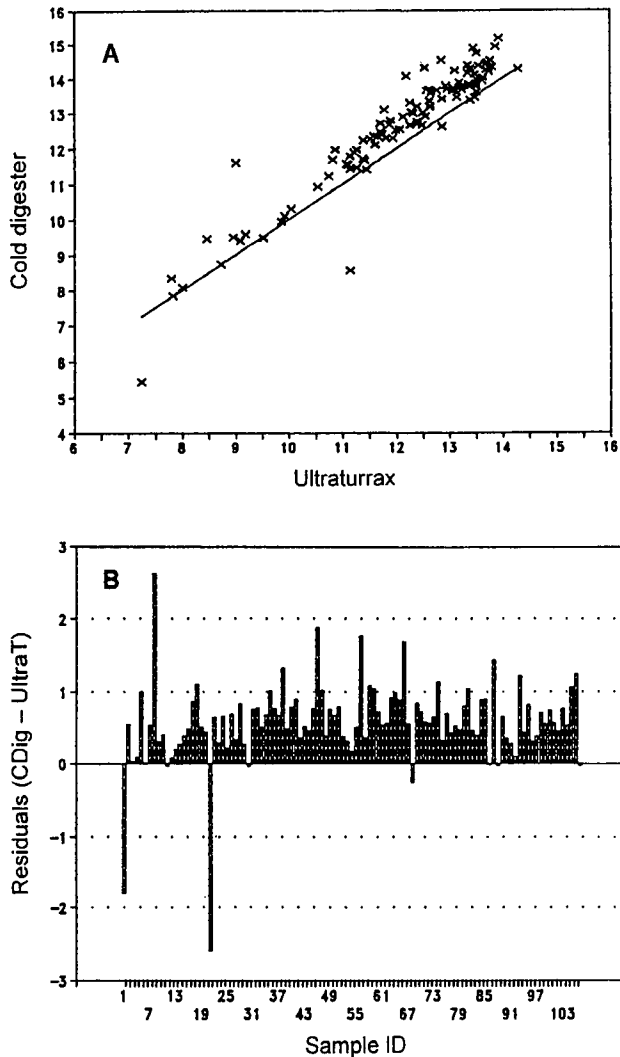


FIGURE 5: (A): Comparison of the Ultraturrax and cold digester procedures for extraction of pol from shredded cane. (B): Residuals plotted against sample number, Differences = (Cold extractor - Ultraturrax).

Comments concerning the comparison include:

- (a) The Ultraturrax consistently produced lower pol % cane than the cold extraction procedure. Extraction efficiency of the Ultraturrax is probably dependent on the levels of fibre in the cane.
- (b) The regression between the two procedures was good ($R = 0,95$).
- (c) The cane deterioration study only contained samples with fairly low pol % cane, (maximum = 14%). The differences due to incomplete extraction by the Ultraturrax can be expected to increase for cane with higher pol levels.

Although the reliability of the SMRI calibration for predicting brix in clean cane from SASEX has not been established, there is strong evidence to suggest that the principal reason for the observed bias is due to the lower extraction

efficiency of the Ultraturrax procedure. The mere fact that NIR was able to detect the bias between the two laboratory methods is a strong indication that NIR can be used for shredded cane analyses.

NIR parameters and their effect on the accuracy of NIR estimates using different sample cells

Using a single coarse-sample cell for reading multiple samples can be tedious. An additional cell to help speed up analysis was purchased from Bundaberg Sugar in Australia. NIR users have been cautioned about using different cells (Ebby 1995). It is important to check that the windows of the cell are optically transparent. This is especially important when one cell is used for calibration development whilst a second is used for prediction work. A study of the difference in the optical properties of the two cells was performed. Duplicate samples of shredded cane were first scanned in the NIRSystems cell. The contents were then packed into the Bundaberg cell. Prediction data from both cells are shown in Table 7 and in Figure 6. A t-test comparison indicated that the mean values from the two cells were identical and in future the two cells will be used in tandem to increase sample throughput.

Table 7

Statistical comparison: Effect of using different coarse-sample cells for predicting brix in 1995 shredded cane samples.

Statistics	Laboratory Data	Cell 1 NIRSystems	Cell 2 Bundaberg
Mean	14,71	14,57	14,51
Standard Error	0,08	0,077	0,076
SD	0,77	0,77	0,76
Variance	0,59	0,60	0,57
Range	2,65	3,18	3,34
Minimum	13,3	13,0	12,8
Maximum	16,0	16,2	16,2
N	100	100	100

T-test, two samples assuming equal variance	
Pooled variance	0,59
Null Hypothesis: (NIRS - Bundaberg) =	0
Degrees of freedom	198
t	0,53
P (T<=t), 2-tail	0,59
t-critical, 2-tail	1,97

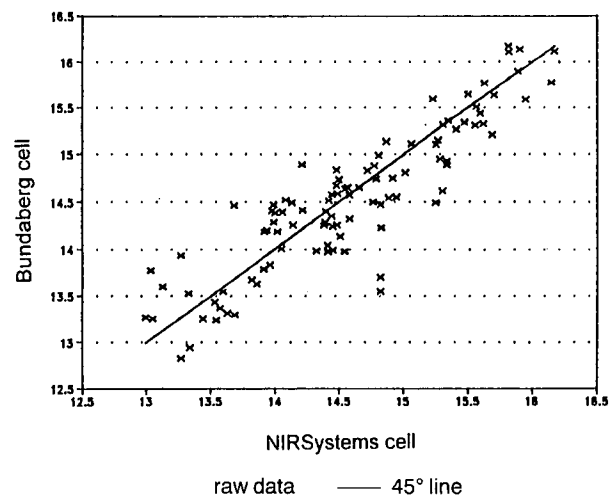


FIGURE 6: NIR coarse-sample cell comparison: NIRSystems versus Bundaberg.

Repeatability of predictions: effect of duplicate scans, duplicate sub-samples and different operators

The expertise of the operator in mixing, sub-sampling the prepared cane, packing the cell and scanning each sample is important in obtaining precise and accurate NIR estimates. For this reason, the following variables were investigated:

- duplicate sub-samples versus duplicate scans for each sample
- operator 1 versus operator 2

Eighty samples from the 1995 study were used to predict brix in shredded cane using the 1994 calibration model. Two-way analysis of variance (with replicates) was carried out and the results of the investigations are included in Appendices 1 and 2. The results of these Anova studies indicate that:

- (a) The means and standard deviations for duplicate sub-samples were identical.
- (b) As expected the repeatability of duplicate scans was superior to the precision of duplicate sub-samples, [errors from sub-samples were virtually 3 times greater than scanning (0,89/0,33 = 2,7)].
- (c) Although there was no significant difference between the means of the two operators, the variance of operator 1 was virtually three times greater than that obtained by operator 2. This sort of scatter will obviously have a deleterious effect on the standard error of prediction and the overall correlation. This finding suggests that new analysts should be trained and then evaluated before long term NIR is done.

Conclusions

- SMRI spectral and analytical data collected in 1994 were used for the development of calibrations for brix, pol and moisture in shredded cane.
- Using NIR and laboratory data, obtained in 1992 from another mill, the 1994 calibrations produced excellent NIR predictions for all three analytes. The predictions were additionally impressive as the calibrations and validations were scanned on different instruments.
- Using NIR and laboratory data, obtained in 1995 from four mills, the 1994 calibrations produced excellent NIR predictions for brix. It was obvious, from the pol estimates, that repeated thawing of the frozen shredded cane samples had resulted in cane deterioration.
- SMRI calibrations for brix produced predictions that were severely biased when clean cane from SASEX was evaluated. The poor results were attributed to differences in the extraction techniques when carrying out pol and brix analyses. The mere fact that NIR was able to detect the bias between the two laboratory methods is a strong indication that NIR can be used for shredded cane analyses.
- When two coarse-sample cells, made by different manufacturers were compared, it was found that the brix results were virtually identical. The use of multiple sample cells is preferred for high throughput NIR work.
- Scatter obtained when using duplicate sub-samples was found to be virtually three times greater than the instrument's scanning error. Replicate sub-samples are essential to reduce sampling errors. Expertise in subsampling shredded cane and packing the NIR coarse-sample cell is essential if errors are to be reduced. A further comparison between two analysts revealed a three-fold difference in standard errors of prediction. Clearly operator technique needs standardising.

NIR has a good chance of being successful for DAC analysis. Reasons for this are:

- Some CTS laboratories have to cope with high sample throughput (15-20 DAC samples per hour). NIR is rapid, can reduce labour costs and can determine brix, pol and moisture simultaneously.
- The errors due to sub-sampling each cane consignment are similar to those obtained from NIR predictions.

It is important to note that NIR is not suitable for the mill balance as sub-sampling cane is not an issue. All mixed juice entering the factory is accurately weighed. In addition laboratory errors on juice are extremely low when compared to NIR errors.

Recommendations

Although it is clear from the 1992 and 1995 NIR prediction results that some bias and slope errors exist, the results are nevertheless most encouraging. The next stage is to increase the tempo of obtaining samples from different mills. Possible avenues of research include:

- to obtain samples from both North and South Coast mills as well as Midland mills, throughout the 1996/97 season. This will allow the development and comparison of global and local calibration models for each analyte.
- installing an instrument at one mill and using existing calibration models to estimate brix, pol and moisture in DAC samples. A validation procedure should be developed to update and monitor the predictions, for example 25% of all samples scanned could be checked using laboratory data.
- assessing the packing technique. The NIR reflectance unit was used for this work. Light scattering and light absorption are sensitive to particle size variations and to packing density. Future tests should pay particular attention to the repeatability of CTS laboratory shredders (mill to mill) and to the packing technique prior to NIR scanning.
- evaluating the efficacy of storing and thawing shredded cane for subsequent analyses.

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Appendix 1

Two-way Anova (with replication). Effect of sub-samples and duplicate scannings on prediction results for brix in shredded cane, 80 shredded cane samples were used to predict brix % cane, 1994 calibration model

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F-value
Sub-samples	1	0,645	0,645	3,56
Samples	79	569,54	7,21	540,6
Interactions	79	14,35	0,182	13,62
Errors	160	2,134	0,013	
Total	319	586,7		

Mean for sub-sample 1 = 13,03; Standard deviation = 0,08
 Mean for sub-sample 2 = 13,12; Standard deviation = 0,08
 Repeatability (between duplicate scannings) = 0,33
 Reproducibility (between sub-samples) = 0,89

Appendix 2

Two-way Anova (with replication). Effect of two different operators on the prediction repeatability for brix in shredded cane. (see Appendix 1 for details)

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F-Value
Operators	1	0,368	0,368	0,65
Samples	79	580,88	7,35	16,42
Interactions	79	44,79	0,567	1,27
Errors	160	71,63	0,448	
Total	319	697,67		

Mean for operator 1 = 12,97; Standard deviation = 0,64
 Mean for operator 2 = 13,04; Standard deviation = 0,25
 Repeatability (between sub-samples) = 1,89
 Reproducibility (between operators) = 2,01