REVIEW OF NEAR INFRA-RED SPECTROSCOPY RESEARCH IN THE SOUTH AFRICAN SUGAR INDUSTRY

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Abstract

Sugar industries world-wide are showing increasing interest in the potential applications of near infra-red (NIR) analysis as a research and management tool in the fields of soil fertility, cane nutrition, cane quality testing and screening for resistance to certain pests and diseases. During the past decade both filter and scanning NIR reflectance spectrophotometers have been used to improve nitrogen use efficiency of sugarcane by matching the crop's N requirement to soil N mineralising potential and plant N status, both properties being determined by NIR. Calibrations were developed and validated for N in leaf samples as well as total N content, organic matter content, N mineralisation potential and texture of soil samples. Well over 70 000 leaf and 140 000 soil samples submitted by cane growers have been routinely tested by the Fertiliser Advisory Service (FAS) using NIR in conjunction with other techniques. Recent developments have centred on comparing the suitability of both filter and scanning instruments for the rapid determination of various constituents in cane juice, shredded cane, bagasse, raw sugar and molasses. Possible new applications for NIR that are discussed include partitioning the N pool in the cane plant, estimating photosynthesis, predicting yield potential and screening for pest and disease resistance.

Keywords: NIRS, near infra-red spectroscopy, sugarcane

Introduction

During the past two decades near infra-red spectroscopy (NIRS) has gained wide acceptance in the food sciences, chemistry and chemical engineering, biochemical, environmental, pharmaceutical and medical fields. The success of NIRS can largely be attributed to its ability to allow rapid quantitative and qualitative analyses of multicomponents in single samples using minimal sample preparation. Despite advances in applying NIR at the research and process levels for cereals, oilseeds and forage assessment, relatively little progress has been made in adopting this technology for routine use in the cane industry. Research has been confined mainly to the USA, Australia and South Africa, and includes the use of both filter and scanning instruments for foliar diagnosis (Meyer, 1983), N fertiliser management (Meyer *et al.*, 1986), cane juice analysis (Meyer and Wood, 1988; Edye and Clarke, 1993), shredded cane analysis (Sverzut *et al.*, 1987; Berding *et al.*, 1989; Brotherton and Berding, 1995; Clarke *et al.*, 1996; Schäffler and Meyer, 1996), assessing soil properties (Meyer, 1989; Meyer *et al.*, 1995), analysis of sugar related products (Schäffler *et al.*, 1993) and predicting varietal resistance to the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Rutherford *et al.*, 1993). This paper is a summary of some past and current research in South Africa, as well as indicating potential new applications. Comparison of NIR spectra for a range of products tested are shown in Figure 1, and Table 1 is a summary of the constituents for which successful calibrations have been developed.

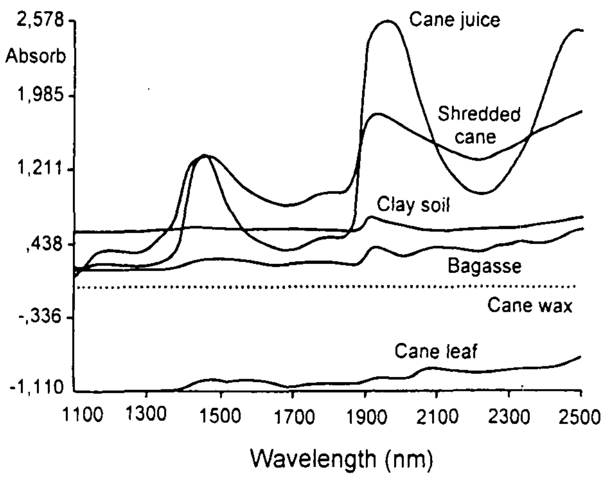

Figure 1. Comparison of NIR spectra for various materials.

Table 1
Summary of working calibrations developed for a range of constituents in various products.

Cane leaf	Soil	Cane juice	Shredded cane	Raw sugar/ molasses
N, P, S, Si	Total N	Pol	Moisture	Pol
Photosynthesis	Org N	Brix	Pol	Brix
Yield	Min N	Sucrose	Brix	Sucrose
Eldana rating	Clay	Glucose	Fibre	Fructose
Mosaic rating	Silt	Fructose	Tannin	Glucose
	Sand	Alcohol	Lignin	Ash
	CEC	Total N	Waxes	Starch
				Invert

Foliar diagnosis

Determination of the N requirement of sugarcane is an important activity undertaken by the South African Sugar Association Experiment Station (SASEX). In 1983, a Technicon 300 Bran filter instrument was first calibrated and validated for leaf N analysis (Meyer, 1983). Fifty leaf samples with N contents ranging from 0,80-3,0% were used to calibrate the instrument.

A further 125 samples analysed by the standard Kjeldahl steam distillation method were used to validate the N calibration (see Table 2). The accuracy and precision of the NIR method was further evaluated by repeatedly analysing 13 reference samples over a period of five days. The small differences between the mean results (0,07%), and the small variation in the N values obtained (CV range 0,8-4,5%), suggested that the NIR method was sufficiently reliable for determining N in leaf samples. In practice, a level of accuracy of ±0,1%, and reproducibility below 5%, are considered to be acceptable in assessing the N status of sugarcane by means of leaf analysis. The NIR method is about 10 times faster than the Kjeldahl procedure.

Some of the more important applications of NIR in leaf analysis concern its use in providing whole crop cycle fertiliser recommendations for sugarcane and in nutrient survey programmes. Since 1983, more than 65 000 leaf samples have been analysed for N content by NIR. The data set is updated regularly and used to assess changes in N availability from soils in the sugar industry (Meyer *et al.,* 1989).

N use efficiency studies

NIRS has provided a rapid means of detecting, through leaf analysis, the relative efficacy of timing, placement and the use of different N carriers in various trials (Meyer and Wood, 1994). Recently leaf NIRS analyses have proved useful in assessing the N requirements of different cane varieties. For many years, fertiliser recommendations in the South African sugar industry have been based on the variety NCo376. Analyses of the standard top visible dewlap (TVD) leaf, covering thousands of samples from variety trials, have shown significant varietal differences in N content. Current trials indicate that the optimum N requirement of important varieties such as N12 and N14 can differ from that of NCo376 by up to 75 kg N/ha. Limited field evidence also suggests that a variety such as N12, which uses N less efficiently than NCo376, may respond more effectively to split N applications. Threshold levels for interpreting leaf analyses may require adjustment for varieties that use N less efficiently.

Quantifying the distribution of forms of plant N in sugarcane is another new research area in which NIRS can be used. Forms include nitrate-N, amino-N, soluble-protein (mainly Rubisco) and proteins having different structures. Total N is often used to assess N sufficiency, although it includes stored N in the form of Rubisco, which is not very mobile. Possibly, as in other crops, nitrate N in cane may be a more sensitive indicator of N sufficiency than total nitrogen. Knowledge of the transformation of proteins in the cane plant from structural to soluble proteins could assist in predicting the effects of N on cane quality, as well as explaining the tolerance of plants to certain pests and diseases. Proline is an amino acid that has been linked to moisture stress in sugarcane (Rutherford, 1989) and as such could be a

Table 2
Examples of calibrations developed for selected constituents.

Product/ constituent	Range	Wavelength (nm)	Calibration			Validation		
			n	R	SEC	n	R	SER
Cane leaf			98			125		
N	0,8-3,0	2186, 1240		0,98	0,11		0,96	0,15
Ash	1,0-9,0	1900, 1860, 1968	61	0,97	0,15	94	0,93	0,23
Silicon	0,5-4,0	1900, 1682, 2448	27	0,89	0,05	27	0,77	0,07
Photosynthesis	15-40 umol/m²/s	2100, 2139, 2190	42	0,90	0,84	30	0,65	2,90
Cane yield	85-210 t cane/ha	2384, 2238, 2448	51	0,91	13,00	191	0,86	14,00
Eldana	20-220	2332, 1754, 2320	51	0,75	17,00	191	0,69	18,00
Soil			200			74		
N mineralisation potential	1-4	2236, 2230		0,86	0,30		0,83	16,00
Total N	0,03-0,60	2050, 1870		0,90	0,01		0,84	19,00
Organic C	0,3-7,0	2050, 1744		0,92	0,46		0,83	19,00
Clay	5-75	1956, 1920		0,94	3,80		0,81	15,00
Cane juice			90			35		
Pol	0,7-13,0	2274		0,96	0,25		0,91	3,20
Brix	1,7-14,0	1366, 2160		0,98	0,15		0,92	3,30
Sucrose	0,7-13,0	2322		0,94	0,29		0,90	3,40
Glucose	0,15-0,80	2342		0,65	0,04		nd	
Fructose	0,15-0,80	2292		0,68	0,05		nd	
Shredded cane			26			136		
Brix	6-21	1434, 2082		0,94	0,24		0,85	3,80
Pol	5-19	1198, 2282		0,93	0,42		0,88	3,90
Dry matter	16-38	2224, 1838		0,88	0,86		0,82	4,70
Fibre	7-14	1376		0,85	0,41		0,80	6,00
Cane stalk			32					
Wax	Qualitative	1940, 1194, 2072		0,65	2,10		nd	nd
Bud scale flavonoids	Qualitative	2180, 1734, 1680		0,75	1,70			

n = number of samples; R = regression correlation coefficient; SEC = standard error of calibration; SER = standard error of prediction; nd = not determined useful indicator when interpreting foliar analyses. High proline is likely to indicate that the crop was stressed at sampling, and that caution is necessary when interpreting leaf analyses.

Soil nitrogen mineralisation potential

Attention was given in 1986 to assessing the merits of NIR for soil testing. Previous work had shown that the N requirement of sugarcane could be estimated more reliably from soil properties such as N mineralisation potential, texture, colour and organic matter content. For advisory purposes a system was developed for placing soils into low, moderate, high and very high mineralising categories (Meyer *et al.,* 1986). Two hundred air-dried and ground (0,25 mm sieve) soil samples, of known mineralising potential, organic matter, total N, and clay content were used to calibrate a Technicon InfraAlyzer 450 instrument. Comparative statistical information obtained for the different constituents (Table 2) suggests that most could be satisfactorily estimated by NIRS. Reliability decreased in the order: clay, organic matter, total nitrogen and N mineralisation rating (Meyer, 1989). Coded soil samples from 21 trials showed that predicted N mineralisation ratings were correct in 17 of the trials.

Sugar products

Analysis of pol and brix in sugarcane is an important analytical service rendered by laboratories in the sugar industry. The standard procedure, based on filtration and clarification of expressed cane juice, is tedious and labour intensive. In 1987, the suitability of NIRS for rapidly estimating cane juice quality components was assessed (Meyer and Wood, 1988). Mixed cane juice samples of known pol, brix, sucrose, fructose and glucose contents were used to calibrate a Technicon InfraAlyzer 450. Regression analyses indicated that brix, pol and sucrose values estimated by NIRS correlated closely with those obtained by conventional methods of analysis.

In 1992, an NIRSystems 6500 spectrometer was used to study analytes in bagasse, shredded cane, direct analysis of cane (DAC) extracts, mixed juice, molasses and raw sugar (Schäffler *et al.,* 1993). Analytes included pol, brix, dry solids, moisture, sucrose, glucose, fructose, invert, ethanol, colour, ash and starch. NIR produced a good estimate for many of the analytes tested (Table 2). More recently an intensive collaborative investigation between the Sugar Milling Research Institute and SASEX, used more than 500 shredded cane samples to calibrate and validate a NIRSystem 5000 spectrophotometer for pol, brix and moisture readings (Schäffler and Meyer, 1996). Calibration R values based on partial least squares regression analysis were better than 0,95 for brix, pol and dry matter, but were lower for fibre (0,89). The NIR predictions for pol, brix and dry matter were acceptable with R values of 0,88 to 0,94, and standard errors of performance from 0,24 to 0,42%. It was concluded that the technique was sufficiently reliable for rapid analysis of sugarcane in plant breeding and agronomy variety trials. NIR is currently under evaluation as an alternative to DAC for rapidly assessing quality in cane consignments in the millyard.

Yield potential predictions

NIR has the potential to detect key constituents such as starches, sugar, cellulose, lignin, proteins, water and amides, and also certain constituents linked with S, Mg, Ca and K. As part of a project to evaluate the merit of NIRS scanning of cane leaves for assessing crop performance, 400 TVD leaf samples, collected from 20 regional variety trials located throughout the cane belt, were used to determine possible relationships between yield parameters, leaf composition and NIRS reflectance measurements. Results from irrigated trials at Pongola showed that NIR absorption spectra of leaf samples from 4-5 month old cane, in the 2 238 to 2 500 nm range, were positively correlated with cane yield (R=0,91). There was a negative correlation with pol% (R=0,82) in 12 month old cane. Accuracy of the calibration equations was tested on five independent data sets and, although the correlation coefficients for validation were generally lower (0,60 to 0,86), the results were sufficiently promising to warrant continuing this field of study.

Of additional interest is that much of the variation in pol could be accounted for by the N and ash content of stalk and leaf. Previous studies have also shown that high N levels in the leaf could be associated with lower sucrose values (Wood, 1979; Gascho *et al.,* 1986) while high ash levels in cane juice were negatively correlated with sucrose content (Mullins and Roach, 1985). NIRS research in Australia has indicated that the ratio of total N to non-structural carbohydrate in whole shoots of wheat may be a key indicator of yield potential (Blakeney *et al.,* 1995). It is possible that this ratio in young irrigated cane may have an additional role as an indicator of crop potential.

Estimating and investigating photosynthetic rates

Measuring photosynthesis in the field is time consuming and weather dependent, and requires considerable skill. Photosynthetic rates were measured on 70 leaf samples taken from a variety trial and using a portable infra-red gas analyser (Inman-Bamber, 1995). The samples were then scanned by NIR, in both a fresh and dried state, in the 1 100 to 2 400 nm region. Stepwise regression analysis showed that photosynthetic rate and internal CO_2 concentrations were highly correlated with NIRS absorption values (R>0,95). The wavelengths that were selected for the calibration equation (2 139, 2 100 and 2 190 nm) were consistent with the third overtone stretching vibrations of C-O, O-H and C-H bonds associated with carbohydrate compounds, as well as second overtone N-H bending modes found in proteins.

Various investigators have demonstrated positive correlations between leaf photosynthetic rate, chlorophyll and soluble protein content (Dornhoff and Shibles, 1976; Hesketh *et al.,* 1981). Recently, leaf photosynthesis in soya beans was positively correlated with leaf greenness, measured non-destructively by means of a handheld SPAD-502 chlorophyll meter (Ma *et al.,* 1995).

Examination of the NIRS data showed that 30% of the variation in photosynthetic rate could be accounted for by variation in leaf N. Photosynthetic response to increasing light intensity is strongly dependent on leaf N (Ludlow et al., 1991; Allison and Haslam, 1993). Inherent differences in yield potential between varieties in many crops may be due to differences in N use, which in turn determine radiation use efficiency (Muchow *et al.,* 1994). The other characteristic that accounted for a significant variation (37%) in photosynthesis was leaf Si content. It has been shown, under normal light, that silica deposited in silica cells and stomatal guard cells could serve as 'windows', allowing more light to pass through the epidermal to the photosynthetic mesophyll tissue (Lau *et al.,* 1978), thus promoting higher rates of photosynthesis and more tillers per plant. This could partly account for the significant relationship that was obtained between cane yield and leaf ash content in the Pongola data set (R=0,68), as silica comprises about 70% of the ash in sugarcane.

Predicting host plant resistance to pests and diseases

There is the prospect that NIRS may prove suitable for screening germplasm from breeding material and wild species of sugarcane for resistance to pests and diseases. NIRS was recently evaluated for predicting flavonoid characteristics associated with *E. saccharina* resistance (Rutherford *et al.,* 1993). This stalk borer is endemic in South Africa and causes costly damage to cane each year. Multiple regression predictive models based on NIRS data from 30 clones of known *E. saccharina* resistance suggested that stalk bud scale flavonoids and stalk surface wax components accounted for up to 55% of the variation in resistance. Work is currently being done to validate these NIRS bud scale and wax resistance models.

Leaf NIRS scanning is also under investigation as a means of predicting host *E. saccharina,* mosaic and smut resistance in cane. Preliminary results using 230 leaf samples from trials on 12 commercial varieties suggest that up to 60% of the variation in *E. saccharina* resistance could be accounted for by absorption of constituents in the NIR region. Further investigation into likely cause and effect relationships suggests that some of the resistance is linked to leaf silicon (R=0,60) and nitrogen (R=0,39) contents.

Leaf Si is a useful indicator of the silicon status of sugarcane (Clements, 1967). It is possible that Si contributes significantly to stalk borer resistance in sugarcane. In Florida, high Si uptake in sugarcane following treatment with a silicate slag served as a deterrent to the stem borer *Diatraea saccharalis* (Elawad *et al.,* 1985). Pot trials are currently being used to investigate the association between host plant Si and N and infestation by *E. saccharina.* NIRS leaf calibrations for Si have been established. Another possible new application of NIR is for detecting resistance to diseases such as mosaic and smut. Preliminary results with 15 cane varieties have shown that the standard ratings of mosaic and smut were significantly correlated with leaf spectral absorbance in the NIR region.

Conclusions

Because NIRS can be used to analyse materials as diverse as soil, plant tissue, shredded cane, cane juice, bagasse and molasses, it is more versatile than any other analytical technique currently available. It has the added advantage of being fast. It is envisaged that in the future NIRS will play an increasingly larger role at SASEX, especially in the routine determination of quality in samples from plant breeding and agronomy trials. In Brazil, 15 mills which process a total of more than 50 million tons of cane, are using NIRS analyses of juice for cane payment purposes based on technology emanating from South Africa. In the FAS laboratory, all soil samples will in future be screened by NIRS for organic matter content.

In view of the rapid advances being made with handheld NIRS units, cane producers may soon be able to use this technology for checking crop N status, monitoring crop maturity, determining when to apply chemical ripeners and planning harvesting programmes. Handheld NIRS units with limited spectral ranges are already being used for various agricultural applications in the USA and Australia. Staff at the Yanco Agricultural Institute in New South Wales are currently evaluating a portable system for monitoring rice quality. NIR monitoring of crops could supply input for crop models by monitoring N, photosynthesis and crop maturity status, and thereby improve the accuracy of crop forecasting. Ultimately, NIR remote sensing from NASA's Airborne Imaging Spectrophotometer to determine yield and quality of sugarcane crops, using NIR calibrations of the crop canopy, will need to be researched.

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