

RAPID DETERMINATION OF NITROGEN IN CANE LEAVES

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Abstract

An evaluation of a new non-destructive technique for the determination of total nitrogen in cane leaves using a Technicon infra-red reflectance analyzer is described. More than 200 leaf samples were analysed and the results indicated that a nitrogen content between 1 and 3% can be accurately determined in less than a minute. Regression analysis indicated that the N values determined by infra-red analysis were well correlated with the Kjeldahl N values ($r = 0,89$). Furthermore, reproducibility was acceptable with co-efficients of variation ranging from 0,8 to 4,5%. The advantages of the new technique are that it is much faster than the present method of weighing, digesting, diluting and dispensing, which is followed by a colorimetric determination using the indophenol blue method, and that the instrument can be handled by an unskilled operator. This method will simplify the determination of nitrogen in plant tissue analysis of a wide range of crops.

Introduction

The determination of the total nitrogen content of sugarcane third leaf samples is one of the most important analyses undertaken by the Fertilizer Advisory Service (FAS) of the SA Sugar Association Experiment Station. Of the 16 elements considered to be essential for sugarcane, nitrogen has the greatest effect on cane growth and juice quality. Early assessment of how effective nitrogen fertilization has been is important to the grower if timely remedial action is to be taken.

Until about seven years ago the nitrogen content of leaf Kjeldahl digestates was estimated by a microdistillation/titration procedure (Bremner¹). The method, although very reliable, was very time consuming and the maximum laboratory output was between 125 and 150 samples per day, leading to long delays in sample analysis at peak periods. The introduction of a semi-automated LKB sample processor into the laboratory for determining P in leaf digestates and A1 and P in soil extracts (Burrows & Meyer²), led to an improved method for determining N in leaf digestates based on the colorimetric indophenol blue reaction (Burrows³). The time to analyse a batch of 150 digestates was reduced from eight hours to a little over an hour. Despite this considerable improvement in methodology, the system was still subject to the hazardous, unpleasant and time consuming procedure of wet digestion of leaf material with concentrated selenised sulphuric acid.

There has been considerable interest recently in the USA and Europe in applying Near Infra-red Spectroscopy to determine the nitrogen and moisture content of plant material. This technique is well established for the determination of protein, oil, carbohydrate and moisture contents of raw materials used in the baking industry. Preliminary investigations conducted by the soil and plant analysis laboratory at the University of Georgia (Wesley *et al*⁴) have shown that the nitrogen contents of maize leaf samples may be determined to within 0,2 units of the nitrogen contents determined by the Kjeldahl procedure. Investigations conducted on crops such as soyabeans, cotton and peanuts have also produced promising results. The method has not yet been evaluated for sugarcane.

The main advantage of this method is the elimination of the time-consuming digestion, so it was decided that its applicability to the determination of nitrogen in sugarcane leaf samples on a routine basis should be investigated. The equipment, the principle of the method and the procedure used to evaluate

the accuracy and precision of the method when used under routine operating conditions, are described in this paper.

Method

Equipment

A Technicon near infra-red spectrophotometer (Model Infra-Alyzer 400) was used (see Figure 1). Once programmed, it is extremely simple to operate. The ground leaf sample is placed in a sample holder 40 mm in diameter, and it slides into the instrument. The result is displayed on a digital readout screen or printer within seconds. The whole operation takes between 15 to 20 seconds per sample.

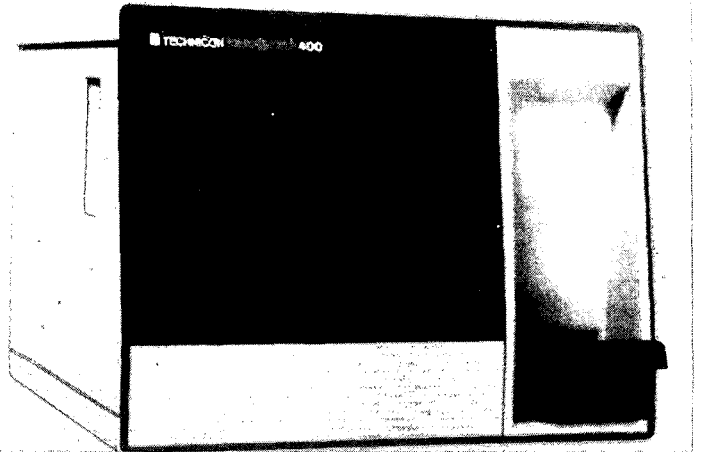


FIGURE 1 The Technicon near infra-red spectrophotometer

Principle of near infra-red reflectance

The infra-red spectrometer uses absorption bands in the near infra-red region to determine the percent protein (nitrogen), oil, carbohydrate or moisture in the sample. This is accomplished by directing narrow bands of light onto the sample and measuring the reflectance (see Figure 2). 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 constituent of interest in the sample.

Absorption of energy coincides with the vibrational energy levels of the molecules composing the sample. The N-H bond in various protein molecules has mainly a bending vibrational mode whereas the carbon-oxygen double bond in starch for example, has mainly a stretching vibrational mode. Stretching vibrations require higher energy than bending vibrations, thus the absorption of electromagnetic radiation occurs at shorter wavelengths. The instrument is programmed to measure the intensity of reflected radiation at the following six wavelengths (measured in nanometers):

- 1 680: used to detect the C-H (stretching) mode
- 1 940: peaks due to the second overtone of the O-H bending mode – used in moisture determination
- 2 100: peak due to the second overtone of N-H bending mode – sensitive to protein and therefore nitrogen differences

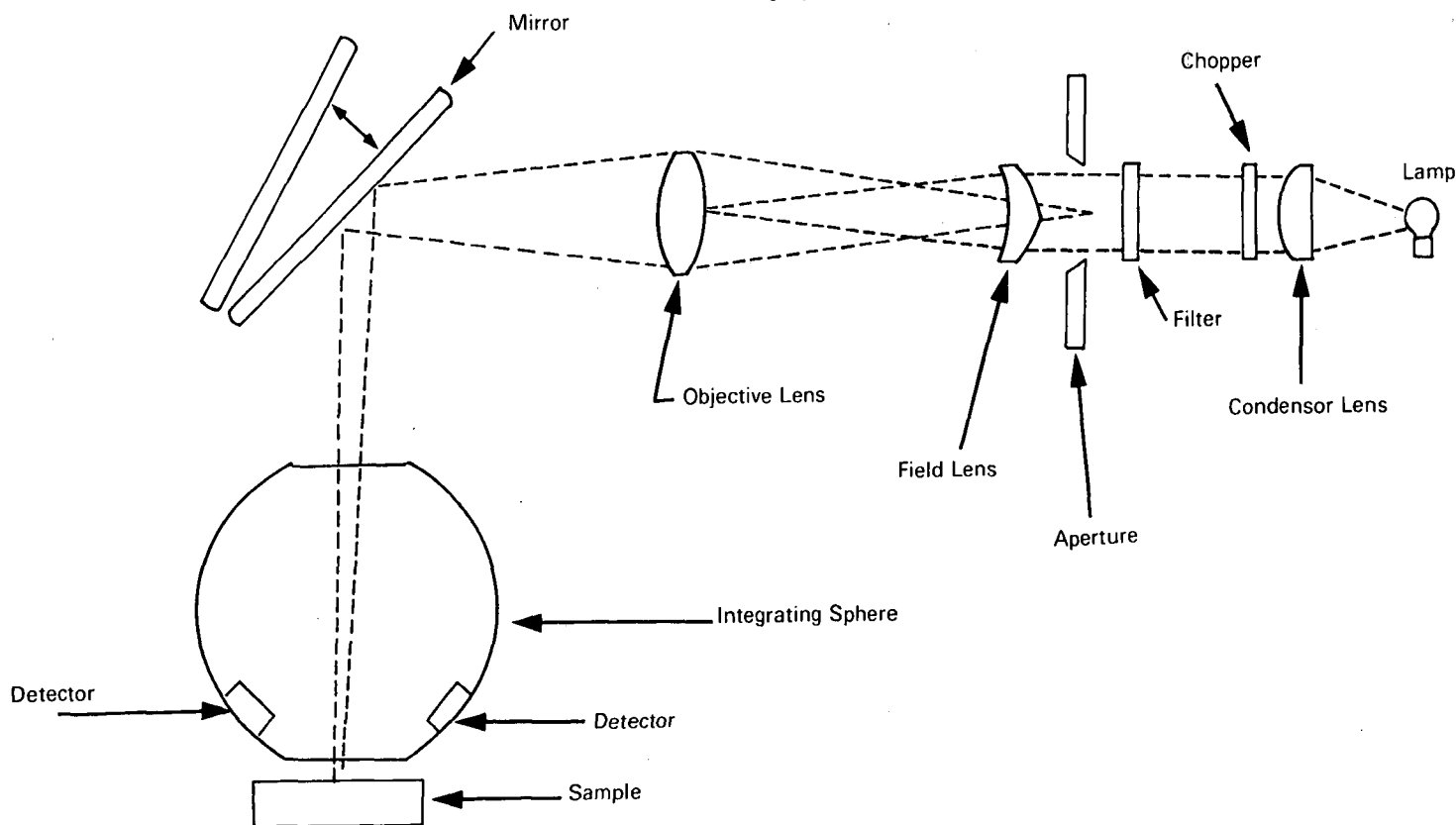


FIGURE 2 Optical system of the infra-red spectrometer

- 4. 2 180: coincides with the C-H and C-O stretching mode vibrations - used in determining protein and oil content
- 5. 2 230: used as a reference wavelength for oil and protein
- 6. 2 310: strong C-H vibrational bending mode - used mainly in determining oil content.

Procedure

The investigation consisted of three parts:

- (i) calibrating the instrument to cover a range of nitrogen contents from 1 to 3%
- (ii) evaluating the accuracy of the method and reproducibility of results in relation to the widely used standard steam distillation procedure for the determination of ammonium ion in Kjeldahl digestates (Bremner¹)
- (iii) evaluating the performance of the instrument in relation to the current routine method based on the colorimetric determination of indophenol blue.

The 300 third leaf samples used in this study were prepared by drying them overnight at 70°C and grinding them to a uniform consistency in a cross beater mill fitted with a 0,5 mm mesh screen. Processing and the interpretation of nitrogen results were carried out on a Hewlett Packard micro-computer (HP85) using the Basic Statistics and Data Manipulation Programme.

Calibration

Fifty leaf samples which had nitrogen contents, ranging from 1 to 3%, were used to programme the instrument which measures the reflected light intensity at the six filter wavelengths and then computes the negative logarithms of the amplitude of each. In the case of protein or nitrogen the final equation that is used is as follows:

$$\% N = K_{1n} \text{Log}_1 + K_{2n} \text{Log}_2 + \dots + K_{6n} \text{Log}_6$$

where Log₁ is the light intensity measured at wavelength 1 680 nm, Log₂ at 1 940 nm etc.

The log numbers and the results of the chemical analysis of the fifty samples were used to determine the K_n constants by multiple regression analysis. Once the values of the constants had been keyed into the instrument no further calibration was required.

Comparison of the infra-red method with the steam distillation method

An initial assessment of the accuracy and precision of this method was made by repeatedly analysing 13 reference leaf samples over a period of five days. These samples had been collected over approximately three years and had been analysed by using the steam distillation method. The results obtained are given in Table 1. The small difference between the mean results (0,07%) and the small variation in the infra-red derived N values for each sample (CV range from 0,8 to 4,5%) suggest that this method is reasonably accurate.

TABLE 1

Variation in third leaf N values over a five day period as determined by the infra-red N analyser

Leaf composite No.	Standard* value	Infra-red analyser N values							Std devn	CV%
		Day 1	Day 2	Day 3	Day 4	Day 5	Mean			
1	1,46	1,53	1,56	1,61	1,59	1,66	1,59	0,05	3,1	
2	1,25	1,23	1,16	1,17	1,18	1,25	1,20	0,04	3,3	
3	1,49	1,50	1,47	1,55	1,55	1,58	1,53	0,04	2,6	
4	1,61	1,71	1,66	1,73	1,77	1,81	1,76	0,08	4,5	
5	1,97	1,92	1,91	1,90	1,93	2,00	2,00	0,04	2,1	
6	1,96	2,05	2,03	2,08	2,06	2,10	2,06	0,03	1,5	
7	1,67	1,72	1,73	1,74	1,73	1,82	1,75	0,04	2,3	
8	1,73	1,78	1,79	1,82	1,83	1,88	1,82	0,04	2,2	
9	1,10	1,19	1,18	1,19	1,18	1,21	1,19	0,01	0,8	
10	2,00	2,04	2,05	2,05	2,09	2,06	0,02	0,02	1,0	
11	1,36	1,44	1,46	1,45	1,47	1,52	1,47	0,03	2,0	
12	1,94	1,91	1,87	1,90	1,91	1,99	1,92	0,04	2,0	
13	1,26	1,39	1,38	1,38	1,36	1,46	1,39	0,04	2,9	
Mean	1,60	1,64	1,63	1,66	1,66	1,72	1,67	0,04	2,3	

* based on mean of 30 determinations by steam distillation method

Because the range in nitrogen values was narrow (1,1 to 2,0%) the study was extended to include 125 samples that had previously formed part of an industry-wide nutrient survey. Their nitrogen contents were determined by the steam distillation method and the values ranged from 1,1 to 3,0%. The relationship between the N contents determined by the infra-red method and those obtained by the steam distillation method are shown graphically in Figure 3. Regression analysis confirmed that the two methods were reasonably well correlated over this range ($r = 0,89$).

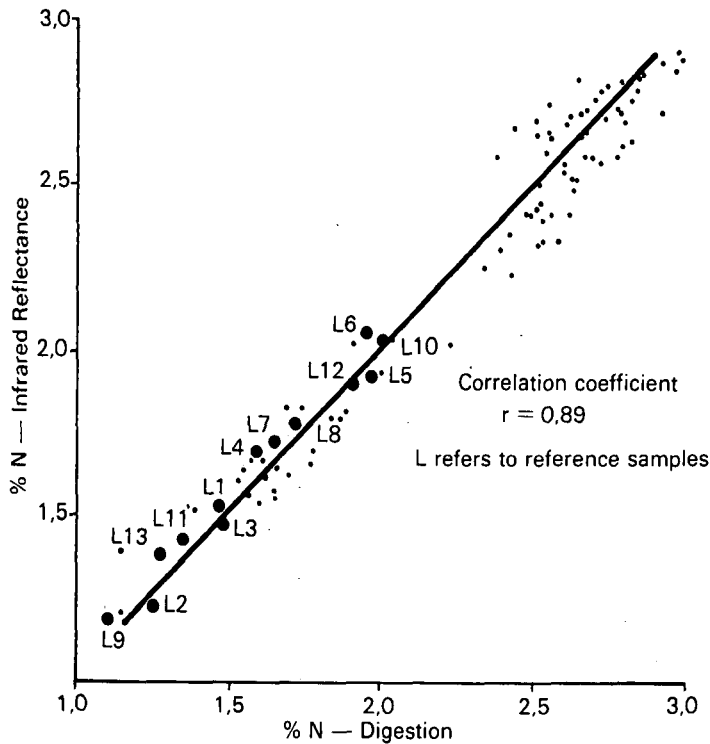


FIGURE 3 Comparison of infra-red with the steam distillation method

Only 12% of the samples analysed by infra-red differed by more than 0,15 units of N when compared with the results obtained by the distillation method (see Table 2). Differences never exceeded 0,25 units.

TABLE 2

Comparison between the infra-red method and steam distillation method for nitrogen (125 samples)

No. of samples	Difference	% agreement
95	± 0,10 and lower	76
15	Between ± 0,10 and ± 0,15	12
11	Between ± 0,15 and ± 0,20	9
4	Between ± 0,20 and ± 0,25	3
0	Greater than ± 0,25	Nil

Performance of the infra-red spectrophotometer under routine conditions

The performance of this instrument under routine conditions in the FAS laboratory was assessed by analysing a

batch of 107 leaf samples, and comparing the results with those obtained with the current FAS procedure based on the Kjeldahl digestion/indophenol blue colorimetric method. To facilitate statistical analysis, samples were subdivided into four nitrogen groups; <1,5%, 1,5 to 2,0; 2,0 to 2,5 and >2,5% N. In general the values obtained with the infra-red method agreed with those of the current FAS procedure (Table 3). An examination of the results for individual samples showed that differences were less than 0,15 units in 80% of the samples analysed. The close agreement is illustrated by the frequency distribution of differences given in Figure 4. This distribution also approximates a normal distribution which implies that the differences are not positively or negatively biased.

TABLE 3

Comparison of N values obtained by infra-red and FAS analysis

N content %	No. of samples	FAS	Infra-red	Difference
		Mean	Mean	
< 1,5	9	1,31	1,36	+0,05
1,5 - 2,0	28	1,68	1,67	-0,07
2,0 - 2,5	14	2,35	2,30	-0,05
> 2,5	54	2,72	2,73	+0,01

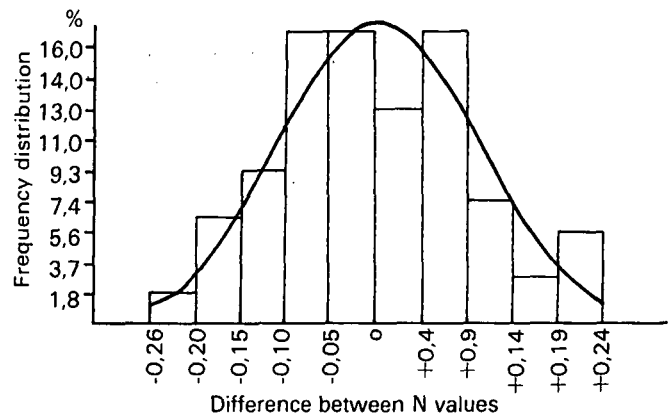


FIGURE 4 Agreement between infra-red and steam distillation method

The same batch of samples was analysed three more times on different days to determine the reproducibility of the method. The results obtained are summarised in Table 4. Co-efficients of variation ranged from 0,5 to 5,5%. These values compare favourably with the values reported by Burrows³ for the colorimetric procedure.

TABLE 4

Comparison of foliar N % results obtained by the routine FAS method and by the infra-red analyser

Observation	FAS	Infra-red N				Mean (CV%)	
		Day 1	Day 2	Day 3	Day 4		
Total sample No.	107	107				—	
Mean N %	2,27	2,26	2,28	2,29	2,30	2,28	
95% confidence interval	Upper	2,37	2,36	2,38	1,39	2,40	2,38
	Lower	2,16	2,16	2,18	2,19	2,20	2,18
Co-efficient of skewness	-0,15	-0,41	-0,38	-0,33	-0,36	-0,39	

The mean N value tended to increase slightly over the four day period but the trend was not statistically significant. Correlation matrix values obtained by regression analysis (see Table 5) confirmed that the N values obtained by infra-red analysis were highly correlated with those obtained by the current FAS method. The better correlation obtained between infra-red results and the indophenol blue results (0,98), when compared with the correlation between infra-red results and steam distillation results (0,89), could be due to the fact that the steam distillation data were obtained more than ten years ago.

TABLE 5
Correlation matrix values between the routine FAS method and infra-red N results

Method	FAS Digest	I-r Day 2	I-r Day 3
I-r Day 1	0,9797	0,9933	0,9932
FAS Digest	-	0,9760	0,9772
I-r Day 2	-	-	0,9961

A time and motion study conducted over a four day period indicated that a batch of 105 leaf samples could be analysed by a single operator in about 80 minutes. This is about six times faster than the present system based on weighing, digesting, diluting, dispensing and determining colorimetrically.

The effect of variation in sample packing when using different operators was also investigated. The results of packing the sample lightly were no different from those obtained when packing it tightly (see Table 6).

TABLE 6
Effect of sample packing on infra-red determined N values

Sample No.	Nitrogen %	
	Standard packing	Highly compacted packing
L1	1,65	1,65
L2	1,30	1,32
L3	1,61	1,60
L4	1,80	1,82
L5	1,98	1,96
L6	2,10	2,09
L7	1,81	1,78
L8	1,83	1,83
L9	1,28	1,29
L10	2,08	2,07
L11	1,52	1,54
L12	1,93	1,94
L13	1,39	1,40
Mean	1,71	1,70

Discussion and Conclusion

The results obtained from this investigation indicate the suitability of near infra-red spectroscopy for determining nitrogen in cane leaf samples on a routine basis. This is an improvement over a method which involves the unpleasant and time consuming acid digestion. The accuracy and precision of this method are comparable with those of the standard steam distillation method and equal to those of the method currently used by FAS.

The new method will result in a labour saving of at least eight man hours per 200 samples. Substantial savings in glassware, chemicals, maintenance and the replacement of corroded equipment are also possible. Other advantages of the infra-red analyser are that:

- it is very simple to operate
- it requires minimal calibration
- samples are not destroyed and may be re-analysed at a later stage
- it is light and portable.

The disadvantages are minor: at least 50 samples are needed to calibrate the instrument. This is the most important step in the whole operation and need be carried out only once. The sample grinding time must be standardised so that the material is of a reasonably uniform consistency. Variation in particle size can affect the infra-red reflectance characteristics of a sample and hence the accuracy of the method. This is particularly important in heterogeneous materials such as grain.

As part of the programme to streamline the leaf testing service for cane growers, this equipment, together with an X-ray fluorescence spectrometer, will shortly be introduced into the FAS laboratory. The new system will allow all the major nutrients and most micronutrients of approximately 200 samples per day to be determined without digestion and by fewer people. The increased capacity will considerably reduce delays during peak periods and will ensure that the demand for leaf analysis by growers will be satisfied for the next ten years.

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