Benefits and analysis of near infrared spectroscopic applications in sugarcane

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Introduction

The near infrared (NIR) reflectance technique has been developed over the last ten years as a rapid prediction method for evaluating protein, moisture and oil contents in grains or cereal.[1–3] The near infrared region is found within the range of 700–2500 nm. Absorptions in this portion of the electromagnetic spectrum are due to harmonics and combinations of bands originated in the middle of the infrared (IR) region, whose absorptions are caused by fundamental vibrations of the C–H, N–H and O–H groups, commonly found in organic products, which makes it possible to quantify various components in one sample. The determination of minerals in leaf tissue and in the soil has been the subject of different studies in NIR technology by different researchers.[4,5] In the case of sugarcane, Meyer[6] found that nitrogen analyses are rapid and precise when using this new technology in South Africa.

In relation to work on sugarcane quality, satisfactory correlations of NIR analyses with conventional analyses of sucrose, fibre, total soluble solids (brix) and moisture have been reported.[7,8] Other authors[9] have established NIR analyses at an experimental level for line-control processes of sugar beet juices in different factories. Calibration of one cane sugar factory could also be utilised in others.

Bearing in mind the importance and technical potential that NIR spectroscopy represents for the Colombian sugar industry, CENICAÑA began a process of studying and implementing NIR technology, applied to cane research.

Materials and methods

Laboratory samples and analyses

A Bran+Luebbe InfraAlyzer 500 was used to calibrate and validate the leaf tissue and soil samples. In order to determine sucrose, brix, fibre and other parameters of cane quality, an NIR Systems 6500 was used. Readings were taken using the reflectance mode and analysed in the range of 1100–2500 nm. Calibrations were made using conventional procedures described for NIR,[1,3] which include the selection of optimal wavelengths for log 1/*R* or log 1/*T* and first or second derivative, combined with statistical regression or correlation analyses.[10]

Leaf tissue analysis

From 146–318 cane leaf (top visible dewlap) tissue samples of different varieties, collected between the ages of 3–6 months, were used for the NIR calibrations and validations. The contents of N, P, K, Ca and Mg were determined in these samples. The primary analyses that served as the basis for these calibrations and validations were done by means of conventional Kjeldahl and colourimetry techniques for N and P, respectively, and atomic absorption for K, Ca and Mg.[6] Multiple linear regression (MLR) and partial linear square regression (PLSR) were used during the calibrations in order to select the best ranges and wavelengths for the validation stage and later routine analyses.

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Cane sugar analyses

The NIR calibrations and validations for determining sucrose (pol) and brix were begun with primary juices from the first milling, hydraulic press or experimental mill, as well as with expressed and diluted juices from direct cane analyses (DAC) using the wet disintegration method and from a mill tandem of a cane sugar factory, respectively.[11] Parallel analyses were made using polarimetry, HPLC and NIR of the juices proceeding from the cane sugar factory and CENICAÑA. For the calibration stage, approximately 315 primary and diluted juice samples were used, which had sucrose (pol) and brix values ranging from 4–23%. For the validation, 318 samples were employed, sampled and analysed under similar conditions. For the calibration and prediction parameters, the NSAS MLR programmes were used, similar to those employed for the leaf analyses, which made it possible to select the best working conditions for routine analyses based on the correlation coefficients and standard errors of calibration and validation.

Results and discussion

Leaf tissue analyses

The results obtained from the NIR analyses for N were correlated with the standard Kjeldahl method. The samples analysed (146) ranged from 1.00–2.91%. In general, NIR values and standard deviations were comparable to those of the primary method (Table 1). The regression analyses confirmed that the two methods were well correlated ($R = 0.98$) in the range studied, which corresponded to the majority of leaf samples from cane of commercial interest (Tables 1 and 2).

The relation between P-content in the cane leaf tissue, determined by means of the NIR technique and that obtained by colourimetry, are shown for both the calibration and validation stages (Tables 1 and 2). As was observed for N, there was a good correlation ($R = 0.86$) for P during the calibrations, using the PLSR model (for the second derivative of the spectrum); nevertheless, the correlation coefficient for the validation of 183 samples was lower than those for N and Ca (Table 2).

The other major elements, such as K, Ca and Mg, had good correlations ($R = 0.77$–0.90) during the calibration between the contents determined via NIR and the standard method of acid digestion followed by atomic absorption analysis. Nevertheless, during the validation of the NIR methodology for

Table 1. Results obtained with NIR calibration for different elements in sugarcane leaf tissue.

No. of samples	Determination	Concentration range (%)	Mathematical model	Correlation coefficient	*SD*
146	Nitrogen	1.05–2.91	MLR 1684–2132 2196	0.98	0.0954
261	Phosphorous	0.11–0.31	PLSR[a] 2nd derivative	0.86	0.030781
288	Potassium	0.92–1.94	PLSR 2nd derivative	0.88	0.109
271	Calcium	0.16–0.55	PLSR 2nd derivative	0.77	0.0574
277	Magnesium	0.09–0.31	PLSR 2nd derivative	0.77	0.0297

[a] Partial linear square regression method of the NSAS programme for the NIR equipment.

Table 2. Results obtained with NIR validation for different elements in sugarcane leaf tissue (no. of samples = 183).

Determination	Concentration range (%)	Correlation coefficient	*SD*
Nitrogen	1.06–2.27	0.90	0.12064
Phosphorus	0.10–0.32	0.570	0.043
Potassium	0.81–1.65	0.37	0.2194
Calcium	0.17–0.51	0.69	0.0648
Magnesium	0.12–0.42	0.50	0.05974

these elements, when employing the best mathematical model (PLSR and second derivative), a better correlation was obtained only for Ca (Tables 1 and 2). On the other hand, observations of time and movement in the laboratory made it possible to conclude that the NIR method is about six times faster that the conventional analytic systems, which are based on digestion, dilution, distillation and titration of each sample.

In general, taking into account the degree of analytical precision required by a fertilisation programme and the recommendations for using fertilisers in sugar cane, the NIR methodology can be used with a high degree of confidence for its precision and analytical reproducibility, particularly in the case of N, an element of great importance in its cultivation.

Cane juice analyses

The statistical information obtained from the calibrations and validations for brix and pol in different cane juices is shown in Table 3. Highly significant correlations were obtained between the NIR

Table 3. Results obtained with NIR calibration and validation for cane juices from first extraction and diluted.

No. of samples	Determination	Concentration range (%)	Mathematical model	Calibration		Validation	
				R	*SE*	*R*	*SE*
315	Brix	9.82–22.98	PLSR[a] 2nd derivative 2000–2350 nm	0.99	0.13	0.99	0.15
315	Pol	8.21–21.21	PLSR[a] 2nd derivative 2000–2350 nm	0.99	0.14	0.99	0.15
166	Sucrose[b]	12.12–22.02	PLSR[a] 2nd derivative 2000–2350 nm	0.99	0.30		
166	Fructose[b]	0.11–0.56	PLSR[a] 2nd derivative		0.83 0.07		
166	Glucose[b]	0.10–0.66	1100–2500 nm		0.80 0.08		

[a] Partial linear square regression method of the NSAS program for the NIR equipment
[b] Primary determinations and correlations based on HPLC

data and the pol and brix values, determined by the conventional methods of polarimetry and refractometry, respectively. Similar to reports by other authors,[7,9] excellent correlation coefficients (R = 0.98–0.99) with low standard errors (0.13–0.30) were obtained for the calibration and validation stages for the cane juices, which makes the implementation of the technology feasible and highly reliable in routine cane-quality analyses. At the same time, the NIR calibrations carried out for juices using HPLC as the primary method were very good for determining both sucrose and other sugars such as glucose and fructose, although the correlation coefficient was lower (R = 0.80) for the latter group than for those reported for sucrose (R = 0.99). The advantages of using NIR technology to analyse cane juices are as follows: The use of toxic clarifiers such as lead sub-acetate is obviated, which contributes to a better environment; rapid reading and availability of results after the calibration and analytical validation stages.

Conclusions

The NIR technique offers considerable analytical advantages once the calibration stage or correlation with an established method for a given constituent or compound has been finished. Among the advantages, the following can be cited:

- It is easy to operate
- It is a non-destructive technique so the samples can be reanalysed later, especially in the case of leaf tissue
- The precision and exactitude of NIR is comparable to that of other established methods for cane juice quality, a good correlation having been found between conventional methods and NIR
- It economises the use of reagents and the time of analyses

Among the disadvantages of using the NIR technique, are the following:

- Prior calibration work for each constituent or compound of a determined material is required
- Variation in particle size of solid samples such as defibrated cane, can affect the reflectance characteristics and consequently the precision of the method, making it necessary to have a good system for preparing or triturating the samples

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