Utilisation of near infrared spectroscopy for determination of the nitrogen content in beet leaves

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Introduction

The need to use an analytical instrument capable of carrying out rapid and sufficiently reliable quantitative determinations, so minimising the work of the operator and the utilisation of chemical reagents, has in recent years steered the interest of the whole world of sugar analysis towards near infrared (NIR) techniques. In fact, by using this type of technique, some analytical problems have been solved, both in the agronomical and technological fields, where measurements on-line and in real time are increasingly requested for the control and computer-based management of plant and equipment.

The experience acquired in the field of production and processing of sugar cane can be considered to be well-established by now. Ever since the 1980s, Meyer emphasised the importance of the use of the NIR technique, either for the routine analysis of nitrogen content[1] and other parameters in the leaves of cane, or as a diagnostic tool to control all the steps of the industrial process.[2] In fact, it could be employed for the analysis of raw material[3] (cane polarisation, fibre and moisture contents) or of juices (dry substance, polarisation, glucose and fructose levels) whilst finding a good agreement between the NIR data and those obtained via traditional analytical methods.

Also in Italy, starting from the 1980s, Vaccari *et al.*[4] suggested the possible use of the NIR technique for the analytical control of various intermediate products of sugar beet processing (brei polarisation, dry substance and total nitrogen contents, polarisation and refractometric dry substance (RDS) of juices such as raw, thin and thick juices, as well as molasses). On the basis of the good results obtained at laboratory level, the NIR technique was directly applied to the control of industrial processing in the Minerbio, CO.PRO.B, sugar factory.[5] The results obtained made it possible to set up good calibrations for the on-line measurements of polarisation and RDS of raw, thin, thick juices, molasses and streams from the Quentin ion-exchange plant. Recently, again at the Minerbio-CO.PRO.B. factory, tests have been carried out on some solid products (fresh and pressed pulp, cossettes, carbonatation sludges) and, even in these cases, encouraging results have been obtained.

Objectives

Based on the experiences mentioned above, we considered the possibility of extending the use of the NIR technique to the beet–agronomical field for the determination of the nitrogen content of beet leaf tissue in the first stages of their development. The importance of such a determination is related to the possibility of being able to manage fertiliser application to beet, knowing the nitrogen content of the leaves at the start of growth i.e. at the 4–6 true leaf stage.[6] If the nitrogen content of such leaves is below a certain limit, the farmers are advised to add nitrogen to the soil; if, on the other hand, the nitro-

Near Infrared Spectroscopy: Proceedings of the 9th International Conference

gen level is above the limit, addition of fertilizer is not necessary or even deleterious. In fact, in the latter case, not only do farmers bear unnecessary costs, but also the problems of environmental pollution of ground water are exacerbated, as well as leading to a worsening of the technological quality of beet due to a build-up of α-amino nitrogen which is not balanced by a proportional sucrose production.

At the moment, the analysis of leaves is mainly carried out using the traditional Kjeldhal method, which is not only time-consuming but also implies the utilisation of chemical reagents. On the other hand, the alternative utilisation of the Dumas technique needs an expensive and mono-analytical instrument, the single analysis of which has no zero costs.

Experimental

Sampling

With the aim of obtaining representative leaf samples, sampling must be carried out following a standardised procedure. The optimal period for sampling is at the 4–6 true leaf stage (the start of growth) bearing in mind that, at this stage, nitrogen fertilisers can be added to remedy possible nutritional deficiencies. The sampling must be carried out from rows of 10–15 plants in a field not larger than two hectares. Using special sampling pliers (ABI Patent) part of each leaf is harvested in the shape of a disc (100–150 plants within 15–20 minutes), so minimising handling by the operator and avoiding excessive damage to the leaves (Figure 1). Then the samples must be microwave oven-dried for about 20 minutes and ground to a uniform small particle size. Each sample, to be suitable for the NIR reading, must be compressed into the shape of a tablet (about 2 g weight; 1.5 cm diameter; 4–5 mm thickness) using a suitable device which operates at up to 200 bar pressure.

NIR readings

A Bran+Luebbe INFRALIZER 450 with 19 filters, available from the Minerbio CO.PRO.B. sugar factory, was used. The reading cell was suitably modified to allow the correct reading of tablets having different thicknesses. Thanks to the perfect polishing of both the sides of the tablets given by the compression device, the tablets were read on both sides. Notwithstanding previous drying of the leaves in the microwave oven, the tablets had a residual amount of water which had to be eliminated using a thermo-balance before the NIR reading to minimise spectral interferences due to different levels of moisture inside the samples.

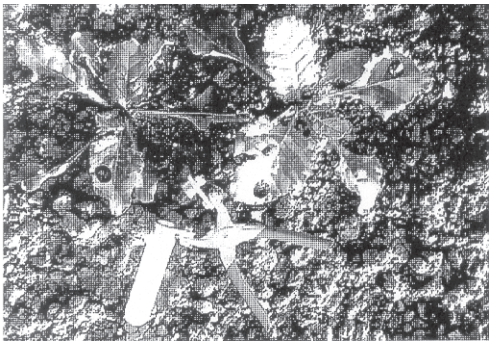

Figure 1. Sampling pliers.

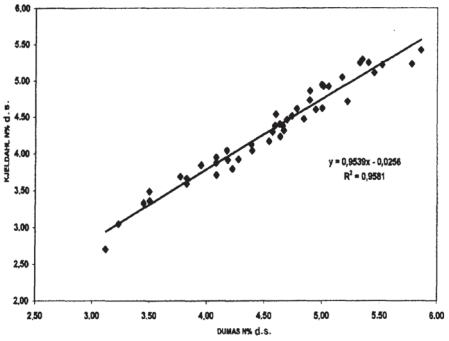

Figure 2. Correlation Kjeldhal-Dumas.

Table 1. Calibration parameters.

Reference method	No. of samples	Range (g/100 g d.s.)	MCC	*SEC*	*F*
Kjeldhal	58	3.05 – 5.11	0.9150	0.1547	290.673
Dumas	51	2.65 – 5.58	0.9745	0.1867	933.731

Calibration

Sixty samples of leaves from the 1997 and 1998 sugar campaigns were used. The samples, after the NIR readings, were analysed using the standard Kjeldhal and Dumas methods. The correlation between the series of data obtained using both of the methods was judged to be satisfactory [$r^2 = 0.9581$ (Figure 2)], although the Dumas data were slightly higher because nitrogen compounds are measured which are not detected by the Kjeldhal method. The best calibration curves obtained using both the standardised methods required the utilisation of two wavelengths; the relevant statistical parameters and the calibration curves are shown in Table 1 and Figures 3 and 4, respectively.

Validation

The validation of the calibration curves was carried out using samples collected in the 1999 campaign. The results, given in Table 2, confirm that the calibration curves obtained in the previous years do not have to be corrected. This latter point is certainly very important because it is confirmed that previously established calibration curves can be used without any need for recalibration or corrections.

Discussion

The possibility of managing the application of nitrogen fertiliser to beet by the analysis of the leaf nitrogen content at the 4–6 true leaf stage of the plant calls for having to carry out a very large number of determinations within a very limited number of days. In fact, farmers, after having collected samples of leaves, need an analytical answer within 24 hours to decide whether or not they have to add fertilisers. If the answer were to be deferred, it would be impossible either to dress the soil, owing to the overgrowing of the leaves, or to modify a deficiency of nitrogen which influences the growth of the plant in its first stage of growth. It is, therefore, clear that the traditional analytical methods present

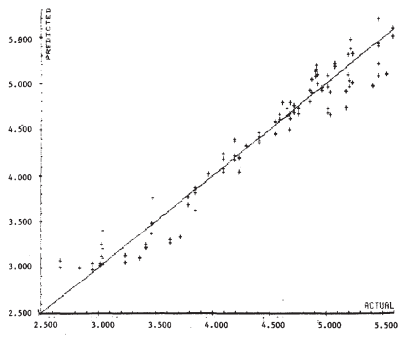

Figure 3. Calibration Dumas.

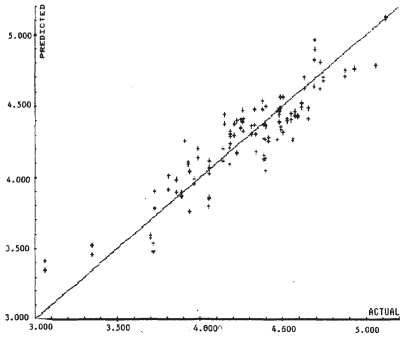

Figure 4. Calibration Kjeldhal.

Table 2. Validation parameters.

Reference method	No. of samples	Range (g/100g d.s.)	MCC	t	t^{9920}
Kjeldhal	20	3.05 – 5.57	0.87	0.084	2.845
Dumas	20	2.85 – 5.78	0.94	0.071	2.845

many disadvantages, principally a long time delay but also involving the employment of large amounts of chemicals which cause disposal problems (Kjeldhal) or high costs and frequent expensive maintenance of the instruments (Dumas).

Conclusion

In conclusion, the NIR technique could be a decisive solution to the problem because it guarantees:

- rapid times of analysis
- abatement of the costs related to the elimination of chemical reagents and simplicity of managing of the sample
- sufficiently reliable data which can be compared with those obtained by the reference analytical methods
- the possibility of carrying out the calibration with the available analytical method (Kjeldhal or Dumas)

An improvement of the method described above could be obtained using a lyophilising device to replace the microwave oven and thus cause fewer changes to the sample from the physical point of view. A further encouragement to proceed could be given by the possibility of using an NIR instrument *in situ* through the using of a reading system based on fibre optical probes which, directly placed on the leaves, might possibly give the required analytical data in real time.

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