Near infrared spectroscopy measurement of sugars and fructans in oaten hay

P.C. Flinn[a] and A.B. Blakeney[b]

[a]*Agriculture Victoria, Pastoral and Veterinary Institute, Private Bag 105, Hamilton, Victoria 3300, Australia.*

[b]*Cereal Solutions, PO Box 201, North Ryde, NSW 1670, Australia.*

Introduction

The export of Australian oaten hay to Japan is now an important industry. The "long fibre" hay is used as a roughage component in diets for dairy cows. Various quality standards, covering both subjective and objective criteria, have to be met by both hay producers and processors. NIR is routinely used to monitor moisture, protein, digestibility and fibre fractions in the hay. However, hay buyers are now also interested in how palatable the hay is to the cows and frequently chew the hay themselves to assess its "sweetness", as well as observe the preference of the cows for different lots of hay. This has led to requests from hay exporters for a rapid test to measure "sugar" content.

Clearly there are many different sugars in forage and a range of alternative tests. The most frequent test is the estimation of total water-soluble carbohydrates (WSC), comprising mainly glucose, fructose, sucrose and fructans. This non-specific procedure usually involves a colorimetric determination using the anthrone reagent[1] and NIR calibrations are routinely used for this measurement.[2]

The purpose of this study was to conduct separate analyses for glucose, fructose, sucrose, fructans and WSC in cereal hay, to develop and compare NIR calibrations and, in due course, to recommend the most appropriate test for the industry to use.

Materials and methods

During the 1997/98 hay season, around 400 samples of cereal hay were scanned on a Foss NIRSystems model 5000 spectrophotometer (Foss NIRSystems, Silver Spring, MD, USA) to obtain reflectance spectra (as log 1/*R*). The samples had been ground through a 1 mm screen in a cyclone mill and small ring cups were used in a spinning sample module. Of these, 96 samples were selected on the basis of spectral characteristics as a representative subset for chemical analysis.

These samples were analysed for fructans by the Megazyme fructan assay procedure (Megazyme International Ireland Ltd, Bray, Co. Wicklow, Ireland). This method uses specific enzymes to hydrolyse fructans to fructose and glucose, which are then estimated by colorimetry using the PAHBAH reducing sugar method.[3] It has recently been accepted as an approved AOAC method. Free sucrose, D-glucose and D-fructose were determined separately using the Boehringer Mannheim enzymic food analysis kit. This kit determines glucose, fructose and sucrose sequentially, using specific enzymes and UV spectroscopy (Boehringer Mannheim GmbH, Mannheim, Germany).

NIR calibrations for these constituents were developed using modified partial least squares regression. Data pre-treatment was accomplished using the "standard normal variate" and "detrend" options

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together with a second derivative math treatment. ISI NIRS-3 software, version 4.0 (Infrasoft International, Port Matilda, PA, USA) was used for collection of spectra and all data processing. Calibrations were also developed for reducing sugars (glucose + fructose), total simple sugars (glucose + fructose + sucrose) and total soluble carbohydrates (glucose + fructose + sucrose + fructans). Calibration success was judged using standard error of cross-validation (*SECV*), coefficient of determination (R^2) and the ratio of *SECV* to standard deviation of the reference data (*SD*).

A second set of samples was spectrally selected from over 800 cereal hays tested during the 1998/99 hay season. These samples are currently being analysed for all the above sugar fractions and will be used to validate the calibrations.

Table 1. Sugar fractions (%) tested in cereal hay samples.

Measurement	*N*	Mean	Min.	Max.	*SD*
Fructans	93	2.69	0.00	9.73	2.29
D-glucose	93	0.82	0.00	2.19	0.54
D-fructose	88	1.48	0.00	3.59	0.87
Sucrose	96	0.34	0.00	2.07	0.40
Reducing sugars	88	2.30	0.15	5.36	1.35
Total simple sugars	88	2.55	0.40	6.35	1.45
Total soluble carbohydrates	90	5.31	0.77	16.08	3.50

N = number of samples
Min. = Minimum
Max. = Maximum
SD = standard deviation of values across population

Table 2. NIR calibration statistics for sugar fractions (%) in cereal hay samples.

Measurement	*SECV*	R^2	*SECV/SD*
Fructans	0.68	0.91	0.30
D-glucose	0.36	0.56	0.67
D-fructose	0.39	0.80	0.44
Sucrose	0.39	0.05	0.97
Reducing sugars	0.64	0.77	0.48
Total simple sugars	0.71	0.76	0.49
Total soluble carbohydrates	1.18	0.89	0.34

SECV = standard error of cross-validation
R^2 = coefficient of determination
SECV/SD = ratio of *SECV* to the standard deviation of the reference data

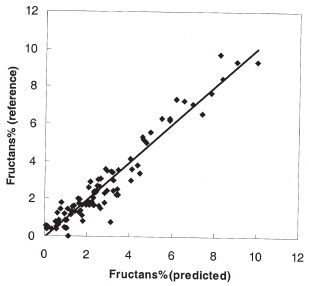

Figure 1. Scatterplot showing the relationship between reference and NIR-predicted values for fructans in cereal hay samples.

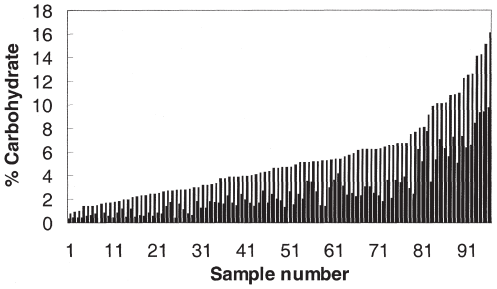

Figure 2. Histogram showing the proportion of total soluble carbohydrates (thin line) as fructans (thick line) for all cereal hay samples tested.

Results and discussion

The mean, range and standard deviation for the various sugar fractions in the hay samples are shown in Table 1. Table 2 shows the NIR calibration statistics for all measurements. Figure 1 shows the relationship between reference and NIR-predicted values for fructans.

The NIR calibration for fructans was highly accurate (low *SECV*, high R^2, *SECV/SD* ratio below 0.3). This was a similar result to previous work with cereal plant tissue samples collected at the stem elongation stage for a fertiliser top-dressing service.[4]

The calibration statistics for fructose, glucose and sucrose were satisfactory, fair and unsatisfactory, respectively. However, most values for sucrose and many for glucose were very low compared to the more significant levels of fructans and fructose. The calibration for total soluble carbohydrates, based on the sum of all sugar fractions measured, was accurate according to the above criteria.

Figure 2 shows the proportion of total soluble carbohydrates as fructans in all the cereal hays tested. It is clear that fructans represent the major proportion of total soluble carbohydrates, but this proportion is quite variable and it is not known if the fructan or non-fructan proportions have more influence on animal preference for the hay.

The most accurate calibration obtained for any of the sugar methods was for fructans and further work is in progress to validate and extend the calibration with samples from a different season. Analysis for WSC using the non-specific anthrone method will also be undertaken, an NIR calibration derived and results compared with the sum of all sugar fractions measured in this study. Future work will attempt to correlate these analyses with animal preference measurements.

Acknowledgement

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References

1. *The Analysis of Agricultural Materials.* Ministry of Agriculture, Fisheries and Food, Tech. Bull. 27. HMSO, London (1973).
2. K.F. Smith, R.J. Simpson and R.D. Armstrong, *Aust. J. Exp. Agric.* **38,** 45 (1998).
3. B.V. McCleary and A.B. Blakeney, *Cereal Foods World* **44,** 398 (1999).
4. V.B. McGrath, A.B. Blakeney and G.D. Batten, *New Phytol.* **136,** 145 (1997).