Analysis of high-moisture material—fibrated sugarcane

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Introduction

Clonal evaluation for sugarcane improvement requires measurements of quality components. The conventional analytical method entails juice extraction from stalk samples, with analyses done on both solids and liquids.[1] In contrast, near infrared (NIR) spectroscopy technology offers a method requiring only fibration to produce a sawdust-like material before spectroscopic analysis. Sugarcane, however, contains about 70% moisture, and so is similar to fresh and processed natural materials such as forages, fruits, sugarbeet, vegetables, fish, meats and silages. An application for meat appeared among the earliest NIR spectroscopy literature,[2] yet contemporary NIR literature has been dominated by applications for dried materials. This imbalance discredits the real advantages offered by direct analysis of fresh materials. Nevertheless, applications for all the high-moisture materials mentioned above have been reported. Our research has focused on development of a NIR application for fibrated sugarcane and has produced promising results.[1,3] Considerations encountered in the development of NIR applications for high-moisture materials are discussed.

Sample integrity

In contrast with low-moisture materials, samples of many high-moisture materials cannot be accumulated and stored. Sugarcane is a biologically fragile material, subject to deterioration by microbial and fungal activity. After fibration, samples must be analyzed within 30 min. Storage of a fibrated cane sample requires rapid freezing of several kilograms sealed to preserve the moisture status. This normally is not done because of the demand on resources.

Sample heterogeneity

A field-plot sample typically consists of nine whole stalks. A sample initially was fibrated using a meat and bone grinder adapted as a standard fibrator for the Australian sugar industry. Variation for component values among units (stalks) within such a sample exists. Experiments optimized a mixing strategy to minimize this variation. Any sub-sample of this mixed sample reveals variation for tissue type (rind, parenchyma and vascular tissue) and particle size within each. The existence of intra-sample heterogeneity was demonstrated.[1] To minimize the resources for optimized sub-sampling, samples now are fibrated using a series combination of two fibrators with different actions.[4]

Abbreviations**: AOV**-analyses of variance; **CCS**-commercial cane sugar; **NIR-**near infrared; **PLS**-partial least squares; **RLA**-routine laboratory analysis; **RMS**-root mean square; **SEC**-standard error of calibration; **SECV**-standard error of cross-validation; **SEP**-standard error of performance; **SNV**-standard normal variate; **VR-**variance ratio.

Sample presentation

Fibrated cane has a sponge-like consistency and will exude juice on surface contact. Condensation can occur on a window surface resulting in localized dilution. This could be important for reflectance spectroscopy. Fibrated cane is pressed against the window of a presentation cell to provide as uniform a surface as possible. However, this pressure has to be controlled to prevent juice expression. This property is a clonal trait and a standard pressure cannot be used. In the transport module of the NIRSystems Inc. Model 6500 spectrophotometer, juice expression can contaminate the ceramic reference. Surfaces of presentation cells become coated with juice during use and require cleaning to avoid serial contamination of samples. Thorough cleaning also prevents build up of microorganisms but requires considerable resources.

Spectral effects of high-moisture

Abrams *et al.*[5] commented that, in high-moisture hays, the moisture absorption band was broad and tended to obscure spectral information of other compounds. Reeves[6] showed that water caused shifts in spectral wavelengths, not related to OH and NH groups and this effect increased with increasing water content. Many sharp peaks found in solid sugars disappeared in solution of these sugars, resulting in large, broad peaks. Reeves[6] considered this contributed to the decrease in accuracy that occurred with high-moisture samples. Trial calibrations with fibrated sugarcane confirmed that the use of low numbers of specific wavelengths was not optimal. Calibration development with PLS regression was preferable. Absorbance values [log (1/*R*)] were above 1.0 in the range 1400 to 2500 nm and approached 2.0 at the upper end. While the linearity of modern instruments is within 1% in this range, for high-moisture materials error will be introduced in calibration transfer between instruments.

Spectral standards

In low moisture NIR applications, stable spectral standards are essential for two reasons:

—to provide check cells for routine monitoring of the analytical process.

—to permit accurate transfer of calibrations between instruments.

Spectral standards typically have characteristics similar to those of the material being analyzed. A series of spectral standards is relatively easy to construct and maintain for low-moisture materials but high-moisture applications would require hermetically sealed, spectrally similar samples. Inclusion of carbohydrates would be required for sugarcane and these are susceptible to deterioration in moist conditions. There is a real need for a series of stable, spectrally diverse, high-moisture spectral standards for application developments for high-moisture materials.

Continuity of calibrations

There are three aspects to maintenance of the continuity of calibrations:

—the transfer of calibrations between, or among, instruments.

—the maintenance of calibrations on a single instrument through a catastrophe requiring a major instrument rebuild or instrument replacement.

—the transfer of calibrations between different sensing modules on the one instrument.

While these are fairly routine for low-moisture materials, any of these procedures are hindered severely in high-moisture applications by the lack of appropriate spectral standards.

In this paper, we report on a calibration experiment in 1994 with a newly acquired instrument and research on transfer of calibrations between instruments for fibrated sugarcane. We also detail precautions required to preserve the integrity of single-instrument installations used for high-moisture material and suggest a procedure for calibration transfer between different sample presentation modules on a single instrument.

Materials and methods

Sugarcane samples were taken from a routine clonal evaluation trial, at BSES Meringa, containing 154 plots in each of two replicates. Analyses were conducted from 25 November to 1 December, 1994. Plot samples were collected daily and processed before deterioration could occur. A nine-stalk sample from each plot was fibrated and mixed. Duplicate RLAs[1] were done on each sample, as previously described,[4] except juice was hydraulically expressed from two sub-samples. Examination of duplicate data ($n = 2 \times 308$) for Brix, CCS, fiber, moisture and polarization reading allowed editing of outliers.

A newly purchased NIRSystems Model 6500 spectrophotometer, with a sample transport module and NR7080 coarse sample cells, was used in reflectance mode. Data were collected in the 600–2500 nm range. The instrument operated in an isolated room at a relatively constant 22ºC and 60% RH. Four cells were filled from each sample. A mean spectrum of 32 scans from each was collected and a sample mean spectrum for the four cells was recorded to a set, first derivative, RMS error. The NIRS3 software package (ISI, Pa) was used throughout this research.

Our 1993 research was conducted with a similar instrument supplied in a collaborative research agreement with NIRSystems Inc., Australia.[3] Standard check cells were not used since our work was in the high-moisture area. However, spectra from a check cell containing glucose powder, maintained in a dessicator, were regularly recorded as a mean of 32 scans. In 1994, the integrity of the instrument used in 1993 could not be guaranteed and so was not suitable for direct standardization with the 1994 instrument. To obtain an indirect comparison of instruments, the glucose check cell was scanned with the new instrument in 1994 under temperature and humidity conditions similar to 1993. Average spectra for 1993 and 1994 glucose spectra were calculated. To investigate other standardization procedures, average spectra for the total 1993 ($n = 917$) and 1994 ($n = 305$) data sets were calculated. As the RLA means for these data sets differed appreciably, data sets with similar ranges and means were derived for each year. Average spectra for the modified 1993 ($n = 154$) and 1994 ($n = 276$) data sets were calculated.

The 1993 data set, an aggregation of four experiments, had been partitioned into calibration ($n = 397$) and prediction ($n = 574$) sub-sets.[3] Standardization files were created with the routine CLONE1 from NIRS3, using the following:

—mean glucose spectra from 1993 and 1994.
—mean spectra for the total 1993 and 1994 data sets.
—mean spectra for the modified 1993 and 1994 data sets.

Three transformed versions of the 1993 prediction data sub-set were created using each of these.

Results and discussion

RLA results 1994

The ranges of RLA results (Table 1) were less than for the more extensive experiments in 1993.[3] The means were higher for Brix, CCS, fiber and pol reading and lower for moisture. The trial was precise, as indicated by the low coefficients of variation, but there was little genetic variation for any component except fiber. Differences among clones were highly significant for all components (Table 1). Data for mass balance, the sum of soluble solids (Brix), fiber and moisture indicated the high accuracy of the component analyses.

Error ratio tests,[1] with values well in excess of 3.0 (Table 2) and correlation and regression statistics indicated the use of duplicate determinations for, and the precision of, all RLAs were very satisfactory.

Table 1. Statistics from AOV for five components of fibrated sugarcane determined by routine laboratory analyses for 154 clones in a two-replicate clonal evaluation trial in 1994, together with statistics for mass balance.

Component	Mean	Range	SE/plot	CV (%)	GCV (%)	"F" clones
Brix (g kg^{-1})	240.9	205.0–268.8	8.6	3.6	3.6	5.1[a]
CCS (g kg^{-1})	173.8	143.2–202.4	6.3	3.6	4.3	6.6[a]
Fiber (g kg^{-1})	142.4	109.2–195.6	6.8	4.8	10.8	21.3[a]
Moisture (g kg^{-1})	656.6	605.1–703.5	9.3	1.4	2.3	12.0[a]
Pol reading (ºZ)	95.3	77.9–109.5	3.7	3.9	4.4	6.1[a]
Mass balance (g kg^{-1})	998.3	992.9–1005.6	2.1	0.2	0.1	1.5[a]

[a]$P \leq 0.01$.

NIR calibrations

In examination of the spectral data using the CENTER option, three spectra were regarded as outliers and deleted. The data set ($n = 305$) was then used for calibration development using modified PLS regression and a range of math treatments. Scatter correction by SNV and Detrend was applied throughout, although tests showed that the value of this correction varied. The best calibrations, as judged on cross-validation criteria, used a second derivative pre-treatment (Table 3). A comparison of the SECV values with the SEP values from the 1993 research[3] suggests the recent calibrations are superior.

Table 2. Error terms from sampling AOV for five components of fibrated sugarcane determined by routine laboratory analyses for 154 clones in a two-replicate clonal evaluation trial, error ratio tests and simple correlation and regression statistics between sub-sample determinations.

	Error component		Error ratio test			
Component	$2\sigma^2_e$	σ^2_s	$2\sigma^2_e/\sigma^2_s$	*r*	*b*	*t*
Brix (g kg^{-1})	73.459	0.396	185.5	0.997	0.991	209[a]
CCS (g kg^{-1})	39.349	0.242	162.6	0.997	0.996	220[a]
Fiber (g kg^{-1})	44.947	1.872	24.0	0.993	0.987	147[a]
Moisture (g kg^{-1})	85.717	0.534	160.5	0.998	0.994	287[a]
Pol reading (ºZ)	13.664	0.028	488.0	0.999	0.996	365[a]

[a]$P \leq 0.01$.

Table 3. Calibration and cross-validation statistics for five components of fibrated sugarcane, developed using modified partial least-squares regression on data treated for scatter correction by SNV and Detrend.

Component	Math treatment	*SEC*	R^2	*SECV*	1 – *VR*
Brix (g kg^{-1})	2, 8, 4	1.275	0.986	1.433	0.982
CCS (g kg^{-1})	2, 4, 4	1.545	0.967	1.871	0.951
Fiber (g kg^{-1})	2, 12, 4	2.547	0.973	2.868	0.966
Moisture (g kg^{-1})	2, 16, 4	1.552	0.991	1.684	0.989
Pol reading (ºZ)	2, 4, 4	0.532	0.988	0.657	0.982

Spectral data transfer

Summary statistics for the RLA Brix values for the prediction data sub-set are given (Table 4; 1993 – rem.). The application of the calibration equation for Brix for the 1994 experiment (Table 3) to the prediction data sub-set, transformed using the standardization file based on the mean glucose spectra, yielded prediction statistics presented in Table 5. While there was a strong, linear relationship between RLA and NIR predicted Brix values, the regression was unacceptable, being strongly biased and skewed. The spectral populations obviously differed as indicated by the large *H* value. The use of a single check cell, of low moisture and absorbance value, as a standardization tool was useless for a high-moisture application. This failure may be related to the limited absorbance range and hence insensitivity to slight non-linearities in log (1/*R*) values above 1.0.[7]

The same calibration equation was applied to the prediction data sub-set transformed using the standardization file based on the mean spectra of total populations (summary statistics in Table 4). This again yielded a similar linear relationship and a regression with a strong negative bias, and strongly skewed (Table 5). The bias was not unexpected given the marked difference in mean Brix for the two standardization populations (Table 4). The *H* value, however, was reduced considerably but still exceeded the threshold value of 3.0. Use of the prediction data sub-set transformed using the mean spectra of the modified populations gave results similar to the previous

Table 4. Statistics for spectral populations of fibrated sugarcane from which mean spectra were derived for standardization, or which were used to assess effectiveness of standardization procedures.

Population	Use	*n*	Mean	Range	*SD*
1993 — all	Stand.	971	220.6	176.2–253.7	13.1
— mod.	Stand.	154	233.3	206.2–253.7	12.9
1994 — all	Stand.	305	240.9	206.1–268.4	10.7
— mod.	Stand.	276	239.2	206.1–253.6	9.6
1993 — rem.	Pred.	574	220.3	176.2–253.4	13.1

Table 5. Assessment of three procedures for inter-instrument calibration transfer, using a calibration equation for Brix developed on 1994 data, and applied to data collected on an independent instrument in 1993.

Standardization method	NIR pred. mean	*SEP*	Bias	Slope	R^2	$\overline{H}$
Glucose check cell	200.8	19.7	19.5	0.87	0.96	46.53
Mean spectra of total populations	240.7	20.7	–20.4	0.85	0.96	3.69
Mean spectra of similar populations	227.5	7.9	–7.2	0.86	0.96	3.74

prediction except that bias was reduced (Table 5). This reduction paralleled the difference in means of the two standardization populations (Table 4). This showed that the spectral populations have to be matched accurately for this technique to be successful. Under these circumstances, the best that can be done is to match the populations by RLA values. However, there may be temporal and/or environmental effects on mean spectra of populations, independent of RLA values. This places the use of average population spectra in doubt.

Standardization options

Recommended procedures for instrument standardization for low-moisture applications are available[8,9] but there has been limited extension of these to high absorbance materials.[10] There are no definite recommendations for standardization for high-moisture applications when appropriate spectral standards are not available. Currently, we see the lack of these as a major deficiency within the technology. Possibly, the best method available is to scan a set of diverse samples in a range of environments, with both instruments, virtually simultaneously. The resultant spectra are used to generate the standardization file which enables spectral data transfer.

For a single-instrument installation, preservation of calibrations requires that a standardization file be established with at least one other instrument. This would require that an instrument involved with high-moisture applications be standardized regularly, by bringing on site a protected master, or "borrowed", instrument, the integrity of which would be guaranteed. The above procedure would then be performed. This will be a symbiotic arrangement offering cross protection against catastrophic failure. Such security already exists for low-moisture applications in the form of the ISI standardization set.

We currently are moving from a R&D situation, using the instrument configuration detailed above, to an at-line development for analysis of trial samples. This uses a remote reflectance probe linked to our current Model 6500 instrument. This will increase throughput of samples and represents an effective, economical NIR application for sugarcane improvement. Transfer of our current calibration data to this new system is highly desirable. Near simultaneous scanning of the same sample set by both modules attached to the one instrument is not feasible. Use of a second instrument is envisaged, initially with both instruments fitted with standard transport modules. The standardization procedure recommend above would be used. Secondly, the "home" instrument, equipped with the remote reflectance probe, would be standardized with the "loan" instrument. Calibration data from the R&D configuration will be transferred sequentially and allow development of calibrations for the at-line system.

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