Optical matching of near infrared reflectance monochromator instruments for the analysis of ground and whole wheat

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Eight near infrared (NIR) reflectance monochromator instruments in Australia have been optically matched (standardised) in order that reflectance spectra can be obtained when the same sample is measured on any of the instruments which are as similar as reflectance spectra obtained by re-packing the same sample on one instrument. This means that data from the different instruments can be merged to produce calibrations based on a larger number of more diverse samples than would be available to any one laboratory, without the need for the actual samples to be moved around the country. In turn, the resulting spectral library and calibrations can be shared. Two methods of standardisation of this type of instrument have been previously reported for ground samples. One method utilises 30 sealed powdered samples of diverse types in order to correct for both slope and bias while the other requires only a single sealed sample to correct for bias. The results of standardisation, using the set of 30 samples and using a single ground wheat, confirms that the single sample standardisation works as well as that performed using the 30 samples. In addition, an extension of the single sample method using whole grain samples is reported. The results confirm that the single sample method can be successfully used to optically match NIR reflectance monochromator instruments of the same brand.

Keywords: monochromator, NIR, reflectance, standardisation, calibration transfer, wheat, whole grain.

Introduction

The Grain Industries Centre for NIR[1] was established in Australia in 1996. One of its chief functions is to promote a collaborative, national approach to near infrared (NIR) research. For example, pooling of sample sets from all the wheat breeding programs in the country will produce a large spectral database which represents a wider range of material than would be available in any one program. It is not intended that individual laboratories use national calibrations. Rather, the aim is to create and share a national spectral library so as to utilise calibration procedures based on population structuring.[2–6] Since all the collaborating laboratories have the same type of monochromator instruments, an important prerequisite was to standardise these instruments so that NIR spectra recorded on any instrument can be merged into one database. This will avoid the need to transport samples over long distances, which would be costly and time-consuming, especially considering the quarantine regulations governing the importation of plant material into individual Australian States.

The Shenk–Westerhaus concept of optical matching of NIR monochromators (referred to as "standardisation") using sealed cups containing samples of agricultural materials was originally introduced in the USA[7,8] and developed further in Europe.[9–11] It has also been used successfully to transfer calibrations for the analysis of petrochemicals between Fourier transform near infrared (FT-NIR) spectrometers.[12] Originally, a quadratic model[7] was used to standardise the analysis of ground samples with an instrument fitted with a spinning sample module. A set of 30 dried, ground samples comprising a wide range of materials including grains, forages and mixed feed was created for this purpose. The constant term corrected for the spectral bias arising from different ceramic references and other undefined sources of photometric variation among instruments. The linear coefficient corrected for variations in wavelength. There was no theoretical reason for the quadratic coefficient although it did serve to improve the standardisation of some older instruments. More recently, a simplified procedure using only a single sealed sample has been developed.[8] This is based on the assumption that wavelength standardisation for each instrument is nowadays accomplished internally using a polystyrene standard and the quadratic coefficient for modern instruments was found to be negligible. Results of recent studies involving three FT-NIR spectrometers have confirmed that the Shenk–Westerhaus method compensates for small wavelength shifts by correcting the band intensities.[12] It is recommended that the photometric bias is best corrected using a sample having a spectrum near to the average of all those expected for routine analysis, i.e. if the aim is to analyse whole wheat then the sealed cell should contain whole wheat. However, the original methodology was developed for ground samples for which the expected absorbance ($\log 1/R$) range is 0.01–0.6 where the photometric response is linear, so simple mathematical procedures can be used to correct the spectra. In the context of this project, the need is for exchange of whole grain reflectance data where the absorbance range is above 1.0 and non-linearity is likely to occur. Under these circumstances, there are no reported data which show that a single sample standardisation would be able to correct sufficiently for the purposes of combining spectral data sets from different instruments. The experiments described in this paper were designed to achieve the optical matching of the instruments within Australia, so that data can be shared across the country and not as a rigorous test of the Shenk–Westerhaus methodology.

Materials and methods

Samples

Four sets of samples were used:

A set of 30 sealed, ground samples (NIRSystems, Silver Spring, USA; part number IH-0328; serial number 06-18-97).

Seven samples of whole US wheat packed into whole grain quarter cups (Check Cell for Transport Systems) and sealed.

Seven samples of ground US wheat packed into black small ring cups (Check Cell for Spinning Cup Systems) and sealed.

A set of 34 loose samples of whole Australian wheat with associated Kjeldahl protein data.

Each set of samples 1–3 was sealed so that the sample surface remained undisturbed during transportation.

NIR measurements

Eight laboratories with eleven instruments (Table 1) were included in the standardisation. The sets of sealed samples were distributed by courier in a star network, i.e. they were scanned on the Reference Instrument before and after each Local Instrument. Instrument 1 (the NIRS 6500 Transport Instrument located in the North Ryde laboratory) was chosen as the Reference Instrument. Before attempting to measure the spectra of the standards on each instrument, the instrument response, wavelength accuracy and repeatability were verified as being within the criteria recommended by the instrument manufacturer. In addition, for the transport instruments, the speed of the transport travel was adjusted to allow 32 scans of the quarter cup in a single upward and downward movement.

In each laboratory, other than North Ryde, the measurements were carried out over a period of four days as follows:

Day 1. ISI Diagnostics and linearisation check.
Day 2. 7 ground wheats, 30 diverse ground samples, 7 whole wheats.
Day 3. 7 ground wheats, 30 diverse ground samples, 7 whole wheats.
Day 4. 7 ground wheats, 30 diverse ground samples, 7 whole wheats.

For the sample transport instruments, the samples in small ring cups were scanned four times with a 90° rotation of the sample cup between each scan; the

Table 1. Locations and types of instruments in the Australian network.

No.	Location	Model/type
1	BRI Australia Ltd, North Ryde, NSW	6500 Transport
2	Agriculture WA, Perth, WA	6500 Transport
3	SARDI Pig & Poultry Production Inst., Roseworthy, SA	6500 Transport
4	University of Adelaide, Adelaide, SA	6500 Transport
5	Pastoral & Veterinary Institute, Hamilton, VIC	6500 Transport
6	Pastoral & Veterinary Institute, Hamilton, VIC	6500 Spinning Cup
7	Pastoral & Veterinary Institute, Hamilton, VIC	5000 Spinning Cup
8	Victorian Inst. for Dryland Agriculture, Horsham, VIC	6500 Transport
9	Victorian Inst. for Dryland Agriculture, Horsham, VIC	6500 Spinning Cup
10	NSW Agriculture, Yanco, NSW	6500 Transport
11	CSIRO Plant Industry, Canberra, ACT	5000 Spinning Cup

Notes:
(a) Instruments 5, 6 and 7 were standardised simultaneously without rescanning the samples at BRI Australia Ltd.
(b). Instruments 5 and 6 are the same instrument with different sampling accessories. Instruments 8 and 9 are the same instrument with different sampling accessories.
(c) Instruments 8 and 9 were standardised simultaneously without rescanning the samples at BRI Australia Ltd.

four spectra for each sample were averaged prior to further data processing.

Each set of data was saved as the average of 32 scans to a separate file which was returned to the reference laboratory together with the diagnostics results and the measured temperature and humidity of the laboratory in which the measurements were carried out. A 4-point Fourier smoothing was applied to the data during data collection.

For the North Ryde Instrument (the Reference Instrument), the samples were scanned on the day prior to dispatch and immediately on return from each local laboratory.

For one pair of instruments (1 and 3), selected at random, an independent set of 34 unsealed samples of whole Australian wheat, selected using the ISI "Select" program[2,3] from a population of 361 obtained from grain receival stations across Australia, was used as an independent test of the effectiveness of the whole wheat calibration transfer. The NIR spectra of these were recorded on each of the two instruments using a coarse sample cell and saved as the average of 32 scans. A calibration for protein content was derived using the remaining spectra measured on instrument 1 and used to determine the protein contents of the same samples when rescanned on instrument 3.

Data treatment and analysis

Data were processed using ISI NIRS 3 version 4.1 software (NIRSystems Inc., Silver Spring, MD, USA). While the Shenk–Westerhaus standardisation method is not software dependent,[12] it was most convenient in this case to use ISI software as all the collaborators were able to apply the Shenk–Westerhaus coefficients using an ISI Standardisation File. The standardisation was carried out over the range 1100 to 2498 nm. The raw log 1/*R* spectra were transformed into the first derivative d(log 1/*R*) / dλ using an 8 nm gap and a 4-point smoothing function. This mathematical function is the default setting in ISI software and as such is adopted by most users. Other mathematical treatments were not investigated in this exercise.

Two methods of standardisation (Single Sample Standardisation and 30 Sample Standardisation) based on the sealed sample sets were used.

30 Sample Standardisation uses the diverse set of 30 ground samples[7] and is the original methodology proposed by Shenk and Westerhaus. In this method, spectra of 30 diverse ground samples are recorded and a point-for-point correction to the wavelength and then the photometric axis calculated. The spectra are first transformed to the first derivative. Individual wavelength corrections between Reference and Local Instrument are calculated by determining the most highly correlated Local Instrument wavelength for each Reference Instrument wavelength, fitting a quadratic model to the highest correlating wavelength and to the two neighbouring wavelengths and using the location of the maximum in the quadratic model as the Local Instrument wavelength. A quadratic model is then fitted to the individual wavelength corrections. The final wavelength adjustments on the Local Instrument are two point interpolations using the two modelled wavelength locations closest to each nominal wavelength on the Reference Instrument. A photometric correction is made by regressing the photometric response of the Local Instrument on to the photometric response of the Reference Instrument. The Local Instrument photometric response is then adjusted using the calculated regression coefficients (the Shenk–Westerhaus coefficients).[7,12]

Single Sample Standardisation compares a single spectrum from a single sample scanned on both the Reference and Local Instrument.[8] This correction function is simply the difference spectrum in log (1/*R*) terms. This method was used for both the set of seven whole wheat and seven ground wheats. In each case, the median spectrum was used to create the standardisation file. The median spectrum is the middle one of each set of seven when plotted on the absorbance log 1/*R* scale. In this case, it was also the spectrum closest in terms of Mahalanobis Distance (*H*) from the mean of the seven spectra.

The standardisation function was calculated as follows. A file was created by averaging the spectra recorded across all three days on each Local Instrument and across three days on the Reference Instrument (scanned prior to standardising instrument numbers 2, 3 and 10) for standardisation.

The results of the standardisation were assessed in terms of comparison between the mean differences in spectra between instruments and between repeat measurements on the same instrument using the six samples from the set of seven sealed samples which were not used to create the standardisation. These were expressed in two ways.

The average root mean square of differences D of $d(\log 1/R) / d\lambda \times 10^{-6}$, corrected for bias either between-instruments or between-repacks at *n* corresponding wavelengths—*RMS(C)*.

$$RMS(C) = \sqrt{\frac{\sum D^2 - (\sum D)^2 / n}{n-1}}$$

This way of expressing the performance of instrument standardisation is based on the spectra alone.

Two-way analysis of variance (ANOVA) on the predicted protein contents using calibrations derived on the Reference Instrument over the same wavelength range and with the same mathematical data treatment as used for the standardisation. 327 samples of whole wheat and 32 samples of ground wheat were used to derive calibrations for this purpose using Kjeldahl as the reference method. This is a more standard way of comparing sets of data but is dependent on the robustness of the calibration used to predict protein content.

In all cases, there were six standard samples to be analysed, each with a different protein content. For the whole grain Single Sample Standardisation, there are seven instruments, one of which (10) was scanned twice (before and after routine servicing), making a total of eight for the purposes of this analysis. For the ground wheat Single Sample Standardisation, there were ten instruments, with instrument 10 scanned twice, making a total of 11 for this analysis. However, the set of 30 standards were not rescanned on instrument 10, so there are only ten instruments for 30 Sample Standardisation.

The whole grain standardisation was tested using a whole wheat calibration developed from a population of 327 samples obtained from grain receival stations across Australia. Thirty-four samples were removed for use as a test set and a calibration developed using the remaining samples. In addition, a repeatability file was created from the set of seven sealed whole grain standards scanned at all sites on the network. The repeatability file[8] is an important component of the calibration, particularly when the calibration is to be used on a number of optically-matched instruments. There are many other differences between the instruments' responses such as room temperature, humidity and operator which cannot be modelled by the standardisation procedure. The use of a repeatability file means that the temperature and humidity variation can be included in the final calibration, which should be more robust.

Results

The spectra of the three sets of sealed standards are shown in Figures 1–3. The *RMS(C)* differences between the instruments for both ground and whole grain before standardisation are given in Tables 2 and 3. The results of the standardisation expressed as *RMS(C)* are given in Tables 4–6. *RMS(C)* is used to assess standardisation because when calibrations use derivatives and/or scatter correction, any spectral offset (bias) remaining after standardisation is ignored by the calibration. Only the non-bias differences *RMS(C)*, therefore, have any impact on calibration accuracy. However, in these experiments, the bias was found to be negligible and the maximum difference between *RMS(C)* and *RMS* without the bias correction term was 5 micro $d(\log 1/R)/d\lambda$ units.

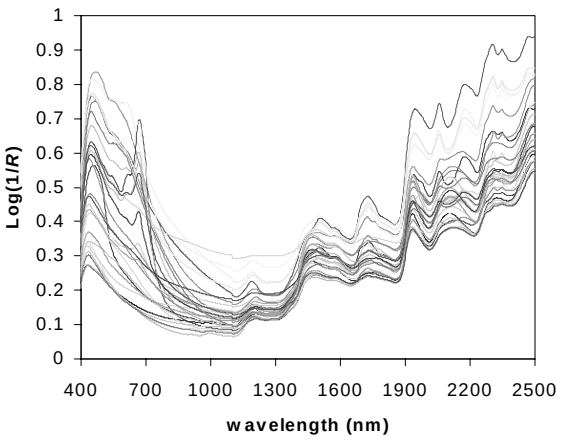

Figure 1. Spectra of 30 diverse ground sample standards.

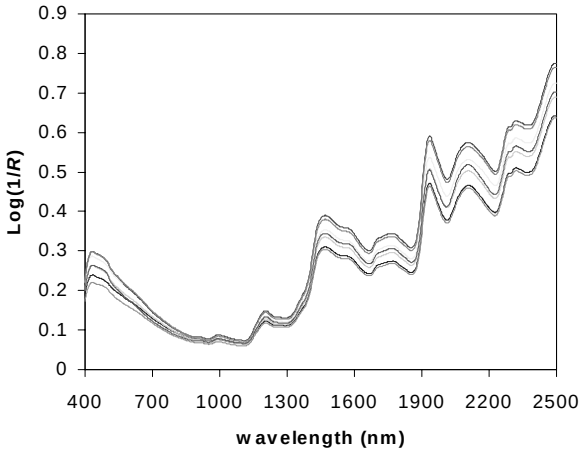

Figure 2. Spectra of seven ground wheat standards.

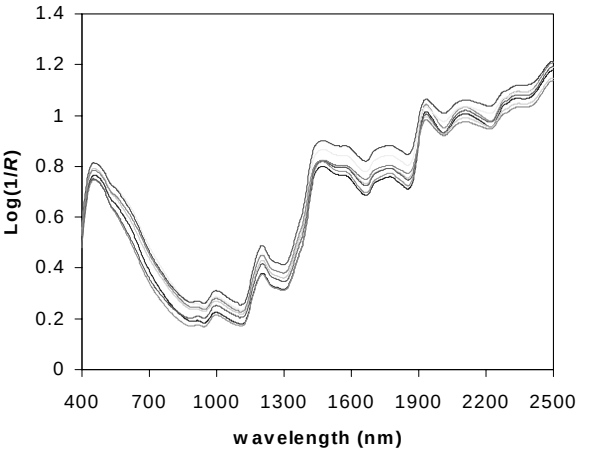

Figure 3. Spectra ofo seven whole wheat standards.

Table 2. Differences between instruments before standardisation measured using six ground standards expressed as *RMS(C)*.

No.	1	2	3	4	6	7	8	9	10
2	230	—	—	—	—	—	—	—	—
3	989	912	—	—	—	—	—	—	—
4	470	352	784	—	—	—	—	—	—
6	647	726	1418	857	—	—	—	—	—
7	375	394	376	508	236	—	—	—	—
8	264	287	883	346	663	356	—	—	—
9	427	433	1104	644	843	372	499	—	—
10	1112	1033	1188	901	1228	1098	1086	1282	—
11	404	353	432	409	222	388	412	446	766

The calibration statistics for the equations used for the ANOVA are given in Table 7 and the results of the standardisation expressed as *F*-statistics calculated from ANOVA are given in Table 8. Tables 9 and 10 and Figure 4 present the results of transfer of a calibration to predict wheat protein content from the Reference to Local Instrument 3. Table 11 shows the nominal wavelengths recorded from the internal polystyrene standard of each instrument.

Discussion

30 Sample Standardisation

The first experiment was to standardise the instruments in the network using the 30 Sample Standardisation procedure based on a set of 30 diverse ground materials.[7] The wide range of photometric response for these sealed samples is shown in Figure 1. The criterion for a successful standardisation is

Table 3. Differences between instruments before standardisation measured using six whole grain standards expressed as *RMS(C)*.

No.	1	2	3	4	5	8
2	368	—	—	—	—	—
3	1166	1010	—	—	—	—
4	552	423	1022	—	—	—
5	598	637	1462	577	—	—
8	481	415	846	454	696	—
10	2278	2182	2398	2066	2302	2327

Table 4. Results of ground sample 30 Sample Standardisation expressed as *RMS(C)*.

No.	1	2	3	4	6	7	8	9	10
2	90	—	—	—	—	—	—	—	—
3	47	89	—	—	—	—	—	—	—
4	83	96	72	—	—	—	—	—	—
6	88	104	99	96	—	—	—	—	—
7	104	98	108	121	83	—	—	—	—
8	56	68	54	81	99	98	—	—	—
9	48	82	53	82	73	80	53	—	—
10	159	203	161	137	159	217	180	175	—
11	125	123	130	125	86	90	118	103	208

Table 5. Results of sample Single Sample Standardisation expressed as *RMS(C)*.

No.	1	2	3	4	6	7	8	9	10
2	45	—	—	—	—	—	—	—	—
3	42	47	—	—	—	—	—	—	—
4	62	57	63	—	—	—	—	—	—
6	71	56	76	50	—	—	—	—	—
7	53	45	59	57	50	—	—	—	—
8	47	44	48	66	74	56	—	—	—
9	49	48	49	58	65	48	47	—	—
10	176	166	183	146	136	168	186	185	—
11	104	93	110	72	58	87	111	104	100

Table 6. Results of whole grain sample Single Sample Standardisation expressed as RMS(C).

No.	1	2	3	4	5	8
2	173	—	—	—	—	—
3	143	160	—	—	—	—
4	205	271	232	—	—	—
5	224	159	254	312	—	—
8	211	167	175	336	215	—
10	434	455	461	389	455	468

Table 7. Statistics for calibrations used in ANOVA.

	Ground wheat	Whole wheat
N	32	327
PLS factors	3	10
Range (%)	7.4–17.1	7.5–16.5
SECV (%)	0.20	0.20
R^2	0.996	0.991

Table 8. Results of 30 Sample and Single Sample Standardisation expressed as ANOVA on protein content.

Description	*F*	Critical *F*
Ground Wheat Unstandardised	34.43	2.026
Ground Wheat Clone 30 Standardisation	16.47	2.096
Ground Wheat Clone 1 Standardisation	0.39	2.026
Whole Wheat Unstandardised	185.757	2.290
Whole Wheat Clone 1 Standardisation	1.213	2.290

Table 9. Test of whole grain standardisation with 34 independent whole wheat samples (results expressed as ANOVA on protein content.

Description	*F*	Critical *F*
Repack on Reference Instrument	0.798	4.139
Reference to Unstandardised Local	182.900	4.139
Reference to Standardised Local	0.073	4.139

Table 10. Results of protein prediction following calibration transfer from Reference to Local instrument.

	Protein
Laboratory Mean	11.15
Laboratory *SD* (%)	2.51
Predicted Mean (%)	10.98
Predicted *SD*	2.49
SEP	0.28
Bias	0.17
Slope	1.002
r^2	0.992
Number of Samples	34
Av. Global *H*	2.22
Av. Neigh. *H*	1.23

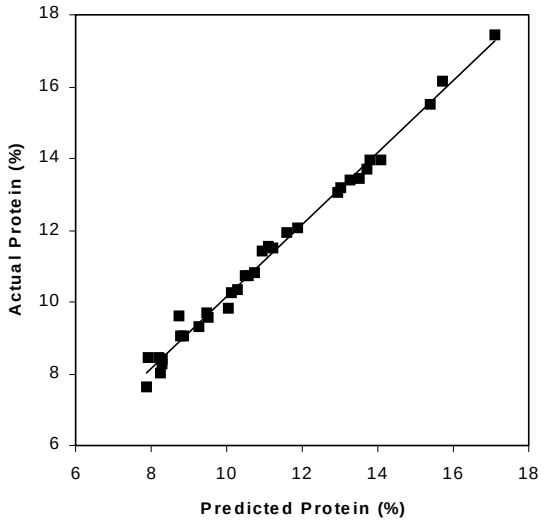

Figure 4. Whole wheat samples scanned on local instrument predicted with calibration developed on Reference Instrument.

for the average *RMS(C)* for the six test samples between each pair of instruments to be equal to or less than that between re-packs on the same instrument.[10] The *RMS(C)* estimated from the replicate scans was

Table 11. Wavelengths of polystyrene standards.

	Wavelength (nm)			
Nominal	1143.63	1681.27	2166.40	2305.93
1	1143.71	1681.02	2166.51	2305.96
2	1143.84	1680.86	2166.66	2305.97
3	1143.59	1681.15	2166.55	2305.84
4	1143.76	1681.02	2166.49	2305.97
5/6	1143.82	1681.01	2166.60	2306.00
7	1143.80	1680.99	2166.57	2305.91
8/9	1143.77	1680.96	2166.56	2305.91
l0A	1143.71	1681.05	2166.46	2305.99
l0B	1143.83	1680.86	2166.58	2305.97
11	1143.76	1680.64	2166.53	2305.85

93 micro d(log1/*R*) / dλ units while the value obtained from re-pack measurements on 15 ground wheat samples was 125 micro d(log1/*R*) / dλ units. The results in Table 4 calculated using the average of measurements over three days on both Reference and Local Instruments show that the *RMS(C)* between pairs of instruments after standardisation was generally close to or better than the value for the re-pack *RMS(C)* and in some cases equal to or better than the replicate scan *RMS(C)* for the sealed standards. Instruments 10 and 11 consistently displayed higher *RMS(C)* values than the other nine instruments.

However, the ANOVA result on the 30 Sample Standardisation (Table 8) shows that while the *F* value is considerably improved compared with the unstandardised result, there were still significant ($P < 0.05$) differences between instruments. This is probably because the 30 Sample Standardisation was designed to achieve the best overall match across a wide range of photometric response.

Single Sample Standardisation on ground wheat

The Single Sample Standardisation method was examined using seven samples of ground wheat.

Their range of photometric responses (Figure 2) was much narrower than for the 30 diverse samples. The *RMS(C)* estimated from the replicate scans for the sealed samples was 80 micro d(log1/*R*) / dλ units while the value obtained from re-pack measurements on 15 ground wheat samples was 125 micro d(log1/*R*)/dλ units. The differences between instruments before standardisation (Table 2) show that some pairs of instruments (for example, 1 and 2; 6 and 11) were better matched than others. The results in Table 5 show that the *RMS(C)* values, following standardisation, were generally much lower than for the 30 sample standardisation and, for any combination of instruments 1 to 9, were within the value for replicate scans of the sealed standards. This means that the spectra of the six sealed ground wheat samples measured on any two of these instruments after standardisation are as alike as the spectra of the standards when measured three times on the same instrument. The *RMS(C)* for instrument 11 was less than the re-pack *RMS(C)*. Comparison of Tables 2 and 5 shows the considerable improvement in optical matching of the instruments achieved by standardisation. Since the results for instrument 10 once again appeared anomalous, the single sample standardisation was repeated following a routine service of the instrument. However, the two sets of results were not significantly different. Furthermore, the ANOVA results (Table 8) confirm that the instruments, including instrument 10, were not significantly different ($P < 0.05$) after standardisation. Therefore, although the spectral response for instrument 10 is the furthest from that of any other, it is still within acceptable limits. Examination of the *RMS(C)* data for instrument 10 before standardisation (Table 2) reveals that it was considerably different to all of the other instruments, perhaps because it is by far the oldest instrument in the experiment. This would suggest that there is a limit to the differences between instruments beyond which the Shenk–Westerhaus standardisation might not be successful.

Single Sample Standardisation on whole wheat

The seven samples of whole wheat had a range of photometric responses (Figure 3) which were well outside the range for the 30 diverse ground samples. Therefore, the 30 Sample Standardisation method is not applicable to this set. The *RMS(C)* estimated from the replicate scans for the sealed samples was 100 micro d(log1/*R*) / dλ units while the value obtained from re-pack measurements, using the set of 34 unsealed samples, was 265 micro d(log1/*R*) / dλ units. The differences between instruments before standardisation (Table 3) again show that some pairs of instruments were better matched than others but no pairs meet the criterion of 265 micro d(log1/*R*) / dλ units without standardisation. Examination of the results given in Table 6 show that, once again, with the exception of instrument 10, the *RMS(C)* values between pairs of instruments were within the re-pack *RMS(C)*. Comparison of Tables 3 and 6 shows the considerable improvement in optical matching of the instruments achieved by standardisation. The ANOVA results (Table 8) confirm that the instruments, including instrument 10, were not significantly different ($P < 0.05$) after standardisation.

Instrument standardisation using the Shenk–Westerhaus method has not previously been attempted for whole grain samples. Therefore, an additional validation of the effectiveness of the standardisation, based on one pair of instruments (1 and 3) selected at random during the course of the experiment, was carried out. Whilst the results from the sealed samples provides a reasonable measure of the effectiveness of the standardisation procedure, the true test was to scan an independent set of samples on two instruments which are standardised and evaluate the performance. The set of 34 grain samples, selected from a population obtained from grain receival stations across Australia, was used for this purpose. These samples were scanned twice (to evaluate the effect of repacking) on the Reference Instrument and then sent by overnight courier for scanning on Instrument 3. The spectra were analysed using the whole grain calibration. The ANOVA results are presented in Table 9. Clearly, there is no significant difference ($P < 0.05$) between the repacks on the Reference Instrument. Similarly, there are considerable differences between the Reference and the unstandardised Local Instrument but they are no longer significant following standardisation. Table 10 and Figure 4 show the result of using a calibration de-

rived for the Reference Instrument to predict the protein content of the 34 samples scanned on the Local Instrument. The *SEP* for protein was acceptable for whole wheat when compared with values reported in the literature[13] and both the global and neighbourhood *H* values[2,3] were within three standard deviations of the global mean, i.e the two sets of spectra measured on the standardised instruments belong to the same population. Although only one pair of instruments has been assessed using this independent test set and it transpired that these were the most closely matched after standardisation, the results demonstrate that it is possible to develop a calibration on one instrument and use it on another in the network.

An important consideration when using a Single Sample Standardisation is the accuracy of the wavelength axis, as this is not corrected by the standardisation procedure. The assumption is that the polystyrene standard in each instrument is identical, and therefore the wavelength (λ) of each instrument can be accurately determined and adjusted using the tilt angle of the grating (θ) as $\lambda = k \sin(\theta + \phi)$ for instrument-specific constants k and ϕ. The recorded polystyrene wavelengths for each instrument used in these experiments is shown in Table 11. If the assumption about the uniformity of the polystyrene standard is true, then Table 11 clearly shows that there is no need to correct for wavelength accuracy by a standardisation procedure. Since the 30 Sample Standardisation corrects for wavelengths differences but the Single Sample Standardisation does not, a significant difference in wavelengths between instruments would result in a better standardisation using the 30 sample method. Since the data presented in Tables 4 and 5 fall well within the instrument manufacturer's wavelength accuracy specification of ± 0.5 nm, it may be concluded that the internal polystyrene standards performed a sufficiently adequate wavelength standardisation.

Conclusions

Eleven Australian monochromator instruments have been standardised so that a calibration for the protein content of whole wheat derived on one instrument may be used without adjustment on another. However, it should be realised that the single sample method is specific to one product and, therefore, a different standardisation is required for each product to be analysed. The 30-sample method has the advantage that it gives rise to a global standardisation which can be used with any ground product. This method does reduce the inter-instrument variance considerably. However, in these studies, it did not perform as well as the Single Sample Standardisation. The results indicate that the wavelength accuracy of the instruments employed in these experiments was not a significant factor during standardisation.

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