Standardisation of near infrared spectra across miniature photodiode array-based spectrometers in the near infrared assessment of citrus soluble solids content

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Introduction

Low-cost silicon photodiode array- (PDA) based near infrared (NIR) spectrometers have found application in the sorting of intact fruit by sugar content. However, while calibration transfer has been reported for relatively dry samples (< 10% water), little work has been published concerning PDA-based instruments using high water content samples (> 70%). PDA-based NIR spectrometers can vary in wavelength calibration and photodetector efficiency. Pixel related photo-detector output can be interpolated to yield a common wavelength scale across instruments. Correction of differences in photometric response between instruments is more difficult, an effect of differences in the signal-to-noise ratio between units associated with output at a given wavelength. Differences in illumination geometries associated with sample orientation relative to light source and detector also contribute to differences in the recorded absorbance spectra of a given sample from two instruments. To accommodate these differences, the absorbance spectra obtained on the slave instrument can be modified to appear as if originating from another instrument. The standard sample(s) used in such an exercise must be similar to the samples on which the predictions are to be used.[1]

A range of chemometric techniques have been applied to calibration transfer for NIR spectroscopy, although no calibration transfer methodology is recommended to suit all applications. We have previously briefly reviewed a number of these techniques, and applied them to the transfer of calibrations between Zeiss MMS1 PDA spectrometers used in the application of non-invasive assessment of SSC (soluble solids content) of intact melon fruit.[2] The techniques were assessed in terms of root mean squared error of prediction (*RMSEP*) (using Fearn's significance testing). Greensill and Walsh[2] concluded that a modified WT method performed significantly better than all other standardisation methods and on a par with model updating.

The following methodologies, involving collection of spectra from a set of 'standards' on both master and slave unit were applied in the previous study:[2] (1) slope and bias correction (SBC), (2) direct standardisation (DS),[3] (3) piecewise direct standardisation (PDS),[4] (4) double window PDS[5] (DWPDS), (5) orthogonal signal correction (OSC),[6,7] (6) wavelet transform-based standardisation

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technique (WT)[8] and (7) a photometric response correction and wavelength interpolative method and (8) a simple method involving wavelength selection. For cases where spectra of the same samples can not be collected on both mater and slave instruments, two methodologies were trialed by Greensill and Walsh:[2] (1) finite impulse response (FIR) and (2) model updating, using the Kennard and Stone[9] algorithm for selection of melon fruit spectra for model updating.

In the current study we trial the same techniques for the application of calibration transfer between PDA spectrometers used in the application of non-invasive assessment of SSC of intact mandarin fruit. In this application a *RMSEP* of < 1% SSC is required.

Experimental method

Standardisation

The performance of standardised calibrations, generated against SSC of mandarin (n = 100, 'Imperial' cultivar from Munduberra, Queensland) fruit tissue, was assessed. Spectra were collected using two MMS1 spectrometers with consecutive serial numbers from two production batches [designated 729, 730 (batch #1), and 845, 846 (batch #2)] giving four spectrometers in total. All samples were allowed to equilibrate to room temperature (27°C) overnight before spectral measurements were made. Wet chemistry was performed on the juice extracted from mandarin halves from each fruit using a commercial citrus juicer to extract juice and a Bellingham–Stanley RMF320 refractometer (~ 0.1% SSC accuracy) to determine associated SSC values. The mean and standard deviation of the SSC value was 9.80 and 0.45, respectively.

Single scans of 30 ms integration time were taken for each spectrum. A maximum count level > 10000 was maintained to minimise any variation in performance due to changing signal-to-noise ratio (SNR) of each system.[10] Spectral absorbance data (using a spectral window 730 to 930 nm) were pre-treated by mean centring. Partial least squares (PLS) multivariate linear regression calibrations were generated against mesocarp SSC using Matlab v5.3 (The Mathworks, Inc., USA) and PLS Toolbox, v. 2.0 (Eigenvector Research, Inc., USA). Calibration performance was recorded for the master instruments in terms of root mean square error of calibration (*RMSEC*), root mean square error of cross-validation [*RMSECV* using leave-one out (LOO) cross-validation segment selection] and standard deviation (STDev) of SSC. Calibration performance in terms of prediction on standardised slave spectra was recorder in terms of *RMSEP*.

The primary assessment for performance of calibrations was made on the significance of the variation in the *RMSEP* following the approach of Fearn[11] ($\alpha = 0.05$ and assuming bias negligible) (see also Snedecor and Cochran[12]). For each comparison of two calibrations, the R^2 of the correlation between residuals (predicted–actual SSC) and the 95% confidence limits on *RMSEP* are reported (Table 1). Since this assessment is always made in pairs, the standardisation technique achieving the best result in the respective data set was assessed against its two nearest neighbours (closest *RMSEP*s) (Table 1).

Algorithms to test each standardisation technique were implemented using Matlab v. 5.3 scripting (The Mathworks, Inc., USA) and the parameters relevant to each technique were incremented to achieve optimisation. Scripts assessing DS, PDS, DWPDS and FIR standardisation techniques used algorithms available in PLS_Toolbox software (Eigenvector Research, Inc., USA) for these standardisation assessments. A new OSC algorithm[7] was used for the OSC technique assessment. Assessment of the wavelet transform technique (WT) was based on a method proposed by Walczak,[8] but differed by the use of DS on the wavelet coefficients instead of directly univariately and linearly regressing one on the other. Wavelet coefficients from the first level decomposition were used in the DS association.

In all cases, except FIR which did not require this parameter, the number of samples used in the standardisation was varied between 3 and 25 to allow an optimum number to be determined. These were selected using the Kennard–Stone algorithm available in the PLS_Toolbox v. 2.0. Window size

Table 1. Significance testing of the results of a citrus population. A comparison of the technique with the lowset *RMSEP* against two nearest neighbours using Fearn's criteria to determine upper and lower significance limits of the *RMSEP* value (refer to Table 2).

Data Set	Method	RMSEP	R^2	Significant
729–730	WT	0.23		
	DS	0.22	0.73	N
	PDS	0.41	0.20	Y
	WT (50)	0.21		
	MU	0.22	0.59	N
729–845	WT	0.26		
	DS	0.2811	0.60	N
	PDS	0.48	0.28	Y
	WT (50)	0.21		
	MU	0.21	0.66	N
729–846	WT	0.28		
	DS	0.37	0.60	
	PDS	0.55	0.27	Y
	WT (50)	0.31		
	MU	0.30	0.75	N
730–845	WT	0.22		
	DS	0.27	0.61	Y
	PDS	0.60	0.1332	Y
	WT (50)	0.21		
	MU	0.24	0.36	N
730–846	WT	0.27		
	DS	0.43	0.36	Y
	PDS	0.41	0.35	Y
	WT (50)	0.33		
	MU	0.29	0.53	N
845–846	WT	0.30		
	DS	0.36	0.47	Y
	PDS	0.38	0.43	Y
	WT (50)	0.33		
	MU	0.26	0.73	Y

for PDS and DWPDS was varied between 3 and 21 (increments of 2). The window size for FIR was ranged from 3 to 41 in increments of 4. The number of OSC components was varied from 1 to 5.

Wavelength range varies slightly among instruments due to small variations in the optical alignment of components on the central glass block. Interpolation to a common wavelength scale was achieved using a cubic spline interpolation technique.

Although the photometric response of these instruments is similar, due to this company's rigorous photodiode selection criteria, differences between instruments with long periods between manufacture dates was observed. The photometric response (mean absorbance spectrum of standardistion sample set) of slave and master was ratioed. A comparison of this transfer technique is made against other proposed transfer techniques.

A technique generally used for updating calibration models with new spectra considered to encompass new variables (for example, new cultivars or growing districts) was used to adapt to new instrumental variables. To assess the capabilities of model updating (MU), increasing numbers of Kennard–Stone selected samples were added to the master data and new models generated. The new model was tested on the original slave data set.

All data sets were subjected to the same data pretreatments (mean centring) and predictive modelling (PLS) with the relevant parameters for both predictive model generation (principal components) and standardisation method implementation (number of samples and/or window size) optimised for each. Calibrations generated used equivalent data preteatment methods which were not optimised for any individual set. Therefore, *RMSECV* and *RMSEP* should not be assessed in an individual context. A 'working' calibration would also require attention to the optimisation of data pretreatment techniques.

Results and discussion

The photometric response of the four spectrometers differed in absolute terms (maximum count) and spectrally (wavelength sensitivity), as illustrated by spectra of a white reference (Figure 1). Spectrometers '729' and '730' were purchased together and since the respective serial numbers (also used as the spectrometer identifiers) are sequential it is assumed that they originate from the same production batch. Spectrometers '845' and '846' were purchased at a later date. While output of all spectrometers varied, the most obvious variation occurred between the photometric response of the 700 series spectrometers, relative to the 800 series (30% higher). The obvious output differences between the two

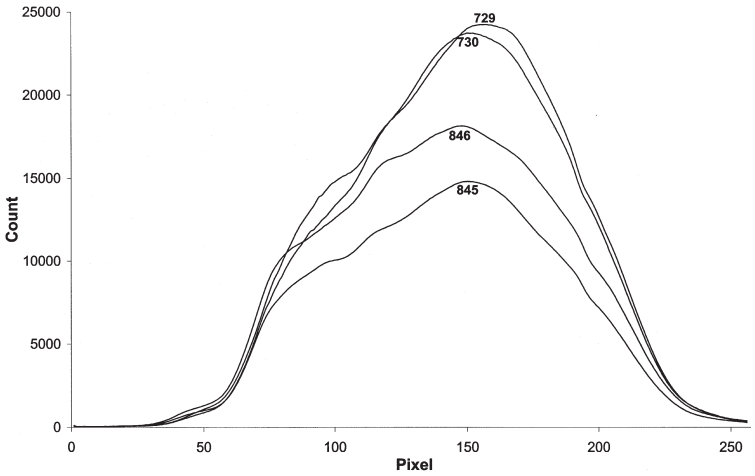

Figure 1. Spectra of the a white reference (teflon tile) acquired with each of four spectrometers used. All operating parameters for the spectrometers (for examples integration time and number of scans) remained constant for the duration of this study. The abscissa is graduated in pixels to highlight the difference in wavelength/pixel allocation between spectrometers as well as difference in photometric response.

Table 2. Performance of calibration transfer process reported in terms *RMSEP* for the prediction of melon SSC using spectra collected on a slave spectrometer and a calibration generated on spectra of the same fruit, collected on a master instrument. Results in bold highlight the standardisation technique with the lowest *RMSEP*. Spectra from the slave (second listed) instrument were transformed to appear as though originating from the master (first listed) instrument spectra. 729, 730, 845, 846 are spectrometer identifiers. The population statistics were = 9.80, *n* = 100 and σ = 0.45.

Spectrometers	729_730	729_845	729_846	730_845	730_846	845_846
RMSEC	0.24	—	—	0.19	—	0.23
RMSECV	0.28	—	—	0.25	—	0.286
Unstandardised	0.50	7.03	8.10	2.51	2.55	0.71
WS	0.65	6.49	7.84	2.68	3.47	0.96
Int + Mod.	4.91	15.11	19.77	11.56	13.21	7.66
DS	0.22	0.28	0.370	0.27	0.43	0.36
PDS	0.41	0.48	0.55	0.60	0.41	0.38
DWPDS	0.40	1.45	0.53	0.46	0.49	0.48
OSC	0.43	0.44	0.47	0.46	0.52	0.55
FIR	0.45	0.45	0.45	0.45	0.45	0.45
WT	0.239	**0.26**	**0.28**	**0.22**	**0.27**	**0.28**
Slope and Bias	0.36	0.80	1.11	3.47	9.913	0.63
WDS (50)	**0.21**	**0.21**	0.31	**0.21**	0.33	0.33
MU(50)	0.22	0.21	**0.30**	0.24	**0.29**	**0.26**

pairs of spectrometers is consistent with the suggestion that these pairs came from different production runs, although the manufacturer (Carl Zeiss GmbH) selects photodetectors (Hamamatsu Q4874) on uniformity to minimise this type of variation. These differences were expected to impact heavily on transferability of calibrations.

Calibrations can be developed using either pixel number or wavelength as the dependent variable. If the pixel number is used, then the standardisation technique must be capable of any misalignment of this variable between spectrometers. Alignment of spectral data from all instruments to a common wavelength scale should overcome this problem, dependent on original wavelength accuracy of the individual instrument. Only the difference in photometric response would remain.

The performance of seven standardisation techniques (SBC, DS, PDS, DWPDS, OSC, FIR, WT), and a wavelength interpolation method with photometric correction were compared using the respective *RMSEP*s (Tables 2). Since WT proved the most successful method in five out of six cases and second most successful in the remaining case, it was compared separately to MU (data presented in the same table) using separately constructed data sets. The sets to be transferred were divided in half, one half used in increasing numbers in the updating process and tested on the unused half (validation set). Of the MU and WT comparison, both performed equally (three of six cases each) , although only one (MU) each proved to be significant, using Fearn's technique. A simple ranking procedure indicated that the relative performance of the techniques to be (best to worst): WT, MU, DS, DWPDS, PDS, FIR, OSC, SBC and Int and Mod.

Of the established standardisation methods, direct standardisation of the wavelet coefficients of the first level decomposition (WT) was demonstrated to be the most efficient for the standardisation of a calibration for the non-invasive assessment of SSC in fresh mandarin fruit samples when used to standardise between MMS1 spectrometers. However, predictive model updating, incorporating 'Kennard–Stone' selected representative spectra of the slave spectrometer, has also been shown to be capable of achieving equally good or better results (in terms of lowest *RMSEP*) with significantly better results in one case. This conclusion is in agreement with our earlier report for calibration transfer for the same instrument for melon spectra.[2]

Model updating has an added advantage over most standardisation techniques of not requiring the measurement of standardisation samples on both spectrometers and allowing the predictive model to evolve to one containing only slave spectra over time. The disadvantage of this method is that a separate model is required for each instrument.

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