Development and use of an "at-line" near infrared instrument to evaluate robustness of melon brix calibrations

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Introduction

Melon-eating quality is indexed by total soluble solids (TSS).[1,2] Other attributes (for example, volatiles and texture) contribute to eating quality, but TSS is often positively correlated with these attributes and high TSS is a prerequisite for good eating quality.[1] Therefore, the ability to grade every fruit for TSS (eating quality), as well as external appearance (shape, size, colour etc.) is desired. As TSS can vary between 4 and 16% w / v and as 80% of TSS is simple sugars (predominately sucrose)[3], a method of measurement of sucrose within intact melons, to a resolution of approximately 1%, is required for a fruit eating quality assurance programme.

Near infrared (NIR) spectroscopy was first applied in a reflectance mode to the measurement of TSS in melons by Dull *et al.*[3] A correlation standard error of prediction (*SEP*) of 1.6% for sliced fruit and 2.2% for intact fruit was reported in this work. Subsequent reports on the use of NIR to assess the TSS of intact fruit show a progressive decrease in the *SEP*, from 2.2%[4] and 1.9%[3] to 0.4%.[5] This improvement reflects change in the instrumentation used and in the optical geometry (light–sample–detector) employed. Systems employing reflectance mode suffer from a background of specular light. However, the optical density of melon fruit makes transmission mode difficult to employ. Aoki *et al.*[5] employed a multiple lamp system, with lamps mounted at 90° to the detector, with respect to the centre of the fruit. NIR technology is now in commercial use in Japan for melon sorting (e.g. Fantech, Mitsui), with a reported *SEP* of 0.5%.

The studies mentioned above report the development of a calibration on one population of fruit only and it is not clear if this calibration is variety–locality–season specific, or is robust across such variations. In a previous study, we[6] employed an NIRSystems 6500 (Silver Springs, MD, USA) reflectance mode spectrometer to consider the robustness of calibrations across melon varieties, growing seasons and growing locations. A combined calibration was useful (i.e. *SEP* below 1% TSS) across time–locality and across several, but not all varieties.

In the present study, we document the selection and optimisation of a spectrometer system suitable, in terms of cost and speed, for the grading of fruit in an at-line setting, with a view to incorporating these results into an in-line setting and report on the robustness of calibrations across varieties, time and locality.

Materials and methods

Cost-effective application of this technology to fruit sorting requires optimisation of (i) instrumentation, (ii) the optical interface between sample and detector and (iii) the calibration population and data treatment.

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Detector attributes

An optical table-based spectrometer was constructed using a Hammamatsu photodiode array, in order to consider the effect of wavelength resolution on calibration. To change resolution, the slit width of the system was altered, with a corresponding change in intensity of illumination of sample to maintain a constant amount of light reaching the detector. The spectrum of an Hg–Ar lamp (Ocean Optics, Dunedin, FL, USA) was used in the characterisation of resolution. In a parallel experiment, the effect of detector signal-to-noise on calibration performance was considered. In this exercise, the signal-to-noise of a Zeiss MMS1 spectrometer unit (Jena, Germany) was altered by changing signal strength or the number of spectra averaged.

In both experiments, a bifurcated fibre optic interactance probe, consisting of eight 400 µm illuminating fibres concentrically arranged about a single 400 µm read fibre (Ocean Optics), was used in conjunction with a tungsten halogen lamp (Ocean Optics, Eerbeek, The Netherlands) to gather spectra of sucrose solution soaked cellulose filter papers (0–20% w / v sucrose) for calibration characterisation.

Two low cost (< A$5,000), miniaturised spectrometers were chosen for comparison, based on the use of different detector technologies (charge coupled device and photodiode array in the Ocean Optics S2000 and Zeiss MMS1, respectively). Spectra of sucrose-soaked filter paper were collected using the two instruments.

Optimisation of an optical configuration for melon calibration.

Melons were obtained from commercial farms (varieties Dubloon, Eastern Star, Hammersley, Highline and Malibu), with spectral collection, juice extraction and TSS determination made on the same day and not more than five days after harvest. A series of trials were undertaken in terms of calibration performance for a range of angles between the incident light on the fruit surface and the area of fruit detected by the spectrometer, with reference to the centre of the fruit. The protocol of sampling for wet chemistry was also considered with respect to calibration performance. Light distribution within a melon fruit was assessed by sequentially cutting the fruit on the axis perpendicular to the lamp–fruit centre and measuring light output over 1 cm^2 areas of the fruit surface using the MMS1.

Calibration development and robustness

Calibrations were developed using WinISIi software, using first derivative data (derivative calculated over four data points), without smoothing or scatter correction. Outlier spectra were removed from calibration population sets using the 3.0 Global H criterion of WinISI software.

Results and discussion

Wavelength resolution

Wavelength resolution of the MMS1 and S2000 instruments is illustrated by the recorded spectra of an Hg-Ar lamp (Figure 1). The MMSI achieved a 13 nm FWHM resolution of the 912 nm peak, while the S2000 achieved a 2 nm

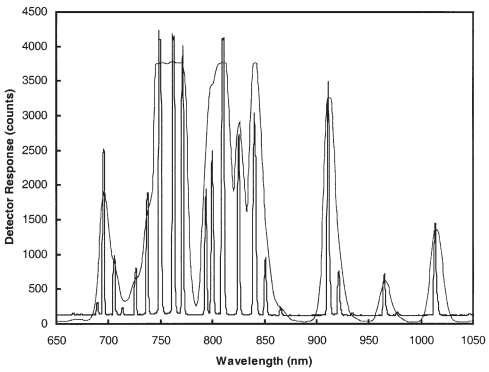

Figure 1. Spectra of a mercury argon lamp acquired with the Zeiss MMS1 (dotted line) and the Ocean Optics S2000 (solid line) spectrometers. Inset illustrates the resolution of the 912 nm peak by the two devices (with detector response normalised to output at this wavelength).

Table 1. Calibration performance of (sucrose-soaked cellulose) in terms of standard error of cross-validation with respect to spectrometer wavelength resolution and signal-to-noise ratio. Means followed by the same letter within the two experiments are not significantly different at a 95% confidence level. The calibration population consisted of 16 sucrose-soaked (0–20% w / w) sets of filter paper (50 sheets thick).

FWHM (912 nm)	Maximum count	Spectra averaged	S/N	*SECV* (°Brix)
7.7	30,300	1		1.04 a
10.6	30,300	1		0.97 a
13.8	30,300	1		0.93 a
16.7	30,300	1		0.93 a
20.0	30,300	1		0.98 a
13.2	2,000	1	1400	2.02 a
13.2	8,000	1	4600	1.29 b
13.2	30,300	1	9700	1.22 b
13.2	30,300	2	15900	1.29 b
13.2	30,300	16	30300	1.46 b

resolution. Given that the second and third overtone bands assessed using NIR are typically broad spectral features, *c.* 50 nm, a resolution of less than 20 nm should not be necessary. This view is reinforced by the wavelength smoothing option typically employed in chemometric procedures.[6] However, a typical MPLS correlation developed on absorbance data has coefficients which can vary widely between spectral data points. This variability hints at a requirement for better resolution.

In practice, decreasing wavelength resolution (characterised at the 912 Hg–Ar line peak) to 20 nm did not significantly decrease the performance of a calibration of sugar solutions on cellulose (Table 1). We conclude that spectral resolution below 20 nm is not a priority characteristic for instrumentation in this application.

Signal-to-noise ratio

The signal-to-noise ratio of the MMS1 and S2000 instruments was characterised by collecting 200 spectra (raw A/D output, 12 bit A/D) of a teflon tile using the interactance probe and light source and calculating a value for mean / standard error of measurement for every spectral data. Light intensity was first adjusted to achieve a signal close to saturation for the two instruments. The MMS1 demonstrated a relative enhancement in the 750–950 nm spectral region, relative to the S2000 [Figure 2(a)]. The signal-to-standard error ratio broadly paralleled the mean signal for both instruments, reflecting the importance of signal shot noise (square root of number of photons received per pixel). However, the ratio of signal-to-standard error of signal of the MMS1 reached a maximum of 40,000, in contrast to only 1,000 for the S2000 [Figure 2(a)]. This result was expected, insomuch as photodiodes deliver a higher signal-to-noise ratio than CCDs at high signal levels.

A similar exercise was undertaken at a low light level, held constant for the two instruments. As expected for a CCD detector relative to a PDA detector, the recorded count from the S2000 unit was greater than that of the MMS1 detector, although only by a factor of two [Figure 2(b)]. This result re-

flects the wider slit width, greater pixel size and lesser pixel dispersion of the Zeiss MMS1 unit. The signal to standard error ratio of the MMS1 was again higher than that of the S2000 [achieving a maximum of 7,000 and 150, respectively; Figure 2(b)]. This result is contrary to that expected on the basis of PDA and CCD detector types and, presumably, reflects differences in electronics between the two systems. Indeed, after initial powering up, detector output decreased slightly for the MMS1 (maximum at 750 nm, with 30 counts decrease on a signal of 30,000, or 0.1% change), stabilising after 1.5 h (data not shown). However, the S2000 unit demonstrated greater fluctuations (*c*. 1% change), with continuing fluctuation after 1.5 h (data not shown). Frequent referencing would be required for the latter unit in a fruit sorting application.

Another source of signal noise is variation in lamp intensity or spectral output. After initial powering up, lamp output changed, with a decrease across most of the spectrum (maximum of 200 counts on a signal of 30,000, or 0.67%), but an increase around 833 nm. Lamp output stabilised after *c.* 1.5 h. These changes are ascribed to changes in lamp chemistry during lamp "warm-up". For all other experiments reported in this study, instrument and lamp were allowed to stabilise for at least 2 h before use.

The importance of signal-to-noise ratio on calibration was investigated by undertaking calibration of spectra with a range of signal-to-noise conditions, collected from cellulose soaked with a range of sucrose solutions. Signal-to-noise ratio was varied by changing the signal level by altering the light level and number of scans averaged per spectrum. The *SECV* of the resulting calibration was significantly affected only below a signal-to-standard deviation ratio of 4,600 (Table 1). We conclude that a single scan, with a count level at 25% or greater of saturation, is adequate for the task of sucrose calibration (0–20% w / v on cellulose matrix) using the MMS1.

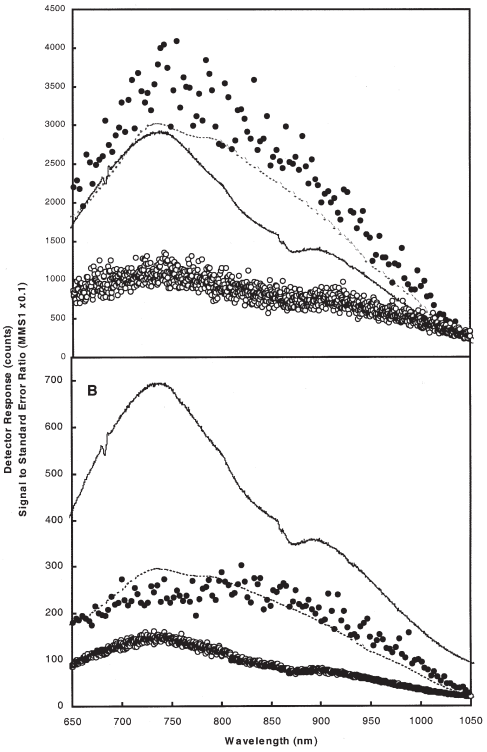

Figure 2. Relative spectral sensitivity (lines) and signal-to-standard error ratio (circles) of spectra collected using the MMS1 (dashed line, solid circle) and S2000 (solid line, open circle) spectrometers. Spectra were acquired using the same integration time (100 ms), light source, fibre optic guides and sample (reference material) for the two devices. Mean signal and mean signal divided by standard error of measurement at each wavelength (*n* = 50) are displayed. Note the scale change for the Zeiss MMS1 signal-to-noise ratio. (a) Light intensity was adjusted so that the output of each detector was near saturation and normalised to output at 720 nm. (b) Spectra were acquired on both instruments at the same, relatively low, light intensity.

On the basis of signal-to-noise ratio it is expected that the S2000 spectrometer would support a poorer calibration than the MMS1. Indeed, the *SECV* of a calibration of cellulose soaked with a range of TSS solutions was three times higher when developed with the S2000, in contrast to the MMS1 (5.4 and 1.8, respectively). The MMS1 was, therefore, adopted for the fruit assessment work reported below.

Optimisation of an optical configuration

Light was diffusely scattered through the melon flesh (mesocarp) but assumed some directionality through the seed cavity of the fruit (Figure 3). The contact angle of the light beam with the fruit surface was essentially irrelevant because of diffuse scattering of light within the fruit, with the detected light level determined by the distance from the illuminated area to the detected area. For convenience, however, lamp and detector were aligned with the centre of the fruit. There is a compromise position between the long path-length of light within the fruit and a high signal. A small angle between detector and lamp allows for a high signal (low noise) but gives a short pathlength in the fruit, with measurement of proportionally more non-edible parts of the fruit, i.e. "skin". A larger angle between detector and lamp provides a longer pathlength, representing more of the edible flesh of the fruit, but results in a low (noisy) signal. However, full transmission mode (lamp-detector angle of 180°) is undesirable on both counts (low signal and measurement of seed cavity attributes as well as flesh attributes). Reasonable signal levels (ie > 25% of detector saturation with a 200 ms integration time) were measured at up to a 60° lamp–fruit–detector angle). With four lamps positioned at 90° increments around the fruit and at an angle of 45° to the detector, a near saturation signal was achieved with an integration time of 200 ms. The choice of this angle was confirmed by the performance of calibrations developed of spectra collected at a range of lamp–fruit–detector calibrations (Table 2).

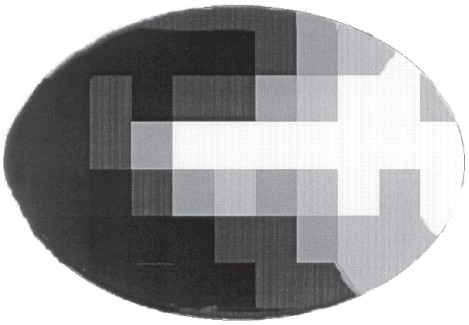

Figure 3. Two dimensional diagrammatic representation (10 mm squares) of light penetration through a rockmelon from an incident light spot (on right side of "fruit"). Data presented as absorbance units, within the following grey scales: (lightest to darkest) 0, 0.61, 1.22, 1.83, 2.44, 3.05 absorbance units.

Sampling and soluble sugar content of fruit

Soluble sugar content was variable within the melon fruit, varying with longitudinal position within the fruit by *c.* 4% TSS, with circumferential position by *c.* 1% TSS and with depth (skin to seed cavity) by *c.* 4% TSS. This observation is consistent with the report of Peiris *et al.*[7] Thus the four lamp

Table 2. Calibration performance (melon-sugar content) with respect to the angle between the illuminated and detected areas of the fruit, with reference to the centre of the fruit. Calibration population *n* = 40, range 7.0–11.9, mean 9.5 °Brix.

Lamp angle (°)	R^2	*SECV*	*SEC*
		(°Brix)	
20	0.19	1.11	0.97
40	0.64	1.24	0.65
60	0.82	0.84	0.43
80	0.38	1.03	0.84

configuration involves detection of light that has passed through volumes of fruit tissue which can be expected to vary in TSS. A variety of sampling procedures were assessed in terms of calibration performance (data not shown). The optimal method involved removal of 0.5 mm cores at a point between

Table 3. Effect of population size reduction using neighbourhood "H" (NH) criterion on calibration performance (melon-sugar content).

NH	Population numbers	*SEP*(*C*) (ºBrix)
0.2	1991	0.85
0.4	1458	0.90
0.6	984	0.82
0.8	647	0.87
1.0	449	0.69
1.2	303	0.77
1.4	232	0.84

Table 4. Performance of a calibration developed on (a) one population (200 spectra) of fruit of variety Dubloon (DubA), (b) five populations (1000 spectra) of fruit of variety Dubloon (DubA-E) and (c) ten populations (2,000 spectra) of five varieties of melon (5var) on the prediction of melon sweetness. Calibration groups for (b) and (c) were selected using a criterion of 1.0 NH. Results marked with an * represent a *SECV* (population data included in the calibration set), while unmarked results represent a true standard error of prediction (*SEP*).

Validation group	*SECV*/*SEP* (ºBrix)		
	Calibration groups		
	DubA	DubA-E	5var.
Dubloon A	0.53*	0.62*	0.72*
Dubloon B	1.33	0.86*	0.93*
Dubloon C	1.28	0.66*	0.75*
Dubloon D	1.17	0.74*	0.79*
Dubloon E	1.13	0.92*	1.03*
Dubloon F	0.93	0.66	0.67
Dubloon A-E	1.42	0.76*	0.75*
Eastern star	1.13	1.11	0.84*
Hammersley	1.13	0.92	1.03*
Highline	0.93	1.16	0.70*
Malibu	0.89	0.66	0.61*

the centre of each of the four illuminated areas and the detected area. Skin and seed cavity material was trimmed from these cores, before pressing to extract juice for the refractometer measurement.

Calibration development androbustness

A calibration developed on a single Dubloon population (n = 200) failed to predict the TSS of other Dubloon populations and of other varieties, as assessed by the standard error of cross-validation (*SECV*) (Table 4). To develop a robust calibration, a data set of five Dubloon populations, varying in time and locality of harvest and an extension of this data set involving a further five populations of four other melon varieties were created. Spectral "redundancy" was reduced by assessing the influence of the WinISI Neighbourhood H criterion on *SECV*. A Neighbourhood H of 1.0 decreased the number of spectra from 1991 to 449 for the five Dubloon and five variety calibration sets, respectively (Table 3).

The calibration developed across five Dubloon populations predicted another Dubloon population and one other variety well (Table 4). However, the performance of this calibration on two further melon varieties was less convincing (Table 4). The calibration developed across ten populations of five varieties performed acceptably across all conditions. These results indicate that a calibration can be relatively robust across varieties, growing region and time.

Acknowledgements

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