VG97109

Further development of NIRS to measure eating quality of melons

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Queensland Department of Primary Industries

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Final Report: Further development of NIRS to measure eating quality of melons

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DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

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SUMMARY

Industry Summary, and Recommendations

A bench top 'at-line' hardware was developed for the assessment of the Brix of melon flesh. This hardware was based on the (Zeiss MMS1) photodiode array spectrometer module (using LabView software code for control and data processing), and using direct illumination of the sample with halogen lamps. This hardware base is common to all known manufacturers of in-line NIRS based fruit Brix grading units (CVS in Australia, Compac in NZ, Mitsui, Fantec and Eminet in Japan).

Calibration work indicated that the technique was sensitive to the variety of melon, and to growing conditions and season. However, the work indicated that a more robust calibration could be developed by including fruit representative of this variation in the calibration set. Such a 'robust' calibration was used to predict the Brix of 'new' fruit (ie. of populations of fruit not included in the calibration set), with a prediction accuracy (standard error of prediction) of approximately 0.7 Brix.

A protocol for updating and checking calibrations was established. This protocol involves assessment of each of two sides of fifty fruit. Spectra are collected and Brix of extracted juice assessed. The predicted Brix is compared to the actual Brix in terms of standard error of prediction (SEP), ratio of SEP to the standard deviation of the population, bias, and correlation coefficient.

In conclusion, the NIRS can be applied to fruit sorting for internal quality attributes, but the technology is in a developmental or 'exploratory-commercial' phase. The technology is applicable to melons, but with less accuracy than achieved with thinner skinned fruits. Application within the Australian horticultural scene would require careful attention to marketing strategy, in order to recoup the high capital and on-going maintenance costs of the NIRS technology, and to the relatively harsh conditions of Australian pack-houses (viz. local specialist support, power supply quality, modem access, temperature control, dust and vibration). The technology must also be presented in a 'user friendly' formal to win acceptance. A commercialisation strategy allowing for this process to occur, while rewarding the original 'investors' (e.g. QFVG), has been initiated.

Technical Summary

A detector platform was chosen and an optical configuration optimised for the non-invasive assessment of the Brix of melon flesh. This hardware was based on the (Zeiss MMS1) photodiode array spectrometer module (using LabView software code for control and data processing), and using direct illumination of the sample with halogen lamps.

The performance characteristics of three commercially available 'miniature' spectrometers based on silicon array detectors operating in the 650 – 1 050 nm spectral region were compared and contrasted with respect to the application of sugar

detection, and criteria established for this application (wavelength resolution, signal to noise of detector, etc.). Calibrations were developed using reflectance spectra of filter paper soaked in range of concentrations (0 – 20 % w/v) of sucrose and of intact fruit, using a modified partial least squares procedure. The MMS1 Si photodiode array spectrometer from Zeiss (Germany) was identified as the spectrometer of choice in terms of cost and calibration performance.

A bench top assembly using the MMS1 for the at-line assessment of melon sugar content is reported in terms of light source and angle between detector and light source, and optimization of math treatment (derivative condition and smoothing function). The recommended optical configuration consisted of four 50W halogen lamps positioned to illuminate the fruit at an angle of 45° relative to the detected region of the fruit and the fruit centre.

This assembly was then used to gather spectra on a range of populations of rockmelons, collected over growing districts and time. The mean Brix of these populations varied from 7.5 to 9.9, highlighting the eating quality issue in this commodity group (a standard of 10 is considered reasonable). Partial least squares calibrations of absorbance data on flesh Brix were developed using the ISI chemoemteric package. Calibrations developed on a single population or small number of populations did not predict well on 'unknown' populations, indicating over-fitting of data. However a calibration developed across a range of varieties, growing districts and times predicted Brix of an unknown populations with a standard error of predict of 0.7 Brix. This result indicates that, with careful selection of the calibration set to represent the range of variation in the population, a single calibration might be developed for all rockmelons.

The approach taken to achieve this robust calibration within this project was onerous, involving a large number of samples. Many of these samples were, however, redundant, being spectrally similar. A recommendation is made to streamline the calibration procedure by identifying those spectrally unique samples using Mahanolobois distance calculations (Neighbourhood distance, NH, in ISI software). This selection step would markedly reduce the number of wet chemistry measurements. A NH criterion of ca. 0.8 is recommended in calibration development.

Publication Schedule

Work was reported in the scientific literature and presented at scientific / technical meetings as follows:

Walsh, K.B., Guthrie, J.A. and Burney, J. (2000) Application of commercially available, low cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. *Australian Journal of Plant Physiology* **00**,000-000 (accepted)

Walsh, K.B., Greensill, C.V. and Guthrie, J.A. (1999) Use of an at-line NIR instrument to evaluate robustness of fruit Brix calibrations. 9th International Conference on Near-Infrared Spectroscopy Verona, Italy. June 13-18.

Walsh, K. and Guthrie, J. (1999) Non-invasive spectroscopy as a tool to measure sink 'status'. International Conference on Assimilate Transport and Partitioning, August 15-20, Newcastle.

Walsh, K.B. and Guthrie, J.A. (1999) Getting it right before they take the first bite – infrared spectroscopy and other developments. Fresh 99, National conference and exhibition for the fresh fruit and vegetable industry. Canberra 2-5 Sept.
Guthrie, J. and Walsh, K. (2000) Calibration robustness with respect to variety and district in the assessment of soluble solids content of melons. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria.

Commercialisation Path and Related Developments

The allied preceding project (FR548) demonstrated the potential for non-invasive assessment of the Brix of melon flesh (mesocarp) using a bench top, laboratory grade instrument (NIRSystems 6500). This work occurred (1995-1997) over a period in which three Japanese companies (Mitsui, Fantec, Maki) adopted similar technology on fruit packlines.

In supporting further work (ie. VG97109), the QFVG melon sub-committee requested that work be undertaken in such a way as to place the melon industry in a position that allowed rapid adoption of NIRS based fruit sorting hardware from any source (ie. any manufacturer), and that the work not be to the benefit of a single manufacturer. The grant proposal for VG97109 therefore indicates that 'all information will be made available through the scientific literature and within the Final Report (to) support the introduction of in-line grading units across the whole melon industry', utilising the hardware platform provided by ' CVS or any other commercial manufacturer'. The rationale here was that published work would stimulate development of appropriate hardware by a range of manufacturers.

In respect of this requirement, all outcomes of the work have been published in the scientific literature and are reported in this Report. However, respecting the cross-fertilisation between the HRDC funding calibration work, and the Australian Research Council Industry Collaborative hardware work with the fruit grading equipment manufacturer, Colour Vision Systems (CVS), a commercialisation strategy has been enacted with CVS to reward the supporters of the HRDC funded work.

NIRS based fruit sorting technology is used in Japan, but only now do these manufacturers feel confident enough in the technology to explore overseas sales. To recoup development costs, pricing is high (ca $250,000 to $500,000 per lane, plus on-going maintenance costs). However, no such sale had occurred by mid 2000. A feature of this project, was therefore to assess the applicability of NIRS technology for the Australian melon industry.

TECHNICAL REPORT

At present grading of internal fruit quality is either subjective or destructive. That is, human / machine vision of the external appearance, or destructive measurements of various attributes of the internal flesh / juice is used to ascertain eating quality of the fruit. In melons the predominate soluble solids are sugars and are the major attribute of eating quality. While a high Brix alone does not fully define melon eating quality, the absence of high Brix makes good quality very unlikely

Bending and/or stretching of certain chemical bonds (C-H, N-H and O-H) causes the absorption of near infra-red radiation. The amount absorbed (or diffusely reflected) at particular wavelengths is proportional to the concentration of the analyte of concern, in this project, total soluble solids (° Brix).

Instrumentation

Work undertaken on the development of 'at-line' instrumentation appropriate to the NIRS assessment has been documented within a manuscript submitted to the Australian Journal of Plant Physiology (Appendix 1). In summary, the abstract of this report is attached below:

The performance characteristics of three commercially available 'miniature' spectrometers based on silicon array detectors operating in the 650 – 1 050 nm spectral region (MMS1 from Zeiss, S2000 from Ocean Optics, and FICS from Oriel, operated with a Larry detector) were compared with respect to the application of non-invasive prediction of sugar content of fruit using near infra-red spectroscopy. The FICS-Larry gave the best wavelength resolution, however, the narrow slit and small pixel size of the CCD detector resulted in a very low sensitivity and this instrumentation was not considered further. Wavelength resolution was poor with the MMS1 relative to the S2000 (eg. FWHM of the 912 nm Hg peak, 13 and 2 nm for the MMS1 and S2000 respectively), but the large pixel height of the array used in the MMS1 gave it a sensitivity comparable to the S2000. The signal to signal standard error ratio of spectra was greater by an order of magnitude with the MMS1, relative to the S2000, at both near saturation and low light levels. Calibrations were developed using reflectance spectra of filter paper soaked in range of concentrations (0 – 20 % w/v) of sucrose, using a modified partial least squares procedure. Calibrations developed with the MMS1 were superior to those developed using the S2000 (eg. coefficient of correlation of 0.90 and 0.62, and standard error of cross-validation of 1.9 and 5.4 %, respectively), indicating the importance of high signal to noise ratio over wavelength resolution to calibration accuracy. The design of a bench top assembly using the MMS1 for the at-line assessment of melon sugar content is reported in terms of light source and angle between detector and light source, and optimisation of math treatment (derivative condition and smoothing function).

Fruit Populations

The 'at-line' unit was used to assess 24 populations of intact rockmelons (each of ca. 200 spectra and related Brix measurements), representing fruit from different locations (three growing districts), of (seven) different varieties, and different harvest times (over two years). A total of 4,661 spectra and Brix assessments were made (Table 1). The melon population mean Brix values, across melon types and varieties, were all less than 10 ° Brix. Thus, no population would have made the US fancy grade (USDA 1968 United States Standards for grades of Cantaloupes F.R.Doc. 61-2272) of 11° Brix and most would have failed to meet even the US number one grade of 9° Brix. As most of the fruit used in the project were packed for market sale we

again conclude that the melon industry has a problem with sugar levels in marketed fruit!

Table 1: Rockmelon Population Statistics.

Date	District	Location	Variety	Spectral Population No.	Range ° Brix	Mean ° Brix	Standard Dev.
15/10/98	North Qld	Clare	Eastern Star	198	5.0-11.5	7.59	1.023
30/11/98	North Qld	Clare	Dublin	356	5.0-10.8	7.65	1.0659
09/12/98	South East Qld	Bundaberg	Eastern Star	300	4.8-11.0	7.98	1.3844
14/12/98	South Qld	Chinchilla	Hammersly	326	4.8-10.7	7.49	1.1716
19/01/99	South Qld	Chinchilla	Dublin	276	5.4-11.0	8.00	1.1127
01/03/99	South Qld	Chinchilla	Dublin	200	5.8-11.0	8.52	1.0618
04/03/99	South Qld	Chinchilla	Dublin				
08/03/99	South Qld	Chinchilla	Dublin	198	5.0-11.5	7.59	1.023
15/03/99	South Qld	Chinchilla	Dublin	172	5.6-11.6	8.38	1.2776
24/03/99	South Qld	Chinchilla	Dublin	170	5.1-12.2	8.57	1.4077
12/04/99	South Qld	Chinchilla	Highline	180	5.8-12.0	8.71	1.1424
21/04/99	South Qld	Chinchilla	Malibu	184	5.1-10.6	7.38	0.9669
06/05/99	North Qld	Gumlu	Dublin	138	5.9-10.3	8.74	0.8686
26/05/99	North Qld	Gumlu	Mission	106	6.2-11.9	9.87	1.2162
27/05/99	North Qld	Gumlu	Eastern Star	72	6.2-10.8	8.92	1.0020
10/06/99	North Qld	Gumlu	Eastern Star	142	6.7-12.1	9.09	1.0892
23/06/99	North Qld	Gumlu	Eastern Star				
24/06/99	North Qld	Gumlu	Eastern Star	160	5.4-12.3	9.3	1.0852
08/07/99	North Qld	Gumlu	Eastern star	180	6.6-11.7	8.87	1.0194
15/07/99	North Qld	Gumlu	Hammersly				
19/07/99	North Qld	Gumlu	Eastern star				
03/08/99	North Qld	Gumlu	Hammersly	160	4.9-12.3	7.63	1.1888
20/08/99	North Qld	Gumlu	El dorado	202	5.3-12.9	9.41	1.6853
15/09/99	North Qld	Gumlu	El dorado				
Total				**2,552**			

Calibration Development

Work undertaken to develop calibrations for melon Brix using the 'at-line' instrumentation was presented at the 8th International Conference on Near Infra-red Spectroscopy held in Essen, Germany, and will be published in the Proceedings of that conference (NIR-99) (Appendix 2). The following report extends that covered in the Conference proceedings.

Of the total 4,661 spectra and Brix assessments made across twenty four populations of fruit, a subset (20%) was randomly chosen from each population and pooled (n = 935, mean Brix 8.71, std. dev. 1.71, range 4.8 – 14.4) to act as a validation set. This data was not used in the development of the calibration. The remaining spectra

(n = 3726, mean Brix 8.67, std. Dev. 1.69, range 4.4 – 15.2) were culled of outliers (using a Mahalanobois distance based statistic from the chemometric package ISI).

The math treatment of data prior to calibration development was optimised by comparing the impact of derivatives, data smoothing and scatter correction (standard normal variance and detrend) (Table 2). The best calibration, in terms of minimum standard error of calibration (SEC) and standard error of cross validation (SECV), and maximum regression coefficient (R^2) and ratio of SECV to the standard deviation of the population (SDR), was obtained with use of a second derivative calculated over four data points, without smoothing and without scatter correction.

Table 2: Optimisation of Math Treatment

Math Treatment	Scatter Correction	SEC	R^2	SECV	SDR
0:0:1	none	0.82	0.75	0.85	1.95
0:0:1	SNV	0.87	0.73	0.88	1.89
0:0:1	SNV and detrend	0.85	0.74	0.86	1.92
0:0:4	none	0.91	0.70	0.91	1.81
1:4:1	none	0.83	0.75	0.84	1.96
1:4:1	SNV	0.85	0.75	0.84	1.96
1:4:1	SNV and detrend	0.86	0.73	0.87	1.90
1:4:4	none	0.88	0.72	0.88	1.88
2:4:1	none	0.82	0.75	0.83	1.99
2:4:1	SNV	0.84	0.74	0.85	1.93
2:4:4	SNV and detrend	0.84	0.74	0.86	1.93
2:4:4	none	0.86	0.72	0.87	1.89

The combined calibration set of 3726 matched spectra and Brix measurements contained much redundant information, as indicated by the 'Neighbourhood Distance' (NH, a Mahalanobois distance measurement, giving a measure of similarity of a spectra to its nearest 'neighbour') routine. Spectra were removed from the calibration set under the criteria of increasing stringency for 'different' spectra (ie. similar spectra removed, Table 3), and partial least squares regression (PLS) calibration performance measured. Calibration performance was not degraded until over one third of the population was removed. In practice, we recommend sample selection by the NH method prior to addition to the calibration set. This strategy should allow for a reduction in the workload of Brix measurements involved in the development of a calibration.

Table 3: Calibration population selection

NH	n	SEP	R^2
0.0	3726	0.93	0.70

0.1	3621	0.92	0.70
0.2	3146	0.95	0.70
0.4	2060	0.94	0.70
0.6	1408	0.97	0.68
0.8	1023	0.99	0.67
1.0	742	1.00	0.66
1.2	577	1.04	0.63
1.4	453	1.23	0.51

The performance of the PLS calibration method was compared to that of multiple linear regression (MLR) and the 'Local' regression method (in which spectra similar to a given sample are selected from a calibration set and a sample specific PLS calibration developed) were compared. All methods were run from the ISI chemometric package. Two forms of Local calibration were used, one (L1) in which the parameters set by Berzaghi et al. (2000) were adopted (300 samples, 40 PLS factors, 10 discarded), and a second (L2) in which the operating parameters were 'optimised' (at 175 samples, 25 PLS factors, 3 discarded). The PLS technique delivered the calibration with the best performance (as assessed using Fearn's criteria for comparisons, Fearn, 1996) (Table 4).

Table 4: Comparison of Regression Techniques. Pairwise comparisons are made, with the technique achieving the superior calibration reported. Nsd – not significantly different.

	SEP	PLS	MLR	L1	L2
PLS	0.93	-	PLS	PLS	PLS
MLR	1.00		-	nsd	MLR
L1	1.01			-	L1
L2	1.25				-

The PLS calibration method is therefore recommended.

Finally, the robustness of the calibration was tested on populations totally unrepresented within the calibration group (Table 5; see also Appendix). A calibration developed on a single population of Dublin performed poorly on other populations (ie SEP> 0.9). A calibration developed across five populations of Dublin fruit predicted an 'unknown' population of Dublin and also Malibu fruit well, but performed poorly on other varieties. A calibration developed across all varieties also performed well on an 'unknown' population of Dublin.

Table 5: Calibration robustness as assessed by comparison of standard error of prediction (SEP) involving fruit groups not involved in the calibration.

Three calibrations are trialed, DubA being based on a single population of Dublin fruit, DubA-E based on five populations of Dublin fruit, and 5var, which was based on the five Dublin populations and a further four populations of other varieties. Population Dublin F was not used in the development of any of the calibrations. Values with an asterisk indicate that those populations were involved in the development of the calibration employed.

Validation group	SECV (° Brix) Calibration groups		
	DubA	DubA-E	5var.
DubloonA	0.53*	0.62*	0.72*
DubloonB	1.33	0.86*	0.93*
DubloonC	1.28	0.66*	0.75*
DubloonD	1.17	0.74*	0.79*
DubloonE	1.13	0.92*	1.03*
DubloonA-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*
DubloonF	0.93	0.66	0.67

We therefore recommend the development of a single calibration for rockmelon, across varieties, growing districts and time, with the criteria for comparison being performance of the calibration on populations of fruit not included in the calibration group.

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Fearn, T., (1996) Chemometric space. *NIR News. Issue 5,* **7**, 5-6.

Guthrie J, Wedding B, Walsh K. (1998). Robustness of NIR calibrations for soluble solids in intact melon and pineapple. *J. of Near Infrared Spectro.* **6**, 259-265.

Walsh KB, Greensill VC, Guthrie JA. (1999). Use of an 'at line' NIR instrument to evaluate robustness of fruit Brix calibrations. *J. of Near Infrared Spectro.* **7**, xxx-xxx.

Technology Transfer

Work was reported in the scientific literature (see also Appendices 1 and 2) and presented at the following scientific / technical meetings:

Walsh, K.B., Guthrie, J.A. and Burney, J. (2000) Application of commercially available, low cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. *Australian Journal of Plant Physiology* **00**,000-000 (accepted) (Appendix 1).

Walsh, K.B., Greensill, C.V. and Guthrie, J.A. (1999) Use of an at-line NIR instrument to evaluate robustness of fruit Brix calibrations. 9th International Conference on Near-Infrared Spectroscopy Verona, Italy. June 13-18 (Appendix 2).

Walsh, K. and Guthrie, J. (1999) Non-invasive spectroscopy as a tool to measure sink 'status'. International Conference on Assimilate Transport and Partitioning, August 15-20, Newcastle.

Walsh, K.B. and Guthrie, J.A. (1999) Getting it right before they take the first bite – infrared spectroscopy and other developments. Fresh 99, National conference and exhibition for the fresh fruit and vegetable industry. Canberra 2-5 Sept.

Guthrie, J. and Walsh, K. (2000) Calibration robustness with respect to variety and district in the assessment of soluble solids content of melons. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria (Appendix 3).

The following publications/reports were produced by the group during a similar timeframe, but were not supported by HRDC-QFVG funding.

Long, R., D. Midmore and K.B.Walsh (2000) Agronomic practice and calibration robustness in the assessment of soluble solids content of melons. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria.

Golic, M. Walsh, K. and Lawson, P. (2000) The effect of concentration and temperature variation on the SWNIR spectra of sugars in fruit. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria.

Greensill, C. and Walsh, K. (2000) Optimisation of instrument precision and wavelength resolution for the performance of NIR calibrations of sucrose in a water-cellulose matrix. *Applied Spectroscopy* **00**, 000-000 (accepted)

Walsh, K.B., C.V.Greensill and G.Brown (2000) A hardware platform for NIRS based, pack-line sorting of melons for soluble solids. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria.

Greensill, C.V. and K.B.Walsh (2000) Calibration transfer between NIR systems with different optical geometries in the NIR assessment of melon soluble solids content. Australian Near Infra Red Spectroscopy Group meeting, April 4-6, Hamilton, Victoria.

Grower groups were advised of project progress through the following presentations and press articles: -

- Presentation to fruit growers at 'Value adding' seminar, Ayr, NQ (July 1997).
- ABC radio interview, 'Country Hour', Ayr, NQ (July 1997).
- Conducted workshop at Stonefruit Growers National Conference, Swan Hill, Vic. (July 1997).
- ABC radio interview, Rockhampton, Qld (September 1997).
- Presentation to Australian Melon Growers National Conference, Bundaberg, Qld, (October, 1997)
- Presentation to QFVG / HRDC, Rocklea Markets, (January 1998).

- Presentation to fruit growers at 'Regional Development' seminar, Bowen, NQ (April 1998).
- Presentation to pineapple QFVG sub-committee and AusIndustry, Rockhampton, Qld, (April, 1998).
- Win TV interview, Rockhampton, Qld (May 1998).
- Good Fruit and Vegetables (August 1998).
- Presentation to QFVG melon sub-committee, Rocklea Markets, (August 1998).
- Presentation to National Citrus Conference, Mundubbera, Qld, (April 1999).
- Presentation to Sweetcorn Workshop, Bathurst, NSW, (May 1999).
- Presentation to QFVG melon sub-committee, Rocklea Markets, August 1999
- Good Fruit and Vegetables (September 1999).
- Fresh 99, National conference and exhibition for the fresh fruit and vegetable industry. Canberra 2-5 Sept., 1999.

Appendix 1.

Application of commercially available, low-cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit (submitted to Australian Journal of Plant Physiology)

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Abstract

The performance characteristics of three commercially available 'miniature' spectrometers based on silicon array detectors operating in the 650 – 1 050 nm spectral region (MMS1 from Zeiss, S2000 from Ocean Optics, and FICS from Oriel, operated with a Larry detector) were compared with respect to the application of non-invasive prediction of sugar content of fruit using near infra-red spectroscopy. The FICS-Larry gave the best wavelength resolution, however, the narrow slit and small pixel size of the CCD detector resulted in a very low sensitivity and this instrumentation was not considered further. Wavelength resolution was poor with the MMS1 relative to the S2000 (e.g. FWHM of the 912 nm Hg peak, 13 and 2 nm for the MMS1 and S2000 respectively), but the large pixel height of the array used in the MMS1 gave it a sensitivity comparable to the S2000. The signal to signal standard error ratio of spectra was greater by an order of magnitude with the MMS1, relative to the S2000, at both near saturation and low light levels. Calibrations were developed using reflectance spectra of filter paper soaked in range of concentrations (0 – 20 % w/v) of sucrose, using a modified partial least squares procedure. Calibrations developed with the MMS1 were superior to those developed using the S2000 (e.g. coefficient of correlation of 0.90 and 0.62, and standard error of cross-validation of 1.9 and 5.4 %, respectively), indicating the importance of high signal to noise ratio over wavelength resolution to calibration accuracy. The design of a bench top assembly using the MMS1 for the at-line assessment of melon sugar content is reported in terms of light source and angle between detector and light source, and optimization of math treatment (derivative condition and smoothing function).

Keywords: charge coupled device, in-line, fruit quality, miniature spectrometer, near infra-red spectroscopy, photodiode array, resolution, sweetness

Introduction

To generalise, in the past half-century breeding and post-harvest physiology programs associated with fruit have focussed on the production issues of quantity, and quality with respect to storage life and visual appearance. The general consumer perceives that eating quality of fruit such as tomato has decreased over this time frame. Agronomic and breeding programs can deliver fruit with improved eating quality, however this goal has not received emphasis because of the difficulty of assessing internal attributes of every item of fruit. Various non-invasive technologies such as nuclear magnetic resonance, chlorophyll fluorescence, acoustics, and near infra-red spectroscopy (NIRS) can be applied to the task of non-invasive assessment of fruit

eating quality attributes. NIRS is the most appropriate technique in terms of speed of assessment and cost.

Near infra-red spectroscopy has been applied to the non-invasive estimation of fruit eating quality. Published reports of such applications have largely involved either the use of research grade near infra-red (NIR) instrumentation, unsuited to packing shed or field use (e.g. Kawano *et al.* 1992; Guthrie and Walsh 1997; instrument value ca. A$100 000) or the use of purpose built spectrometers, unavailable for general application (e.g. Jaenisch *et al.* 1990; Bellon *et al.* 1993; Peiris *et al.* 1997). During the mid to late 1990s, however, several low cost (<A$10 000), miniature (spectrometer size < 500 cm^3), array spectrometers capable of operation up to 1050 nm became commercially available. Osborne *et al.* (1998) reported the use of the Zeiss MMS1 for the prediction of sugar content of kiwifruit, and Bellon *et al.* (1996) reported the use of an Oriel Instaspec 2 to predict sugar content of apples, under laboratory conditions. Mowat and Poole (1997) employed an Ocean Optics S1000 and a laptop PC to discriminate between field populations of kiwifruit. These spectrometer modules are also finding use in a range of other instrumentation of interest to physiologists (e.g. portable spectroradiometers).

The choice of instrumentation for the task of non-invasive assessment of fruit quality is made difficult by a lack of published specification requirements for this task. We have contributed to this field with a consideration of the wavelength resolution and signal to noise requirements of the task (Greensill and Walsh, 1999). In the current manuscript we review the design requirements for this application, compare commercially available spectrometer modules that have been used by different with respect to these criteria, and report on the design of an optical system suited to the assessment of melons.

Infra-red (IR) radiation is strongly absorbed by organic molecules, with the wavelength of absorption characteristic of the molecular bond. Overtones of the fundamental band (IR) frequencies, particularly those arising from R-H stretching modes (O-H, C-H, S-H, N-H, etc.), cause absorbance in the NIR region of the spectrum, although this absorbance is typically 10 – 1 000 times weaker than that of the fundamental band. IR peaks are narrow and diagnostic, and thus instrumentation capable of high wavelength resolution is desirable. In contrast, peaks in the NIR spectra are broad, up to 100 -150 nm wide. However, as radiation sources are readily available to deliver high intensities in the NIR region and as detectors sensitive to this region have a relatively low noise, NIRS lends itself to the quantification of organic constituents.

NIRS has been used in many fields, with most work carried out in the region of 1100 – 2500 nm (PbS detector). However, strong absorbance by water at around 1600 nm has restricted use of the technique to dry materials and to reflectance optics. Hydrated objects are characterised by complicated hydrogen-bonding interactions between water, sugar, protein, etc., which complicate the spectra obtained. The application of short wave NIR (700-1100 nm) is promising because : (1) the bands are ascribed to the third and fourth overtones of O-H and C-H stretching modes and are expected to be separated due to anharmonicity, (2) lower absorbance at these wavelengths allows for transmission optics, and (3) the corresponding instrumentation is low-cost and suited to process control, and portable enough for *in situ* field measurements. The

ability to collect and interpret spectra of hydrated objects using short-wave NIR has blossomed in the past decade, with advances in detector arrays, fibre optics and personal computing power.

Unfortunately, due to the complexity of NIR spectra (band overlaps), relatively sophisticated chemometric procedures (data processing such as derivatives, and data analysis using multiple linear, partial least squares or neural network regression techniques) are required for spectral analysis. Spectral data can easily be over-fitted in the regression analysis. The resultant calibration is useful for the predictions within the populations from which it was developed, but can fail in use on new populations (ie. the calibration is not robust). Instrumentation drift over time can also result in prediction failure, and differences between instrument units can preclude calibration transfer between instruments.

The design of the spectrometer can be rationalised with respect to the application. For example, given the broad character of the absorption peaks in the NIR region, it is possible that spectral resolution may be traded off to increase light sensitivity (i.e. a wider slit, or wider pixels). Pixel dispersion is calculated as the range of wavelengths divided by the number of pixels. Spectral resolution may be determined by pixel dispersion, but is otherwise a function of slit width as well as the quality of the dispersive element (e.g. density of lines on grating). Further, the dispersive element may be chosen with transmissivity characteristic, rather than wavelength resolution, as the primary feature. The type of detector should also be considered with respect to the application requirements. Silicon detectors are sensitive into the NIR up to about 1 100 nm, while indium gallium arsenide (InGaAs) detectors are useful over the 900 to 1 700 nm spectral region. However, silicon detectors are preferred for reasons of cost and signal-to-noise. Photodiode silicon detectors are approximately 100 times less sensitive to light than charge coupled device silicon detectors, but the higher saturation level of the photodiode support a 10 fold higher maximum signal to noise ratio for this detector, relative to CCD detectors (i.e. 10 000 cf. 1 000). Overall, CCDs are preferred for very low light applications, while photodiodes are the better choice for accurate absorbance measurements when higher light levels are available (Oriel 1997). However, signal per detector pixel can be increased by increasing the height of the pixel and slit, by focussing light from a high slit onto the array or by summing columns in a two-dimensional array.

The specification requirement for a spectrometer to support NIRS assessment of fruit in an in-line or field setting includes high signal to noise ratio, relatively high sensitivity (particularly if complete transmission spectroscopy is intended), and tolerance to vibration and dust. In-line application also requires the capacity for rapid spectral acquisition, with assessment of up to 10 pieces of fruit per second. Scanning grating instruments, with light detected by a single detector, are too slow in this respect, and are also vibration sensitive in terms of wavelength calibration. A stationary dispersive element and a fixed detector array can be very robust in terms of wavelength reproducibility, and very rapid in terms of spectra acquisition. Therefore, the typical spectrometer for the in-line sorting of fruit will consist of an entrance slit (with an inverse relationship between spectrometer sensitivity and wavelength resolution), a dispersive element (prism, grating or acousto-optical tunable filter, AOTF), a fixed array detector (linear silicon or indium-gallium-arsenide photodiode array, PDA; or linear or two dimensional charge coupled device array, CCD), and an

analogue to digital conversion device (usually 8 to 16 bit, i.e. a grey scale of 256 to 65536 levels, typically up to the dynamic range – maximum signal / detection limit – of the instrument). A grating is the usual choice for the dispersive element, blazed at a wavelength in the NIR to maximise the efficiency of light transmission in this range, although the spectrometer used by Peiris *et al.* (1997) utilised an AOTF as the dispersive element. The detector is usually either a silicon photodiode array (PDA) (as in the Zeiss MMS1, as used by Osborne *et al.* 1998; and the Oriel Instaspec 2, as used by Bellon *et al.* 1996), or a linear CCD array (as in the Ocean Optics S1000, as used by Mowat and Poole 1997). Bellon *et al.* (1993) described the application of a two dimensional CCD array, comprised of 500 by 582 pixels (pixels 17 by 11 μm). A grating was used to disperse the light such that rows represent spectra, and columns were averaged to increase the signal to noise ratio. The Oriel FICS unit is capable of accepting various detectors, but is optimally used with a 2 500 μm high (PDA) detector. As costs decrease, InGaAs arrays will offer potential, operating over the wavelength range 900 – 1700 nm.

The spectral response, and the stability of this response, of a spectrometer will be affected by the spectral output of the light source, transmission and reflection characteristics of the optical path within the spectrometer (e.g. entrance slit width, grating groove density), the stability of the mounting of the optical components (with respect to vibration and thermal expansion coefficients), the spectral response of the detector, and the stability of the electronics. The effect of trade-offs between wavelength resolution and decreased light levels at the detector (e.g. narrower entrance slit), and between signal to noise ratio and detector sensitivity (i.e. photodiode cf. CCD) encountered in the choice of NIR instrumentation, deserve attention with respect to the task of assessing the sugar content of intact fruit.

In this manuscript we evaluate three commercially available, low cost NIR spectrometers which differ in terms of the aforementioned parameters, with respect to the non-invasive measurement of Brix (sugar content) of melon fruit. We also report on the optimisation of an optical configuration suited to the assessment of melons, and the optimisation of chemometric processing technique. A field portable unit has subsequently been based on this design, and melon spectra collected across growing district and time to explore calibration robustness issues.

Materials and methods

Spectrometer Description

Three commercially available miniature spectrometers, with gratings chosen for operation in the NIR, were acquired – the Zeiss MMS1 (Zeiss, Germany), the Ocean Optics S2000 (Dunedin, Florida, USA, distributed through LasTek, Adelaide, Australia) and the Oriel Fixed Image Compact Spectrometer (FICS, model 77443), using a Larry linear CCD array (distributed through LasTek, Adelaide, Australia).

The Zeiss MMS1 (Monolithic Miniature Spectrometer), released in 1994, consists of a block of glass (UBK 7) with the imaging grating directly replicated onto one surface. The body thus acts as the dispersive element, and also images the entrance slit onto the diode array by varying groove density and using curved grooves to correct coma and flatten the focal curve to optimise use of the flat detector structure (6 mm long).

The refractive index of the material (UBK 7) used in the construction of the body is higher than that of flint glass, giving greater angles of refraction and thus enabling the unit to be reduced in size. With the monolithic construction, the grating is immovable and thus vibration tolerant and protected against dust, and the spectrometer is relatively tolerant of temperature changes (wavelength drift of 0.012 nm/K specified). A fibre optic cross section converter is employed, with a linear arrangement of 30 quartz fibres (each 70 μm wide) acting as slit for the instrument. Thus slit width is not alterable. A Hammamatsu diode array (S3904-256Q, 256 elements, each 25 x 2500 μm, 6 mm total length) is used as the detector. With detection of wavelengths between 300 and 1150 nm, the MMS1 has a pixel dispersion of 3.3 nm/pixel. Order sorting filters are applied during manufacture to different regions of the array to eliminate detection of second order spectra over this wide wavelength range. A 12 bit analogue to digital conversion device was used, under the control of Zeiss software. The Zeiss software supplied with the MMS1 employs a smoothing function for its graphical display, but not on saved data, as used in the calculations of mean and standard error of signal in this study.

The Ocean Optics S2000, released in 1997, has increased sensitivity relative to the original 1992 release (S1000). A 2048 element linear CCD array (each 12.5 x 200 μm, 4 mm total length) is employed, with only the mid-section used to minimise problems with field distortion. To optimise use in the NIR region, an order sorting filter (550 nm) was factory installed. With the grating (#14, blaze 1000 nm, 600 l/mm) and the slit width (50 μm) chosen (factory installed), a 3 nm resolution is specified. With a nominal wavelength range of 632 to 1278 nm, and a number of blackened pixels, the S2000 has a pixel dispersion of 0.36 nm/pixel. A 12 bit analogue to digital conversion device was used, with data acquisition controlled by Spectra Array (LasTek, Adelaide, Australia).

With the Oriel, the slit is mounted on a slide, and so can be varied. A 25 μm slit was used in this study. A 2048 element linear 'Larry' CCD array (12.5 x 200 μm) was employed as the detector. With the grating (blaze 1000 nm, 600 l/mm) chosen, a wavelength range between 300 and 1150 nm was detected, giving a pixel dispersion of 0.41 nm/pixel. A 12 bit analogue to digital conversion device was used, with data acquisition controlled by Spectra Array (LasTek, Adelaide, Australia).

Spectrometer comparisons

The performance of the spectrometers was compared in terms of wavelength resolution and stability, relative spectral sensitivity, relative detector sensitivity, signal to noise ratio, stability over time and variation in temperature and calibration performance. To achieve these comparisons, wavelength calibration was undertaken using a mercury argon lamp (HG1, Ocean Optics), and spectra were acquired of a reference material (WS-1, halon reference, Ocean Optics) and of samples (i.e. Whatman #1104 filter paper saturated with a range of concentrations of sucrose, and intact melon fruit).

Spectra of filter paper soaked with sucrose solutions were collected using a reflectance probe with six illumination fibres and one read fibre (all fibres 400 μm in diameter, R400-7, Ocean Optics). The read fibre was directed to a spectrometer, while the illumination fibres were connected to a 6 W tungsten halogen light source

(LS-1, Ocean Optics). SMA connectors (NA 0.22) were used to connect these items. Spectrometer temperature was varied by placing the spectrometer within the oven of a Fisher Gas Partitioner (Model 1200) and within an ice box, and monitored by a thermocouple placed in contact with the spectrometer body. Temperature was ramped from ambient (22°) to 0 °C, and then increased to 45 °C, at approximately at 0.2 °C/min.

NIRS calibration technique

Two populations (n = 40 (two spectra per fruit); n =210 (one spectra per fruit); combined Brix range 5.4 to 11.2) of rockmelons (cv. Dubloon) were sourced from the Bowen-Burdekin (North Queensland) region in November 1998 for use in the comparison of instrumentation. A further ten populations (total n = 1991, two spectra per fruit, Brix range 4.4 to 12.2) of rockmelons (four cultivars, from various growing regions) were obtained during 1998 and 1999 and spectra collected using the purpose built instrument described below. This larger data set was used in the consideration of the optimal data treatment for calibration, with the optimal data treatment then used for the calibrations involving the comparison of instrumentation reported in this manuscript. Spectra acquisition and wet chemistry occurred within three days of harvest at the 'slip' stage (fruit breaking away from peduncle). Spectral data was acquired of an area of the fruit equidistant between point of attachment of peduncle and corolla, but not of an area which had been in contact with the ground during fruit growth. Where two spectra were acquired per fruit, spectra were acquired from opposite sides of the fruit. Juice was extracted of 40 mm diameter plugs of fruit mesocarp tissue underlying the assessed areas, with soluble solids concentration assessed using an Erma (Tokyo, Japan) digital refractometer. While a range of ca. 6 Brix units was recorded between different fruits (population ranges reported above), a range of ca. 2 units was also recorded within the central region of a given fruit (i.e. around the 'equator' of the fruit). Brix of prepared sucrose solutions was also measured using the Erma refractometer.

Zeiss and Spectra Array files were converted to JCAMP format, and imported into the chemometric package ISI (version 3.0, Infrasoft International, PA, USA). Spectral outlier were defined using the ISI critical 'H' statistic (distance of spectral sample to population mean) set to a value of three. Analysis involved a modified partial least squares (*MPLS*) procedure using raw, first or second derivative absorbance data. Standard normal variance (*SNV*) and detrend were used for scatter correction. The effect of the number of data points used in the derivative calculation ('gap') and the number of data points used in a smoothing routine offered in the ISI software was considered. As suggested in the ISI manual, calibrations were compared primarily on the standard error of cross validation (*SECV*) statistic, where SECV should not be greater than 20% greater than SEC, and attention given to the *1-VR* (variance ratio) statistic.

Optimization of optical configuration for the non-invasive assessment of melon fruit sugar content

Philips halotone (12V, 50W, 50° light spread, aluminium reflector) lamps were used as light sources and the Zeiss MMS1 unit used as the detector. Reflectance (in which specularly reflected light is received by the detector) and partial transmission optical

arrangements were trialed, as the optical density of the fruit prevented full transmission optics. The core configuration consisted of the detector fibre optic positioned to view the 'top' of the fruit (a position on the fruit equidistant from peduncle and blossom ends which was not an area of the fruit which had rested on the ground during fruit growth). Lamp(s) positioned to illuminate the fruit at some (varied) distance from the area seen by the detector (ie. varied angle to detector relative to centre of fruit).

The intensity of light received by the detector and the calibration performance (for prediction of mesocarp Brix) was considered with reference to the following variables : (i) the angle of incidence of light onto the fruit surface, (ii) the angle between detected area and illuminated area with reference to the centre of the fruit (i.e. distance between detected area of fruit and illuminated area), (iii) the number of lamps employed, (iv) the distance from detector fibre optic to fruit, the presence of a shroud between detector fibre optic and fruit surface and (v) the duration of illumination (with respect to temperature of fruit).

Results and discussion

Wavelength accuracy and resolution

The instruments were calibrated using a mercury argon lamp, and spectra of the mercury argon lamp acquired at near saturation count at the 842.5 nm emission line (Fig. 1). The spectrum acquired with the MMS1 unit demonstrated a poor wavelength resolution relative to either the S2000 (Fig. 1) or the Oriel unit (data not shown). Second order spectral peaks were recorded with the S2000, but not the other instruments (e.g. at 1080 and 1155 nm, data not shown). The peak at 912.3 nm was chosen for further characterisation as it was isolated from other peaks in the MMS1 spectrum. Spectra were acquired with the count of this peak at near saturation and normalised between instruments. The line width (full width at half maximum, FWHM) of the Oriel and S2000 was 1.2 and 2.1 nm, respectively, an order of magnitude superior to the MMS1 result of 13.1 nm. These results are consistent with the slit widths, pixel dispersion and geometries of the three units.

Array spectrometers have a reputation for wavelength precision, relative to instruments in which the monochromator is a moving grating (and therefore sensitive to mechanical disturbance). The wavelength calibrations of the MMS1 and S2000 were checked periodically over a period of 6 months. During this period the instruments were used in air conditioned laboratories, but were subject to mild shocks and temperature fluctuations during transport between laboratories. No recalibration was necessary over this period for either instrument (i.e. measured position of 912.3 spectral line of HgAr lamp did not vary by more than 0.3 nm). However, the FWHM of array spectrometers can be sensitive to temperature, as differential expansion of materials within the spectrometer changes the geometry of the light path. The monolithic construction of the MMS1 should be advantageous in this respect. Wavelength accuracy and FWHM was stable for both the MMS1 and the S2000 over the temperature range expected in a packing shed environment. The FWHM of the MMS1 was estimated at between 13.03 and 13.2 nm, with no consistent change as the temperature of the spectrometer was varied between 4 and 45 °C (data not shown).

The FWHM of the S2000 varied between 2.06 and 2.12 nm as temperature was varied over this range, tending to increase with temperature (data not shown).

Relative spectral sensitivity

The three spectrometers employed silicon based detectors, and so are expected to show decreasing sensitivity through the region 700 – 1100 nm, with no response beyond 1100 nm. However, the spectral sensitivity of the instrument can be altered by doping of the silicon in the detector, by use of coatings over the surface of the detector elements, and with respect to the spectral efficiency of the grating (primarily determined by the blaze wavelength). Spectra were acquired using the three instruments of the reference material in reflectance mode, using the interactance probe and a tungsten halogen light source. Spectra with a maximum count level near saturation were acquired for each spectrometer, and spectra compared after normalization to the count at 730 nm (Fig. 2). The MMS1 was more sensitive than the other instruments over the wavelength range 750-1050 nm, and particularly over the region 800 – 900 nm. The Oriel-Larry unit was more sensitive at wavelengths between 650 and 700 than at 720 (data not shown). The spectrum acquired using the MMS1 was also smoother than equivalent spectra acquired with the FICS or S2000. An increase in count after 1060 nm was recorded with the S2000, a result interpreted as a second order spectra (the S2000 unit employed a 550 nm primary cut-off filter).

Spectra were recorded of the HgAr lamp using the MMS1 spectrometer, while altering the temperature of the spectrometer between 0 and 45 °C (data not shown). The measured count of a 'dark ' region of the HgAr lamp spectrum (870 nm) increased with temperature by a count of 0.33 per °C (on a count of 29 at 0 °C, linear regression, r^2 = 0.913). The measured count of the 912 emission line was more responsive to temperature, increasing by a count of 10.96 per °C (on a count of 2757 at 0 °C, linear regression, r^2 = 0.913). Thus detector spectral sensitivity and dark current are changing with instrument temperature. These changes may be accommodated in a field application by minimising detector temperature change, and by collecting reference and dark spectra at the same instrument temperature as experienced while collecting sample spectra.

Relative detector sensitivity

Reflectance spectra of a reference material under halogen lamp illumination were acquired using the three spectrometers at a range of probe heights (i.e. different illumination levels) but the same acquisition time per spectrum (100 ms). Regression relationships were established between the readings of the three instruments. Detector response was recorded at 735 nm, as the wavelength at which highest counts were recorded in the MMS1 and S2000 units, and also a wavelength likely to be used in calibrations developed for the sugar content of fruit (e.g. Guthrie *et al.*, 1997). The S2000 gave count readings 2.65 times higher than that of the MMS1 (r^2 = 0.998) (e.g. Fig. 2B), with a saturation count reached at only 25% of the range of the MMS1. In contrast, the slope of the Oriel – MMS1 regression was only 0.019 (r^2 = 0.996) (data not shown).

The sensitivity of a CCD array to light, in terms of electrons/count, is reported to be ca. 150 times greater than that of a PDA (Oriel 1997). The low sensitivity of the

Oriel FICS assembly was primarily due to the design of the instrument optics to focus light onto a 2500 μm height (PDA) array, not onto a (CCD) detector array only 17 μm tall, as used in this study. The relatively high sensitivity of the MMS1, as a photodiode array, relative to the S2000, as a CCD array, is explained by the size of the pixels in the two arrays, and also by the degree of pixel dispersion in the two units. The MMS1 pixel (25 x 2500 μm) has an area 400 times greater than that of the S2000 CCD (12.5 by 12.5 μm). Also, the effective slit width of the MMS1, at 70 μm (diameter of fibre optic), was greater than that employed in the S2000 (50 μm).

'Signal to Noise' Ratio

Fifty reflectance spectra of the reference material were recorded at near saturation levels, and at a low light intensity (peak counts of >300), for each instrument. The mean was divided by the standard error of the count at each wavelength as an estimate of the signal to noise ratio. With spectra recorded at near saturation levels, the maximum signal to noise ratio, recorded at peak signal count at 735 nm, was approximately 40 000, 1 000 and 4 000 in the MMS1, S2000 (Fig. 2A) and Oriel units (data not shown), respectively. At a low light level of approximately 10% of full scale; the maximum signal to noise ratio was approximately 3 000, 250 and 400 for the MMS1, S2000 (Fig. 2B) and Oriel instruments (data not shown), respectively.

The total pixel noise in the signal from either the photodiode or CCD array can be approximated as the square root of the sum of squares of the followings three components, (a) read out noise, which is due to amplifier and electronics, (b) shot noise from the signal itself, equivalent to the square root of the signal, and (c) the shot noise of the dark current, which is dependent on exposure time and very dependent on temperature. The spectral shape of the noise (mean/standard error) values followed that of the signal, reflecting the importance of the signal shot noise to the total noise. The signal to noise ratio should be better for a CCD than a PDA for the operating range of the CCD, reflecting a lower readout noise, but the maximum signal to noise ratio of the PDA (achieved at higher signal levels) is expected to be an order of magnitude greater than that of the CCD (ca. 10 000 cf. 1 000, respectively; Oriel 1997). The results obtained with respect to maximum signal to noise of the PDA and CCD detectors was as expected however, the signal to noise ratio was also higher for the PDA based spectrometer than with the CCD based instruments for light levels within the range of operation of the CCD. The low noise of the MMS1 at the lower light level is therefore attributed to a low read out noise, relative to that expected for a PDA.

Bellon *et al.* (1993) estimated the signal-to-noise ratio of their CCD based system by dividing the spectrum of a reference material by the standard error of 10 reflectance ratios (spectrum of reference material divided by a reference spectrum of the same material) at each assessed wavelength (rather than by the standard error of the repeated raw spectra). Assuming spectra were acquired at near saturation levels, and given the use of an eight bit A/D card (i.e. saturation at a count of 256), the report of a maximum signal to noise ratio of 90 000 is equivalent to a ratio of 360 (i.e. 90 000 / 256) in terms of the current study. Thus the signal to noise ratio achieved by Bellon *et al.* (1993) was similar to that obtained with linear CCD arrays in the current study. This is surprising, in that as Bellon *et al.* averaged data over 512 rows, noise should

have been decreased by a factor of the square root of 512 (22.6) over that of a single pixel. The difference is attributed to noisy electronics.

Stability of spectrometer and lamp output

Using a light source which had been activated some 3 h earlier, the output of the MMS1 and S2000 was recorded with respect to time from instrument activation (Fig. 3A, 4A). For the MMS1, counts generally decreased (e.g. at 750 nm, by 25 counts on 3 500) (Fig. 3A, 4A). The MMS1 was considered stable within 60 min of activation. In contrast, the S2000 was relatively unstable, fluctuating by up to 3% of initial response, and not stable even after 90 min from activation. The stability of instrument response is a critical parameter in consideration of the frequency of referencing required, or the preference for a dual beam over a single beam operation.

Using a MMS1 spectrometer which had been activated some 3 h earlier, the spectral output of a tungsten halogen lamp was recorded with respect to time from activation (Fig. 3B, 4B). Spectral output decreased by ca. 5%, across most wavelengths, but increased, by ca. 2%, at 833 nm. Most changes were complete within 30 min of lamp activation. These spectral changes are attributed to the chemistry of the tungsten halogen lamp during a warm up period following ignition. Parallel data were collected with the S2000, with trends similar to that reported above (Fig. 4B). The stability of the lamp output is also a critical factor in consideration of the frequency of referencing required in an application.

Choice of spectrometer for the application of non-invasive sorting of fruit by NIRS

The application of fruit sorting by near infrared spectroscopy requires an instrument which is relatively sensitive to light, in order to capture spectra of fruit in transmission or interactance modes without use of an unduly high incident radiation load (with the attendant sample heating problems). The instrument must be sensitive over the spectral region 700 – 1 050 nm (or higher), and the detector response must be relatively stable. As noted earlier, wavelength resolution below 10 nm is probably not necessary (e.g. Greensill and Walsh, 2000).

The Oriel-Larry unit gave the best wavelength resolution of the three instruments. However, its sensitivity was poor, and on this basis the instrument was eliminated from consideration. The S2000 gave better wavelength resolution and detector sensitivity than the MMS1. However, the relative response of the MMS1 in the near infrared (750 – 1 000 nm) region was higher than in the visible region than the S2000 (Fig. 2). Further, the signal to noise ratio of the MMS1 was an order of magnitude higher than the S2000, both at high light levels (i.e. near detector saturation) and at a low light levels (within the detection range of the CCD) (Fig. 2).

To compare the spectrometers for their application to the task of assessment of fruit by NIRS, we captured spectra of filter paper saturated with sugar solutions of varying concentration in one experiment, and of a population of honeydew melons in a second experiment. The MMS1 supported better calibrations than the S2000 for both the sugar solution and fruit populations (Table 1). We conclude that the attribute of wavelength resolution was not important to the calibration process, relative to the

attribute of signal to noise ratio. Of the three instruments considered, we recommend the MMS1 for use in the application of fruit sorting by NIRS.

Optimising optical configuration and instrument parameters for fruit sugar content calibration

Light angle relative to fruit and detector

The intensity of light detected was not dependent on the angle of incidence of the light beam on the fruit surface (data not shown). This result is explained in terms of the diffuse transmission of light through the fruit, with incident radiation scattered within the fruit such that the angle of incident illumination has little effect.

As expected, the intensity of light detected at a given wavelength (800 nm) decreased as the light beam was moved away from the detected area. The decrease in detector response, R (counts), was described with reference to the distance between the centre of the illuminated area and the detected area of the fruit, D (mm). This exercise was undertaken for an optical arrangement involving a single 50 W halogen lamp and the detector aligned to the centre of the fruit and positioned at 10 cm from the fruit surface (eqn. 1). The exercise was repeated incorporating a 45 mm diameter cylindrical shroud between the detector and the fruit surface, to eliminate specular radiation (eqn. 2).

$R = 18525\ e^{-0.0668\ D}$ ($R^2 = 0.943$) (light without shroud) eqn. 1

$R = 64646\ e^{-0.085\ D}$ ($R^2 = 0.994$) (light shrouded) eqn. 2

Thus, moving the detector from 40 to 50 ° with respect to the light source decreased the observed count from ca. 24 to 4% and 7 to 2% of maximum signal (i.e. detector saturation; 200 ms integration time) for the non-shrouded and shrouded arrangements, respectively.

We had expected that, at lesser angles of light source to detector, higher levels of radiation would be monitored, but that the 'path' of this light would be primarily through exocarp and outer mesocarp tissues. Therefore, increasing incident light to detector angle should allow for proportionally more spectral information on the tissue of interest, the edible mesocarp. However, at some incident light to detector angle, the disadvantage of decreased light transmission (i.e. decreased signal to noise ratio) must outweigh this advantage. Also, as the angle between incident light and detector is increased (particularly beyond 90°); it is expected that proportionally more of the detected light will have penetrated the seed cavity and carry spectral information about seeds, as well as about mesocarp tissue.

Spectra were acquired at four lamp-detector angles for 40 fruit, using a shroud between lamp and fruit (Table 1). Calibration standard error of cross validation (SECV) increased as lamp angle increased, except at 80 °. However, very poor regression coefficients were recorded for spectra collected at 20 ° and 80 °. We conclude that spectra collected at 40 ° supported the best calibration. An angle of 45° between incident light and detector was adopted in further characterisation of the optical system.

Number of lamps

Increasing the number of lamps, positioned at 45 ° in the vertical plane, with respect to the detector, and at 90 ° intervals in the horizontal plane, with respect to other lamps, was expected to increase the available signal to the detector, and decrease the signal to noise ratio. Aoki *et al.* (1996) reported an optical arrangement employing 16 lamp positions around the equator of the melon fruit, with the detector viewing an area of the fruit at 90 ° to this plane. However, the use of more lamps will also increase the volume of the fruit 'sampled' by the light, which may degrade a calibration based on the analysis of a relatively small tissue sample in the primary analytical method.

Increasing the number of lamps from 1 to 4 had little consistent effect on calibrations developed using the full data set of Brix in melon (Table 2). However, calibrations developed with outlier data removed demonstrated a consistent decrease in SECV with an increase in number of lamps, although the calibration coefficient was not consistently increased with lamp number. Four lamps were employed in further characterisation of the optical system.

Detector 'shrouding' and distance of detector to fruit

Calibration performance was degraded by removal of the shroud between the detector and the fruit surface, in terms of R^2, *SEC* and *SECV* (Table 2). The placement of a 40 mm high, 45 mm diameter collar on the fruit under the detector supported a calibration which was comparable to the arrangement employing a shroud between detector and fruit surface (Table 2). These results are consistent with the interpretation that the detection of specular and emergent light that has interacted only with the very surface layers (top few millimetres) of the fruit surface degrades calibration performance. Calibration performance was slightly improved in terms of both calibration R^2 and *SECV* when the distance between fruit surface and detector was allowed to vary in response to fruit size (i.e. by ca. 50 mm). The detector fibre optic has a numerical aperture of 0.22, and will thus image an increasing area of the fruit surface as distance between the probe and the fruit surface is decreased. This could result in an increased detector count, offset by a decrease in light intensity. If the field of view of the detector overlaps the areas of direct lamp illumination of the fruit surface, specular reflection will also reach the detector.

Number of scans per spectra

The SNR of spectra will improve proportionally to the square root of the number of scans averaged per spectrum. Calibration SECV decreased with increased number of scans, although this improvement was marginal and the regression coefficient decreased between four and 16 scans. Increased scan time will lead to sample change through heating, which could alter spectral characteristics and thus calibration performance (Guthrie and Walsh 1999). However, when fruit were held under the lamps for a period of 3 min, (fruit internal flesh increased in temperature by less than 1 °C, while skin temperature rose by greater than 20 °C), calibration performance was not significantly impacted. Averaging of four scans per spectra was adopted in further characterisation of the optical system.

Calibration maths

The optimal math treatment of spectral data is expected to be specific to the instrumentation and the application. For example, scatter correction routines (standard normal variance and detrend in the ISI software) are typically applied to reflectance spectra of samples with a rough, light scattering surface. First and second derivative procedures are useful to remove changes in spectral baseline level and slope, and to highlight spectral features. The optimal value for the 'gap' (number of data points) over which the derivative is calculated will depend on the band width of the spectral feature of interest, and the noise and wavelength resolution of the instrumentation used. Data smoothing routines can also be useful in the reduction of noise and elimination of redundant spectral information. An empirical 'test it and see' approach is generally used to establish the best math treatment for a given application. For example, Guthrie and Walsh (1997) established that a math treatment involving second derivative over four data points and smoothing over four data points was optimal in the calibration of sugar content in intact pineapple fruit using NIRSystems 6500 reflectance spectra.

Calibration math treatment was optimised for the populations used in the comparison of optical geometry, with a standard treatment adopted (as in Table 2). The optimal optical configuration was then used to collect spectra of a large number of fruit (see below). We report here (Table 3) an exercise in comparison of math treatments on this larger population, which yielded similar conclusions but more marked differences than obtained with the smaller population sets.

Using the ISI chemometric package, outlier spectra were detected and removed from the data set. Approximately 5% of spectra were removed from populations, resulting in a consistent improvement in calibration performance (Table 2). However, as removal of outlier data results in inconsistent population structure, this option was not employed when evaluating math treatments (Table 3). The scatter correction routines (standard normal variance and detrend) decreased calibration performance in terms of *SECV* and R^2 (Table 3). We attribute this result to the optical geometry of the system employed, which was effectively a transmission rather than a reflectance system.

Various signal filtering routines may be used to remove noise from the acquired spectra (e.g. Fourier transform, box car averaging, Butterworth filter). Osborne *et al.* (1998) reported the use of a Butterworth filter improved the development of calibrations of kiwifruit Brix, based on spectra acquired using a Zeiss MMS1 spectrometer. As the Savitsky-Golay signal filter was available within the Zeiss MMS1 test software, we filtered spectra acquired at a lamp – detector angle of 40°. The calibration developed with this data was severely degraded in terms of regression coefficient and *SEC* (Table 2), and this treatment option was not considered further.

Calibration performance was marginally enhanced by the use of derivatised data (second derivative superior to first or no derivative). The best calibrations achieved using 'raw', first and second derivative data yielded a *SECV* of 0.79 (no smoothing), 0.79 (derivative gap of 4 data points, no smoothing) and 0.77 (derivative gap of 4 data points, no smoothing), respectively. We attribute the relative lack calibration response to the use of derivatives to the transmission optical geometry of the system

employed. Derivatives should offer more value in reflectance systems in which baseline shifts between samples can be large.

Calibration performance was degraded as the gap over which the derivative was calculated was increased from 4 to 8 to 10 (Table 3). Calibration performance was also degraded by smoothing of data. The Zeiss MMS1 has a pixel resolution of 3.3 nm, and thus smoothing or derivative calculated over four data points involves averaging of data over a 13 nm spectral range, equivalent to the wavelength resolution of the instrument. Further averaging will involve loss of spectral information.

Calibration performance was not improved by averaging (smoothing) over four data points, a result attributed to the high SNR of the MMS1 detector. Smoothing over a greater gap than used in the calculation of the derivative is expected to result in a loss of information, and a degradation in calibration performance was observed.

Based on the above observations, we recommend a data treatment of second derivatives over a gap size of four, with no scatter correction or smoothing, for this instrumentation and optical configuration.

Design of field unit for consideration of calibration robustness

In summary, the following instrument design is proposed for the non-invasive assessment of melon using a low cost, commercially available spectrometer module : the Zeiss MM1 spectrometer used with a shroud (45 mm diameter, 100 mm length) between the detector fibre optic and the fruit surface, and four 50 W tungsten halogen lamps and lamps mounted at 90° intervals, positioned 100 mm above the fruit and aligned with the approximate centre of the fruit (i.e. angle of 45 ° between light incidence and detected area of fruit). These features have been adopted in a 'luggable' or 'at-line' NIR system that can be transported between pack-houses. This unit incorporates a spring loaded platform to keep the fruit firmly against the detector shroud, while allowing for ease of fruit change over. The outside dimensions of the unit are 400 (width) by 400 (depth) by 550 (height), accomodating one melon at a time, and locating the spectrometer and other electronics above the sample chamber.

The following operational parameters are recommended for the use of this hardware : an integration time of 200 ms, as required to achieve a detector response at ca. 50% of saturation, with detector and lamp powered up two hours before use to ensure instrument stability. Averaging of four scans per spectra is recommended to improve signal to noise ratio. A 'default' data treatment of second derivative calculated over four data points, without further data pre-treatment, is suggested. We have subsequently developed a LabView (National Instruments) based spectral acquisition and analysis package which allows application of calibrations to give predictions in a real-time basis.

This system described was benchmarked in terms of calibration performance against two commercial research-grade NIR spectrometers, operated over the full NIR wavelength capability of these instruments and in reflectance mode (Table 2). The purpose built instrumentation supported a calibration equivalent to that achieved using the Perten DA7000, and slightly inferior in terms of *SECV*, although superior in terms of R^2, to that achieved by the NIRSystems 6500. The purpose built instrumentation

was operated over a reduced wavelength range, and is expected to be compromised in terms of hardware performance as a low cost alternative. The equivalent performance of the purpose built instrumentation is ascribed to the low SNR and optimisation of the system in terms of optical geometry of incident light, sample and detector. We will employ this system to collect spectra of melons of various cultivars, growing districts and seasons to further develop the consideration of Guthrie *et al.* (1998) of calibration robustness. Equipped with a robust calibration, this instrumentation should be useful in physiological, agronomic and breeding programs targeting melon fruit soluble solids content.

Acknowledgments

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Figure Legends

Fig. 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).

Fig. 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 such 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.

Fig. 3. The stability of (A) detector response and (B) light source, as indicated by change in spectrometer response (interactance optics, reference sample) for the wavelength range 300 – 1100 nm with time from instrument and lamp activation, respectively. Data expressed as the difference in the A/D card output to that of the first spectra acquired (at 1 s after detector and lamp activation, respectively). Spectra were obtained using a halogen light source and teflon as a reference sample.

Fig. 4. The stability of (A) detector (MMS1, squares; S2000, circles) response and (B) light source, as indicated by change in spectrometer response (interactance optics, reference sample) at 750 (open symbols) and 833 nm (closed symbols) with time from instrument and lamp activation, respectively. Data expressed as a percentage of the first record (ca. 1 s after detector and lamp activation, respectively). Data is of the same experiment as presented in Fig. 3.

Figure 1

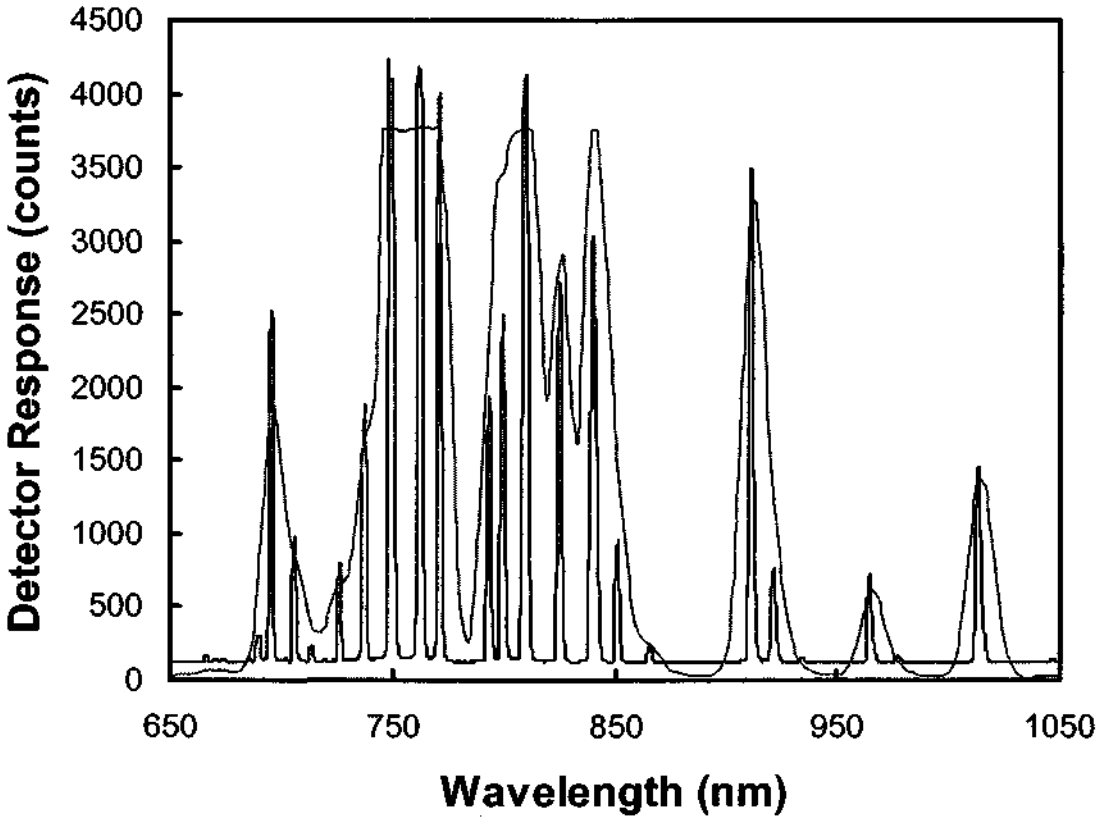
Detector Response (counts)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
650
750
850
950
1050
Wavelength (nm)

Figure 2

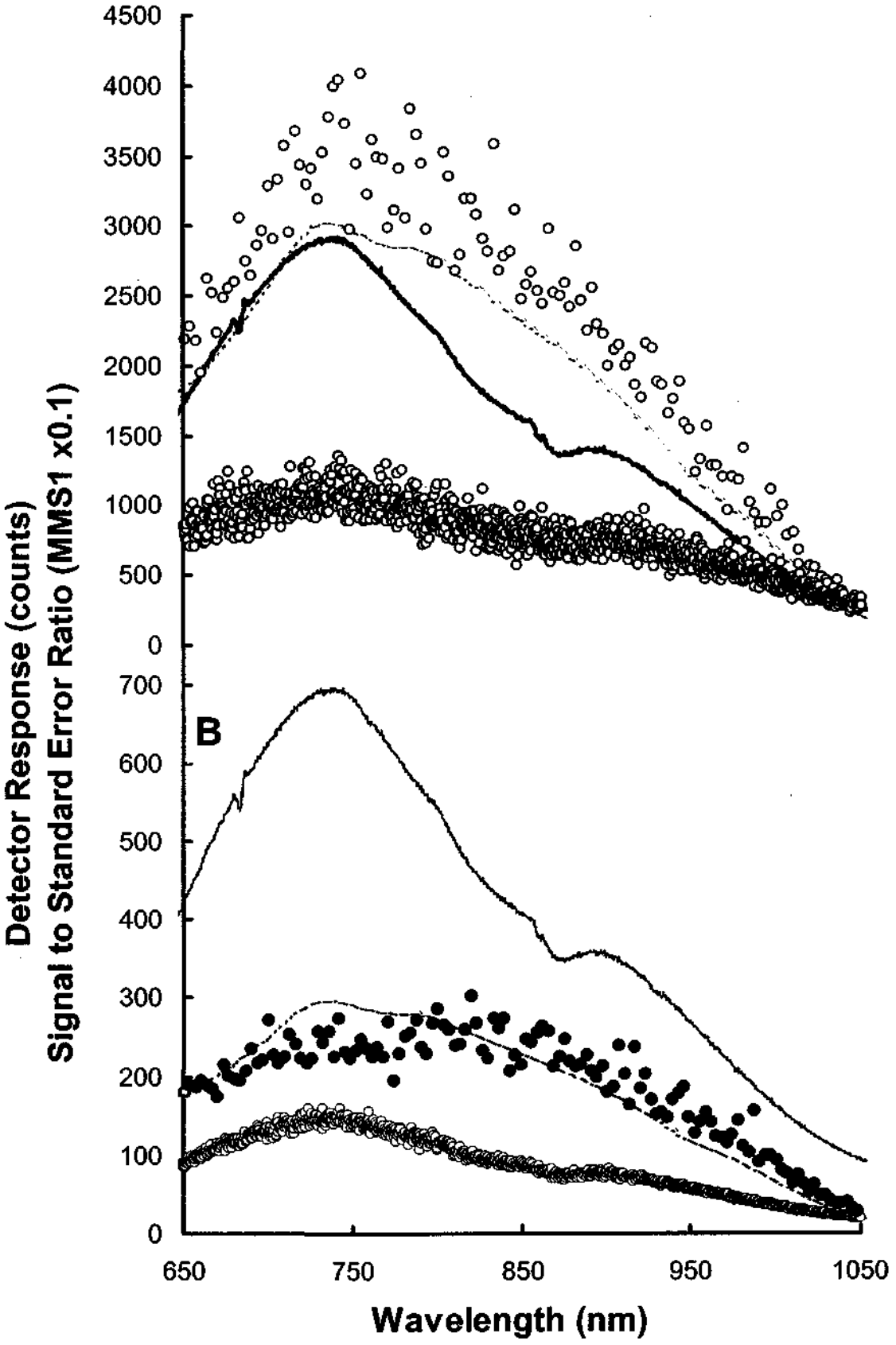
Detector Response (counts)
Signal to Standard Error Ratio (MMS1 x0.1)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
700
600
500
400
300
200
100
0
B
650
750
850
950
1050
Wavelength (nm)

Figure 3

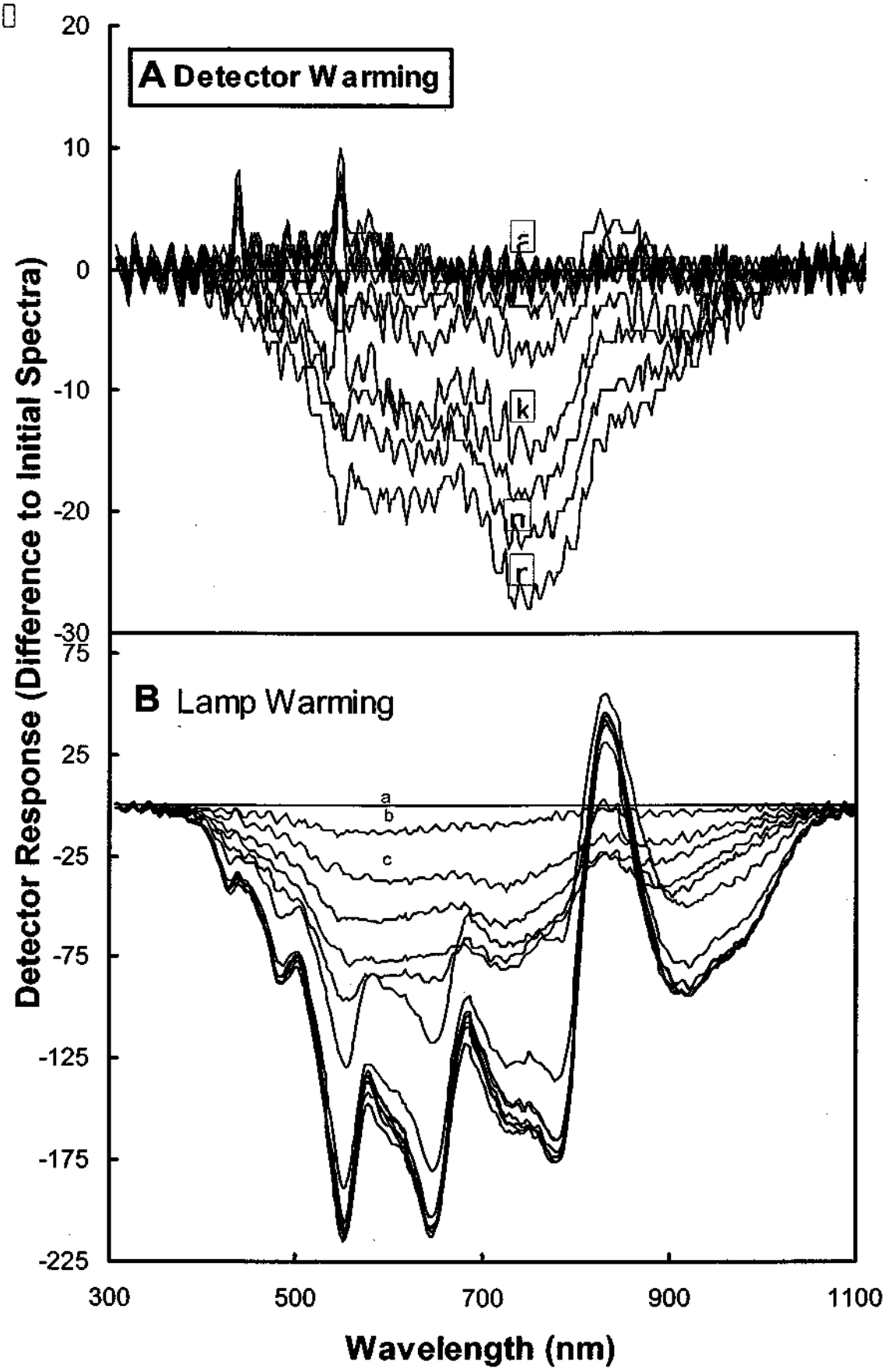
A Detector Warming
B Lamp Warming
Detector Response (Difference to Initial Spectra)
Wavelength (nm)
20
10
0
-10
-20
-30
75
25
-25
-75
-125
-175
-225
300
500
700
900
1100
a
b
c
k
n
r

Figure 4

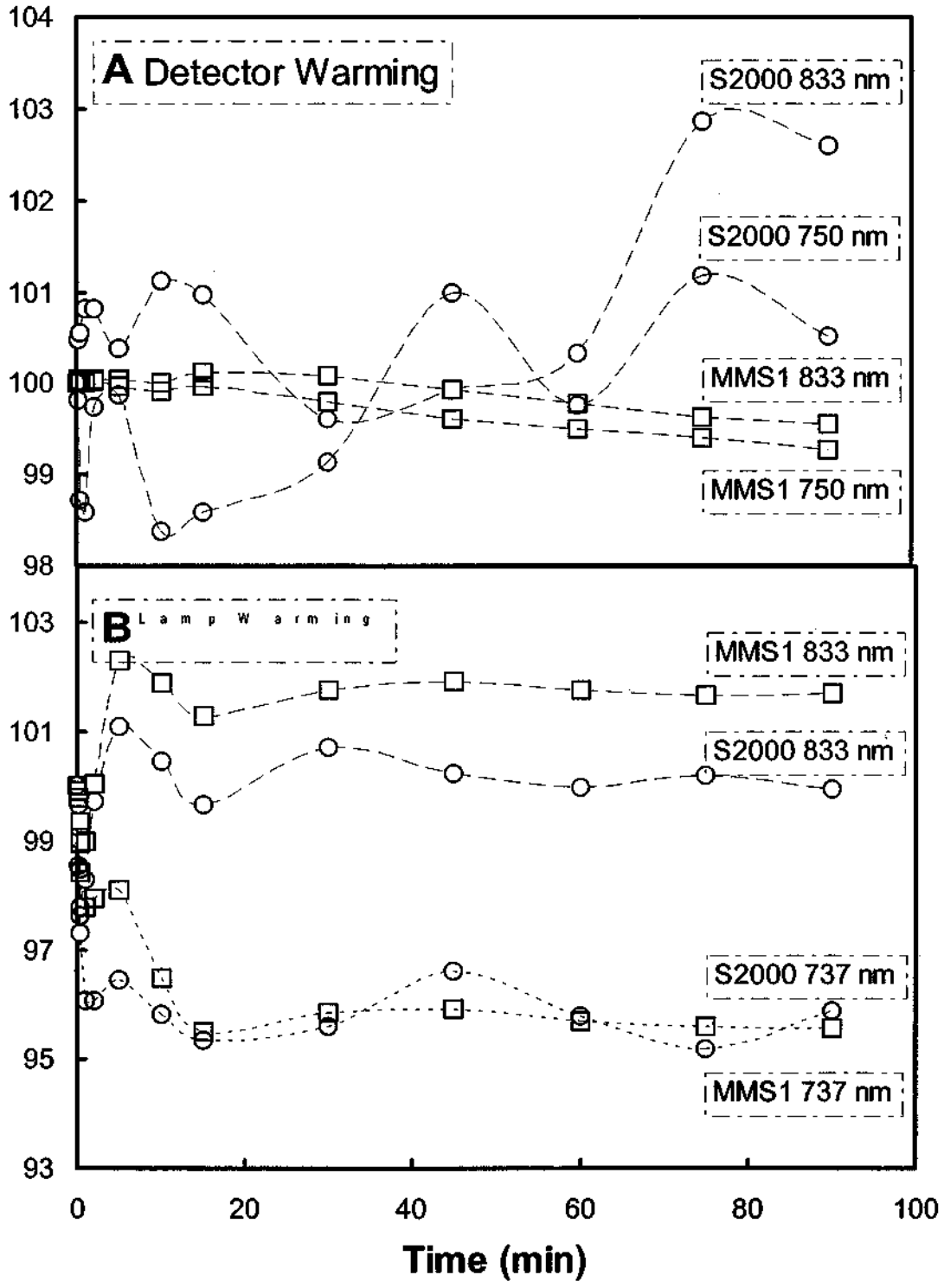
A Detector Warming
S2000 833 nm
S2000 750 nm
MMS1 833 nm
MMS1 750 nm
B Lamp Warming
MMS1 833 nm
S2000 833 nm
S2000 737 nm
MMS1 737 nm
104
103
102
101
100
99
98
103
101
99
97
95
93
0
20
40
60
80
100
Time (min)

Table 1. Calibration (R^2 and *SEC*) and validation (*SECV*) regression parameters (modified partial least squares procedure) of spectra collected with two spectrometers (MMS1 and S2000) using the same optical and sample presentation system (fibre optic interactance probe).

Calibrations were performed using the second derivative (gap size of 4) of unsmoothed data from the wavelength range 700 – 1050 nm. Scatter correction was not employed. Four spectra were acquired of each of 16 filter paper bundles, each saturated at different concentration of sucrose, at approximately 1.5 °Brix intervals between 0 and 20.

Instrument	Terms (°Brix)	R^2	*Sec*	*Secv*
MMS1	2	0.904	1.73	1.85
S2000	2	0.619	3.44	5.40

Table 2. The effect of lamp-fruit-detector angle, a signal filtering routine, number of lights, number of scans averaged per spectrum acquired, and the presence of a shroud between lamp and fruit surface on the calibration of Zeiss MMS1 spectral data (700 – 1050 nm) to melon flesh Brix, in comparision with two reflectance mode bench top NIR spectrometers.

Calibrations were developed for spectra (n = 40) acquired of a population of fruit (mean 9.5, standard deviation 1.7 °B) for various lamp – detector angles and for three spectrometers, and for spectra acquired of a population of 208 fruit (mean 8.3, standard deviation 1.2 °B) for conditions varying with respect to the number of lights, presence of a shroud, and number of scans averaged. The default configuration consisted of shroud between detector and fruit, four lamps mounted to illuminate the fruit at 40° with respect to the detector, and averaging of four scans per spectrum. Data of treatments marked with an asterisk has been repeated for ease of data comparison. For condition 16b, fruit were held under the lamps for 3 minutes before scanning. A data treatment of scatter correction (SNV and detrend), second derivative gap size of 4, with no data smoothing was adopted for all calibrations. The Savitzky-Golay filtering routine (SG) was applied to spectra acquired in the assessment of 40° lamp angle. MPLS regressions were performed with all data, and with removal of outlier data as identified using the WINISI chemometric package critical 'H' of 3. The MMS1 was used with a shroud, four lamps illuminating the fruit at 45°, and four scan averaging in the spectrometer comparison exercise. Spectral data over the wavelength range 700 – 1 050 nm, 700 – 1 700, and 700 – 2 300 nm was used from the MMS1, Perten DA 7000 and NIR Systems 6500 spectrometers, respectively.

Attributes		All Data				Outliers Removed			
	n	Terms	R^2	*SEC*	*SECV*	Terms	Outliers	R^2	*SEC*	*SECV*
Lamp Angles										
20	40	1	0.14	0.99	1.15	1	2	0.19	0.97	1.11
40**	40	3	0.64	0.65	1.18	3	0	0.64	0.65	1.18
40SG	40	3	0.05	1.16	1.40					
60	40	4	0.76	0.52	1.96	4	2	0.82	0.43	0.84
80	40	1	0.15	0.99	1.26	4	1	0.38	0.84	1.03
Number of Lights										
1	210	7	0.64	0.71	0.90	7	3	0.68	0.68	0.86
2	210	2	0.43	0.92	0.95	6	11	0.61	0.74	0.85
4*	208	6	0.63	0.73	0.88	6	5	0.66	0.69	0.83
No. of Scans										
1	210	2	0.45	0.89	0.92	2	3	0.44	0.89	0.92
2	210	2	0.43	0.92	0.95	2	6	0.46	0.88	0.91
4*	208	6	0.63	0.73	0.88	6	5	0.66	0.69	0.83
16a	212	5	0.57	0.78	0.87	6	5	0.63	0.72	0.82
16b	210	6	0.60	0.76	0.84	6	6	0.61	0.74	0.81
Shroud/ pathlength										
Shroud on*	208	6	0.63	0.73	0.88	6	5	0.66	0.69	0.83
Collar on	210	6	0.70	0.66	0.81	7	7	0.75	0.58	0.67
Shroud off – fixed path	213	2	0.45	0.89	0.91	2	9	0.47	0.87	0.89
Shroud off – variable path	196	4	0.50	0.84	0.93	3	15	0.51	0.82	0.87
Spectrometer Comparison										
MMS1**	40	3	0.64	0.65	1.18	3	0	0.64	0.65	1.18
6500 (- 2300)	40	1	0.21	0.95	1.07	2	4	0.51	0.79	1.06
6500 (- 1100)	40	4	0.59	0.69	0.97	3	3	0.71	0.55	0.84
Perten (- 1700)	40	3	0.64	0.65	1.18	3	0	0.64	0.65	1.18
Perten (- 1050)	40	1	0.97	0.19	1.07	1		0.16	0.99	1.10

Table 3. The effect of data treatments (derivative condition and gap size, smoothing, scatter correction) on the calibration of Zeiss MMS1 spectral data (700 – 1 050 nm) to melon flesh Brix.

Spectral data is of a population of 1991 fruit (mean 8.1, standard deviation 1.26 °B). Values marked with an asterisk represent cases were a smoothing window larger than a derivative gap window.

Data Treatment			Scatter Correction (SNV and Detrend)				Nil Scatter Treatment			
Derivative	Gap Size	Smooth	Terms	R^2	*SEC*	*SECV*	Terms	R^2	*SEC*	*SECV*
0	0	1	15	0.55	0.85	0.86	16	0.61	0.78	0.79
0	0	4*	16	0.52	0.87	0.87	16	0.55	0.84	0.86
0	0	10*	15	0.44	0.94	0.95	15	0.48	0.91	0.94
0	0	20*	15	0.31	1.0	1.1	16	0.39	0.98	0.99
1	4	1	15	0.58	0.81	0.84	16	0.64	0.76	0.79
1	4	4*	15	0.50	0.89	0.90	15	0.55	0.85	0.85
1	4	10*	16	0.50	0.89	0.91	15	0.49	0.90	0.91
1	4	20*	15	0.34	1.02	1.04	15	0.40	0.98	0.99
1	10	1	15	0.52	0.87	0.90	15	0.55	0.84	0.87
1	10	4	16	0.50	0.89	0.91	15	0.49	0.90	0.91
1	10	10*	15	0.42	0.96	0.97	15	0.44	0.94	0.96
1	10	20*	14	0.30	1.05	1.07	16	0.39	0.98	1.00
2	4	1	13	0.59	0.81	0.84	15	0.65	0.74	0.77
2	4	4	13	0.54	0.85	0.88	14	0.59	0.80	0.83
2	4	10*	13	0.49	0.90	0.91	14	0.54	0.86	0.88
2	4	20*	12	0.34	1.02	1.03	14	0.41	0.97	0.98
2	8	1	13	0.50	0.89	0.91	14	0.54	0.86	0.87
2	8	4	12	0.44	0.94	0.95	14	0.49	0.90	0.92
2	8	8	15	0.44	0.94	0.96	14	0.45	0.93	0.95
2	8	10*	12	0.39	0.99	0.99	14	0.43	0.95	0.96
2	8	20*	14	0.29	1.06	1.07	14	0.30	1.05	1.06
2	10	1	14	0.50	0.89	0.91	16	0.56	0.83	0.85
2	10	4	14	0.45	0.93	0.95	16	0.48	0.91	0.90
2	10	20*	15	0.29	1.06	1.07	15	0.31	1.04	1.05

Appendix 2.

Presentation to the 9th International Conference on Near-Infrared Spectroscopy Verona, Italy. June 13-18, to appear in NIR 99 (Proceedings of this conference).

Use of an 'at-line' NIR instrument to evaluate robustness of fruit Brix calibrations

K.B. Walsh, C.V. Greensill and J.A. Guthrie

Abstract

Melon eating quality is largely dependent on soluble sugar content, which can be non-invasively assessed using near infrared spectroscopy. Instrumentation requirements for this application are explored, with resolution of 20 nm and signal to noise (standard deviation) ratio of 4,600 indicated to be adequate for an Si array spectrometer based system. Light distribution through the melon from an incident spot is described, with diffuse scattering through the flesh (mesocarp) layers but directionality evident within the seed cavity. An optical system using the Zeiss MMS1 photodiode array spectrometer was optimised in terms of optical geometry, with reference to fruit and lamp(s). This system was used to collect spectra of a range of melon varieties, growing localities and growing times. Calibration sets were trimmed on the basis of global and neighbourhood Mahalanobis distances. For example, one calibration set containing 1991 spectra was reduced to 449 with use of a NH of 1.0, with no loss of prediction precision (SECV). A calibration developed on a single population (variety-time) gave poor predictions of populations of the same variety harvested at other times or locations. A calibration developed over five time-locations of the one variety predicted sugar content of other populations of the same variety well, and populations of one of four other varieties. A calibration developed over five varieties proved to be acceptably precise (SECV ca. 0.8 %TSS) and robust.

Keywords: Charged couple device, photo-diode array, fruit, non-invasive, near infra-red spectroscopy, sugar content

Introduction

Melon eating quality is indexed by total soluble solids (TSS) (Mutton *et al.*, 1981; Dull *et al.*, 1989). Other attributes (e.g. volatiles, texture) contribute to eating quality, but TSS is often positively correlated with these attributes, and high TSS is a prerequisite for good eating quality (Mutton *et al.*, 1981). 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 (predominantly sucrose) (Dull *et al.*, 1992), a method of measurement of sucrose within intact melons with a resolution of approximately 1% is required for a fruit eating quality assurance program.

Near infrared spectroscopy (NIRS) was first applied to the measurement of TSS in melons by Dull *et al.* (1989), operating in a reflectance mode. 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 of the use of NIRS to assess the TSS of

intact fruit show a progressive decrease in the SEP, from 2.2% (Birth *et al.*, 1990) and 1.9% (Dull *et al.*, 1992) to 0.4% (Aoki *et al.*, 1996). This improvement reflects change in the instrumentation used, and in the optical geometry (light-sample-detector) employed. Reflectance mode systems suffer from a background of specular light. However, the optical density of melon fruit makes transmission mode difficult to employ. The Aoki *et al.* (1996) system employed a multiple lamp system, with lamps mounted at 90° to the detector, with reference to the centre of the fruit. NIRS technology is now in commercial use in Japan by companies (e.g. Fantec, Mitsui), with a reported SEP of 0.5%.

However, 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 (Guthrie *et al.*, 1998) employed a NIRSystems 6500 reflectance mode spectrometer to consider the robustness of calibrations across melon varieties, growing seasons and growing locations. A combined calibration was useful (ie. 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 in-line setting, and report on the robustness of calibrations across varieties, time and locality.

Materials and Methods

Two low cost (<$5,000) miniaturised spectrometers were chosen for comparison, based on use of differing detector technologies (CCD and photodiode array in Ocean Optics S2000 and Zeiss MMS1, respectively).

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, 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 a Hg-Ar lamp (Ocean Optics) was used in the characterisation of resolution. A bifurcated fibre optic interactance probe, consisting of eight 400 μm illuminating fibres concentrically arranged about a single 400 μm read fibre was used in conjunction with a tungsten halogen lamp (Ocean Optics) to gather spectra of sucrose solution soaked cellulose filter papers for calibration characterisation. Melons were obtained from commercial farms, with spectral collection and juice extraction and TSS determination made on the same day, and not more than 5 days after harvest.

Results and Discussion

Wavelength Resolution

Wavelength resolution of the MMS1 and S2000 instruments is illustrated by the FWHM of the 912 nm peak of the Hg-Ar lamp spectra. The MMS1 achieved a 13 nm resolution, while the S2000 achieved a 2 nm resolution (Fig. 1). Given that the second and third overtone bands assessed using NIRS are typically broad spectral features, ca. 50 nm, a resolution of less than 20 nm should not be necessary. This

view is reinforced by the spectral averaging option typically employed in chemometric procedures (e.g. Guthrie *et al.*, 1998). 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 developed using the two spectrometers. Indeed, calibration performance was degraded at the highest wavelength resolution employed (7.7 nm), representing diffraction and stray light issues at the narrow slit width employed. 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 two 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 (Fig. 2A). 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 signal to standard error ratio of the MMS1 reached a maximum of 40,000, in contrast to only 1,000 for the S2000 (Fig. 2A). This result was expected, insomuch as photodiodes deliver a lower signal to noise ratio than CCDs.

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 (Fig. 2B). This result reflects the wider slit width 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; Fig. 2B). This result is contrary to that expected on the basis of PDA and CCD detector type, 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 (Fig. 3). However, the S2000 unit demonstrated greater fluctuations (ca. 1% change), with continuing fluctuation after 1.5 h (Fig. 3). 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 ca. 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 of cellulose soaked with a range of sucrose solutions. Signal to noise ratio was varied by changing signal level by altering 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 calibration purposes using the MMS1.

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 five 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 penetration through 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. Light re-emission from the fruit was assessed by measuring light emission from a 5 cm diameter area of the fruit surface while illuminating a shrouded 10 cm^2 area of the fruit.

Light was diffusely scattered through the melon flesh (mesocarp), but assumed some directionality through the seed cavity of the fruit (data not shown). The contact angle of the light beam with the fruit surface was essentially irrelevant because of the diffuse scattering, with the detected light level determined by the distance from illuminated area to detected area. For convenience, however, lamp and detector were aligned with the centre of the fruit. There is a compromise position between 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 allows for a longer pathlength, representing more of the edible flesh of the fruit, but give a low (noisy) signal. However, full transmission mode (lamp-detector angle of 180^0) 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^o lamp-fruit-detector angle). With four lamps positioned at 90^o steps around the fruit, with an angle of 45^o between the fruit detector, a signal near detector saturation (at 200 ms integration) was achieved. 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: 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
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

Sampling and Soluble Sugar Content of Fruit

Soluble sugar content was variable within the melon fruit, varying with longitudinal position within the fruit by ca. 4% TSS, with circumferential position by ca. 1% TSS, and with depth (skin to seed cavity) by ca. 4% TSS. This observation is consistent with the report of Peiris *et al.* (1999). Thus the four lamp 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 was assessed in terms of calibration performance (data not shown). The optimal method involved removal of 0.5 mm cores at a point between the centre of each of the four illuminated areas and the detected area and trimming of skin and seed cavity material from these cores, before pressing to extract juice for the refractometer measurement.

Calibration Development and Robustness

Outlier spectra were removed from calibration population sets using the 3.0 Global H criterion of WinISI software. A calibration developed on a single Doubloon population (n=200) failed to predict the TSS of other Doubloon 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 Doubloon 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 Neighbourhood H criterion on SECV. A Neighbourhood H of 0.2 and 0.80 decreased the number of spectra from 1997 to 647 for this calibration sets, without loss of prediction accuracy (Table 3).

The calibration developed across five Doubloon populations predicted another Doubloon population well, and predicted population of 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 10 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.

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Table 1: Calibration performance in terms of standard error of cross validation with respect to spectrometer wavelength resolution and signal to noise ratio.

FWHM (912 nm	SECV	Treatment	S/N	SECV
7.7	2.02 a	2K counts	**1400**	**2.02 a**
10.6	1.29 b	8K counts	4600	1.29 b
13.8	1.22 b	32K counts	9700	1.22 b
16.7	1.29 b	2 scans	15900	1.29 b
20.0	1.46 b	16 scans	30300	1.46 b

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

NH	Population Number	SEP(C) (° Brix)
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 variety Dublin (DubA), (b) five populations (1000 spectra) of variety Dublin A (Duba-E) and (c) ten populations (2,000 spectra) of five varieties of melon (5var) on prediction of melon sweetness. Calibration groups for (b) and (c) were selected using a criterion of 1.0 NH.

Validation group	SECV (° Brix) Calibration groups		
	DubA	DubA-E#	5var.#
DubloonA	0.53*	0.62*	0.72*
DubloonB	1.33	0.86*	0.93*
DubloonC	1.28	0.66*	0.75*
DubloonD	1.17	0.74*	0.79*
DubloonE	1.13	0.92*	1.03*
DubloonF	0.93	0.66	0.67
DubloonA-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*

Appendix 3
Presentation to Near Infra-red Spectroscopy Group, Horsham, Vic. April, 2000.

Calibration robustness with respect to variety and district in the assessment of soluble solids content of melons

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Near infra-red (NIR) spectroscopy has been used for the non-invasive assessment of intact fruit for eating quality attributes such as total soluble solids (TSS) content. However, little information is available in the literature with respect to the robustness of such calibration across varieties, growing location, or growing conditions (but see Peiris *et al.* 1998, Guthrie *et al.* 1998 and Walsh *et al.* 1999). Using a bench-top 'at-line' unit developed on the same hardware base (viz. detector type, as described in Walsh *et al.* 2000), we have collected spectra from fruit populations through the 1998 and 1999 growing seasons. The optimal calibration procedure for this application involved a second derivative function over a gap size of four points, with no scatter correction or smoothing. Calibration robustness across varieties, growing districts and time of season, and the performance of a combined population set calibration (e.g. SECV = 076, ratio of standard deviation of population / SEP = 1.8), is reported. Optimisation of calibration population structure (using the ISI protocols of neighbourhood and global distances) resulted in the elimination of over 75% of the initial data set. The use of the ISI Local Calibration routine resulted in an improved calibration performance.

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