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Comparison of three near infrared spectrophotometers for infestation detection in wild blueberries using multivariate calibration models

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A near infrared (NIR) spectroscopy system for rapid, automated and non-destructive detection of insect infestation in blueberries is desirable to ensure high quality fruit for the fresh and processed markets. The selection of suitable instruments is the first step in system development. Three diode array spectrophotometers were evaluated based on technical specifications and capacity for larva detection in wild blueberries (*Vaccinium angustifolium*) using discriminant partial least squares (PLS) regression models. These instruments, differing mainly in wavelength range and detector type, comprised two spectrophotometers with scanning wavelength ranges of 650–1100 nm and 600–1700 nm and an imaging spectrograph with the scanning range of 950–1400 nm. The assessed factors affecting predictions included signal-to-noise ratio, wavelength range, resolution, measurement configuration, spectral pre-processing and absorbance bands related to infestation. The scanning spectrophotometers demonstrated higher signal-to-noise ratios with infestation prediction accuracies of 82% and 76.9% compared to the imaging spectrograph with 58.9% accuracy. Resolution, spectral pre-processing and measurement configuration had a lesser effect on model accuracy than wavelength range. The 950–1690 nm bands were identified as important for infestation prediction. In general, NIR spectroscopy should be a feasible technique for rapid classification of insect infestation in fruit.

Keywords: NIR spectroscopy, insect infestation, non-destructive detection, fruit, blueberry, instrument comparison

Introduction

There is an increased consumer interest in the potential health benefits from regular consumption of blueberries (*Vaccinium* sp.) due to their antioxidant properties shown to be correlated to inhibition of cancer cell proliferation.[1] A major quality concern for the blueberry industry is the internal infestation of fruit by the blueberry fruit fly larvae.

For the last few decades, near infrared (NIR) spectroscopy has shown considerable promise for the non-destructive analysis of food products and is ideally suited for on-line measurements in the agrofood industry due to its advantages: minimal or no sample preparation, versatility, speed and low-cost of analysis.[2] Most of the well-known applications of NIR spectroscopy in fruits[3] have focused on the quantitative prediction of chemical composition, internal damage and ripening stage in various fruits such as: kiwifruit,[4,5] apple and mango,[6,7] cherry,[8] grape,[9] plum and nectarine[10] and dates.[11]

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The limited number of reports of NIR technology on blueberries have focused on predicting firmness,[12] soluble solids content and bioactive compounds (anthocyanins, flavanoids, phenols and ascorbic acid),[12,13] as well as detection of internal foreign substances by NIR imaging.[14] However, to the best of our knowledge, the potential of NIR technology for the non-invasive detection of larvae infestation in individual blueberries has not been addressed in the literature.

NIR spectroscopy has been successfully applied for the prediction of internal insect infestation in grains. Ridgway and Chambers[15,16] reported using reflectance mode NIR spectroscopy to detect external and internal insect infestation of wheat kernels. They attributed their success to the detection of insect protein and chitin and moisture differences in the infested wheat samples. Dowell *et al.*[17] were able to detect larger larvae of three different species using an automated NIR system. Another application[18] resulted in the detection of parasitised rice weevils internal in wheat kernels.

The accuracy of a spectroscopic measurement relies mainly on the technical specifications of the instrument and on sample presentation. The type of light source and detector technology, measurement principle and configuration, signal-to-noise ratio, wavelength range and resolution are among the most important technical parameters. The selection of an instrument suitable for on-line blueberry measurements involves additional constraints: it should be small in size, deliver robust performance in vibrating environments and operate in reflectance mode. These requirements make it more difficult to decide which instrument should be preferred for infestation detection in blueberries.

Previous studies of fruit and grain testing using NIR have largely involved research grade instruments, with a wavelength range between 500–2500 nm, which are often unsuitable for a manufacturing environment.[6–8,17–20] However, multiple studies have reported successful application of low-cost, shortwave (500–1100 nm) NIR instruments for fruit quality assessment.[9,21–23] Walsh *et al.*[22] compared three instruments with diode array detectors and a 650–1050 nm wavelength range and recommended instruments with high signal-to-noise ratio, high sensitivity, rapid spectral acquisition and tolerance to vibration and dust for on-line assessment of melons. Perez-Mendoza *et al.*[24] compared two NIR spectrophotometers, a diode array and a scanning monochromator, and one FT-NIR interferometer with wavelengths spanning the 400–2500 nm range for determining the number of insect fragments in flour from infested wheat. They identified wavelengths contributing to detection believed to correspond to protein, lipids and starch NIR absorbance. Paz *et al.*[25] compared a scanning monochromator, a diode array and a combination instrument within the 400–2500 nm range, which showed similar accuracy in measuring the quality of apples. Resolution is also an important technical characteristic. Schimleck *et al.* demonstrated that the quality of the calibrations for various wood properties was affected to different degrees as resolution was decreased.[26]

NIR detection of internal larvae in blueberries presents a different matrix compared to most previous fruit and grain studies, requiring the assessment of optimal spectrophotometer specifications. Possible challenges include strong water absorbance at 1400–1600 nm which may overlap some infestation-specific absorbance in aqueous fruit samples compared to grains, variability due to the large genetic diversity of wild blueberries and different stages of larva development at the time of measurement. On the other hand, spectrophotometers with a wavelength range of up to only 1100 nm might not include sufficient wavelengths correlated to internal infestation in fruit.

The present study assessed the feasibility of detecting larvae in Maine wild blueberries (*Vaccinium angustifolium*) using one photo diode array (PDA) and two charge-coupled device (CCD) spectrophotometers, selected for their speed, robustness and the lack of moving parts. The specific objectives of the study were: (1) to compare three commercially available NIR spectrophotometers based on their attributes, i.e. wavelength range and resolution, optical setup and signal-to-noise ratio (S/N) with respect to their classification capacity of larvae infestation in individual blueberries for a potential in-process-line application; and (2) to assess the feasibility of using NIR spectra in partial least squares (PLS) models, after different spectral treatments, as a means for infestation classification of blueberries. We also present explanations of NIR wavelength bands likely to be related to larvae infestation in blueberries.

Materials and methods

Instruments

Three NIR systems were investigated: the low-cost, miniature system, Ocean Optics SD2000, the research grade Perten DA7000 and a custom-built NIR imaging spectrograph, Oriel MS-257. Their main technical differences included wavelength range, resolution and detector type. The technical specifications of these instruments are summarised in Table 1.

The SD2000 is a vis-NIR system from Ocean Optics with a silicon (Si) CCD detector and a factory installed longpass filter (OG590) blocking wavelengths < 590 nm. It utilises an L2 lens and a 25 µm wide slit providing a nominal bandwidth of 650–1100 nm. The useful wavelength range, excluding high noise bands, in this analysis was 650–1090 nm. The light source and collimating lens were positioned approximately 40 mm from the sample and reflected light was collected at 45° from the incident light via a single-strand optical fibre. The light source and the receiving fibre were mounted on a hemispheric aluminium chamber, coated with non-reflective black paint, blocking ambient light during measurements.

The optics of the Perten DA7000 include a sensor module comprised of two PDA detectors, Si and indium–gallium–arsenide (InGaAs), and a source module comprised of a tungsten halogen lamp, chopper and chopper motor which eliminate ambient light interference. The DA7000 operates

Table 1. Basic technical characteristics of three commercially available spectrophotometers: the Ocean Optics SD2000, the Perten DA7000 and the Oriel MS-257.

Property	Instrument		
	SD2000	DA7000	MS-257
Manufacturer	Ocean Optics, Inc., Dunedin, FL, USA	Perten Instruments, Springfield, IL, USA	Oriel Instruments, Stratford, CT, USA
Detector type	2048 pixel CCD silicon array detector	76-pixel silicon diode array (400–950 nm) and 76-pixel InGaAs diode array (950–1700 nm)	InGaAs CCD camera 320 × 240 pixels
Dispersive element	Grating #14, 600 lines mm^{-1}	Stationary holographic grating	Grating #3, 600 lines mm^{-1}
Wavelength range in the study (nm)	650–1100	600–1690	950–1390
Resolution (nm)	0.92	0.5	0.2
Light source	360–2000 nm tungsten halogen lamp	360–2000 nm tungsten halogen lamp	360–2000 nm tungsten halogen lamp
Angle between source and detector fibres (degrees)	45	0	45
A/D convertor type	12-bit	12-bit	12-bit

in dual beam, i.e. the reference energy from the source is constantly recorded along with the sample energy. However, a spectrum of Spectralon standard material (Labsphere, North Sutton, NH, USA) with > 99% diffuse reflectivity was also recorded to account for background effects resulting from optical windows and spectral attenuation in the reference fibre. A bifurcated fibre bundle, comprised of source and detector fibres integrated into one cylindrical probe (0° angle), was directed vertically downward and positioned approximately 10 mm above the sample.

The MS-257 from Oriel Instruments used a model SU-270D InGaAs CCD camera from Sensors Unlimited Inc. (Princeton, NJ, USA) with exposure time F = 16 ms and nominal resolution 1.1 nm pixel^{-1}. The light source was focused on the sample in down-view mode. Reflected light was collected by two optic fibres each mounted at a 45° angle from the direction of incident light and approximately 5 mm from the sample. The two fibres were 600 µm in diameter with a numerical aperture of 0.22.

Spectrophotometer comparisons

As an estimate of signal-to-noise ratio, approximately 20 spectra of reflectance standards were collected at near saturation levels for each instrument. A Spectralon disc for the SD2000 and MS-257 and a Spectralon pill for the DA7000 instruments were used. The average spectrum was divided by the standard deviation of the intensity counts at each wavelength.

Measured spectral wavelength resolution was varied by reducing the number of data points. The original number of points, 219, was consecutively reduced by factors of 2, 4 and 8 in the DA7000 spectra. SD2000 spectra with 1505 original number of points were reduced by a factor of 7 to 215 points, for comparison with DA7000 models and by factors of 14 and 25.

Fruit and artificial infestation

Fresh stems with ripe Maine wild blueberries were collected during the harvest season and kept in vials with water in ovipostion cages with laboratory-raised blueberry maggot flies to induce artificial infestation. After one week, the stems were removed from the cage and were held for seven to ten days at 23–25°C to allow the fly larvae to mature to a typical size similar to field conditions. Blueberries were then sized using a sizing template. Subsets of blueberry samples were measured with each of the three NIR instruments recording absorbance spectra [log(1/*R*)]. This laboratory method developed by Drummond[27] provided approximately 50% larvae infestation ratio in the final subsets.

Near infrared spectroscopy

The samples sets for the three instruments contained different numbers of fruit sample: 404 (SD2000), 553 (DA7000) and 92 (MS-257). The number of samples in the MS-257 set was limited due to fewer infested fruit available at the time and fruit damage during the shipment process. All spectra were collected in diffuse reflectance mode with the blueberry stem end facing the light source. Three replicate spectra per blueberry, each an average of 32 scans, were collected with the SD2000 and then averaged. One spectrum from each fruit, an average of 15 scans, was recorded with the DA7000 spectrophotometer. Two NIR images, one image from each fibre, for each fruit were recorded with the MS-257 system. The images were then processed and averaged resulting in one spectrum per fruit in the calibration model. After NIR measurements were taken, all berries were dissected under a light microscope to determine larvae presence.

Calibration models and data treatment

Absorbance spectra of individual fruit were imported into the GRAMS PLSPlus/IQ software (version 6.00, Thermo Galactic Industries Corporation, Salem, NH, USA). Partial least squares (PLS) regression models were built including the reference microscopic data. PLS was implemented in the form of discriminant PLS models using arbitrary categorical variables representing membership or non-membership of the infested fruit group rather than continuous analyte concentrations.[28] Non-infested and infested blueberries were arbitrarily assigned a value of 1 and 2, respectively. Spectral data were regressed on the assigned reference values after mean centring and variance scaling. A blueberry was classified as infested if its predicted value was above 1.5 and classified as non-infested if the value was below 1.5.

Spectral pre-processing included multiplicative scatter correction (MSC) and first derivative using Savitzky–Golay derivation.[28] The optimum number of PLS factors (independent variables) was selected on the basis of minimised standard error of cross-validation (*SECV*), *F*-tests of the *SECV* and examining the regression coefficients plots. Outliers were identified by examining the raw spectra, actual vs predicted plots and spectral and concentration residuals plots in the PLSPlus/IQ software. Cross-validation was performed with random subsets of ten spectra (SD2000 and DA7000) and two spectra (MS-257) at each pass until all sample spectra had been included in calibration and validation sets. The accuracy of the models was evaluated by the percentage of correctly classified samples and *SECV*.

To establish statistical significance, PLS models with random subset cross-validation were repeated ten times for each type of pre-processing and instrument. Average percentages of correctly classified samples (prediction ratios) and *SECV* were reported for each model type. The prediction ratios and the *SECV* between the different instruments and pre-processing were compared statistically, based on the *t*-test with significance level $\alpha = 0.05$.

Results and discussion

Instrument signal-to-noise ratio

The maximum signal-to-noise (S/N) ratio was recorded for each instrument: 31,650 intensity (A/D) counts at 682 nm for DA7000, 80 counts at 1050 nm for SD2000 and 54 counts at 988 nm for MS-257 (Figure 1). The DA7000 S/N profile was noisier than the profiles of SD2000 and MS-257 but its maximum average S/N was two orders of magnitude higher at approximately 26,000 counts. The spectral shape of the noise for DA7000 and MS-257 generally followed that of the spectral signal (not shown), reflecting the importance of the signal shot noise.[22] For the SD2000, the noise shape was different to the spectral shape (not shown) indicating more important electronics read-out noise.[22] As expected, the PDA detector (DA7000) had a significantly higher S/N than the two CCD detectors (SD2000 and MS-257).

Walsh *et al.*[22] estimated the S/N of three systems by dividing the mean of 50 spectra by the standard deviation for each wavelength at near saturation levels. Their reported S/N were 40,000, 1000 and 4000 for the PDA and two CCD units, respectively. The S/N for their PDA unit was slightly higher than the

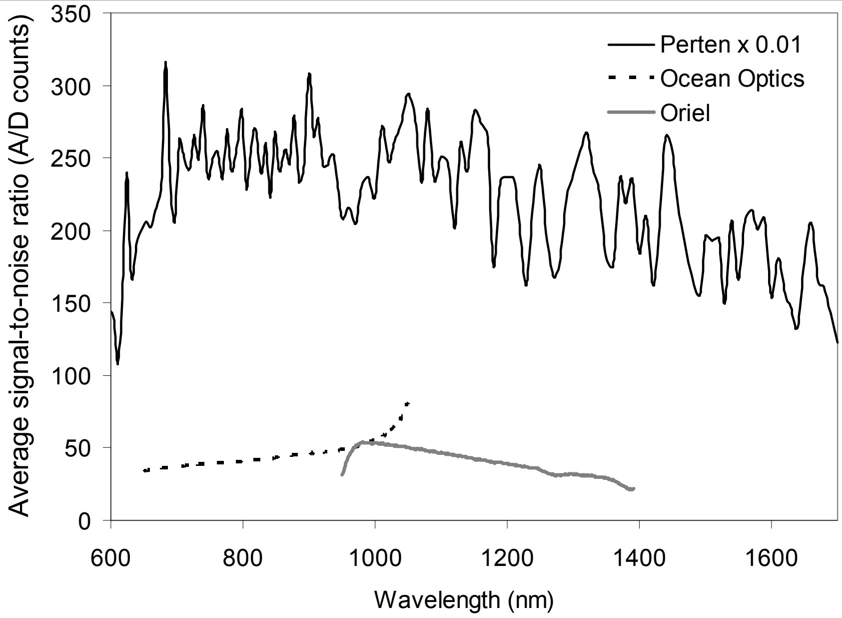

Figure 1. Signal to standard error ratio of spectra collected with the Ocean Optics SD2000, Perten DA7000 and Oriel MS-257 spectrophotometers.

S/N value for the DA7000 and can be attributed to the smaller number of spectra (28) used in our calculations. The differences in S/N of the CCD units are difficult to explain without considering factors such as detector electronics, light source and number of spectra. Overall, in both studies, the PDA units showed considerably higher S/N than the CCD units.

Calibration models and infestation prediction capacity

The results of the PLS discriminant models are summarised in Table 2. Superscript letters (a, b, c etc.) indicate statistically significant differences between models based on instrument and pre-processing type ($\alpha = 0.05$).

When comparing PLS models with no pre-processing, the Perten DA7000 demonstrated the highest prediction ratio of 81%, followed by the Ocean Optics SD2000 with 75.6% and the Oriel MS-257 with 58.9% (Table 2). PLS models with applied MSC from the DA7000 and the SD2000 instruments yielded results which were not significantly different at ($\alpha = 0.05$), while the MS-257 model was inferior. Prediction results after 1st derivative were significantly better in the DA7000 models, no difference in the SD2000 models and lower in the MS-257 models. Overall, the DA7000 models were most accurate, followed by the SD2000 and MS-257 models. The inferior MS-257 models were likely to be due to observed higher noise in the sample spectra and the small number of samples used in the calibrations.

Comparison of pre-processing methods showed that MSC led to slight model improvement for the SD2000 and first derivative led to slight improvement in the DA7000 models. This is likely to be due to the fact that the 45° angle optical configuration in the SD2000 configuration is more prone to light scatter and MSC corrects some of the scatter effects in the spectra. In contrast, the first derivative removes spectral baseline effects and corrects for baseline shifts due to the variation in fruit size in the 0° angle optical configuration of the DA7000. Pre-processing had no effect on accuracy for the MS-257 models. In general, pre-processing led to prediction differences of 0% to 2.2%, which might be considered insignificant in an industrial application. These results are similar to the findings of other authors who observed that spectral pre-processing had some effect on PLS predictions, but statistical significance was often not established.[20,29]

The models with no pre-processing for SD2000 and DA7000 were repeated after reducing the number of spectral points and for different wavelength ranges to study the effects of spectral resolution and wavelength range on model accuracy (Table 3). Model accuracy was almost not affected by reduced resolution for the DA7000 and slightly affected for the SD2000 models after reducing the number of points to 61. Thus, resolution seemed to have a minimal contribution to model accuracy and indicated that reducing the number of wavelength points can potentially be used to improve the speed of an on-line system. Comparing the models with the same wavelength ranges from SD2000 and DA7000 showed similar accuracy for the 650–1050 nm. DA7000 performed slightly better than SD2000 at the 650–850 nm range and better in the 850–1050 nm range likely due to the less noisy InGaAs than the Si detector in the longer range. These results also indicated that both the 45° and the 0° scanning configurations provided equivalent performance over the same wavelength range. DA7000 models in the 950–1390 nm and 1390–1690 nm range were as accurate as full wavelength models suggesting that wavelengths longer than 950 nm provide more infestation related information. Overall, both the Si and the InGaAs detector seemed to provide similar prediction accuracy at their respective wavelength ranges.

The reported infestation prediction ratios in this study are slightly lower than some reports on infestation prediction in wheat kernels.[17,18] However, the difficulty in such comparisons stems from the fact that there are no other blueberry infestation studies and blueberries present a different matrix than kernels. Larvae infestation is also expected to have a different effect on the fruit chemical and physical properties. Larva feeding inside the fruit disrupts the internal matrix which affects fruit shape and firmness. Due to the high water content in blueberries of 85–87%[30] compared to 10–13% in wheat, there is a likely overlap of infestation related wavelength bands

Table 2. PLS infestation prediction results from the Ocean Optics SD2000, Perten DA7000 and Oriel MS-257 instruments. The superscript letters (a, b, c, d, e, f and g) indicate statistically different prediction levels ($\alpha = 0.05$).

Instrument	Ocean Optics SD2000			Perten DA7000			Oriel MS-257		
Total number of spectra (non-infested, infested)	404 (233, 171)			553 (398, 155)			92 (34, 58)		
Pre-processing	None	MSC	SG1	None	MSC	SG1	None	MSC	SG1
Number of PLS factors	9	7	5	9	9	6	5	5	5
Total correct prediction (%)	75.6^{a}	76.9^{b}	76.3^{ab}	81.0^{c}	77.8^{db}	82.0^{e}	58.9^{f}	58.0^{f}	47.5^{g}
SECV	0.42	0.42	0.41	0.41	0.42	0.39	0.53	0.52	0.60

SECV: standard error of cross-validation; None: no pre-processing; MSC: multiplicative scatter correction; SG1: Savitzky–Golay first derivative

Table 3. Effects of wavelength range and resolution on PLS prediction results for the Ocean Optics SD2000 and the Perten DA7000. Model parameters and data sets were identical to the models without pre-processing in Table 2.

Instrument	Wavelength range (nm)	Wavelength points	Number of PLS factors	Total correct prediction (%)	*SECV*
Ocean Optics SD2000	650–1100	1505	9	77.9	0.42
	650–1100	215	9	76.9	0.42
	650–1100	108	9	77.4	0.41
	650–1100	61	9	75.4	0.42
	650–850	96	10	71.9	0.45
	850–1050	96	9	72.9	0.43
Perten DA7000	600–1690	219	8	80.1	0.41
	600–1690	110	8	79.9	0.41
	600–1690	55	8	79.9	0.41
	600–1690	28	8	79.8	0.41
	650–1050	81	11	77.4	0.40
	650–850	41	8	76.7	0.40
	850–1050	41	9	79.0	0.41
	950–1390	89	8	81.7	0.39
	1390–1690	61	6	80.3	0.40

SECV: standard error of cross-validation

with the strong water absorption bands around 1450 nm. High moisture is known to dramatically reduce prediction accuracy if the calibration model is detecting infestations based on the high moisture content of the larvae.[17] In addition, the high genetic diversity in wild blueberries compared to cultivated fruit or grains, variation in larvae maturity and larvae location inside the blueberry introduce additional variability in the spectral data possibly reducing prediction accuracy.

Wavelength regions

Plots of PLS factor weights indicating absorbance bands contributing to prediction for SD2000 and DA7000 models

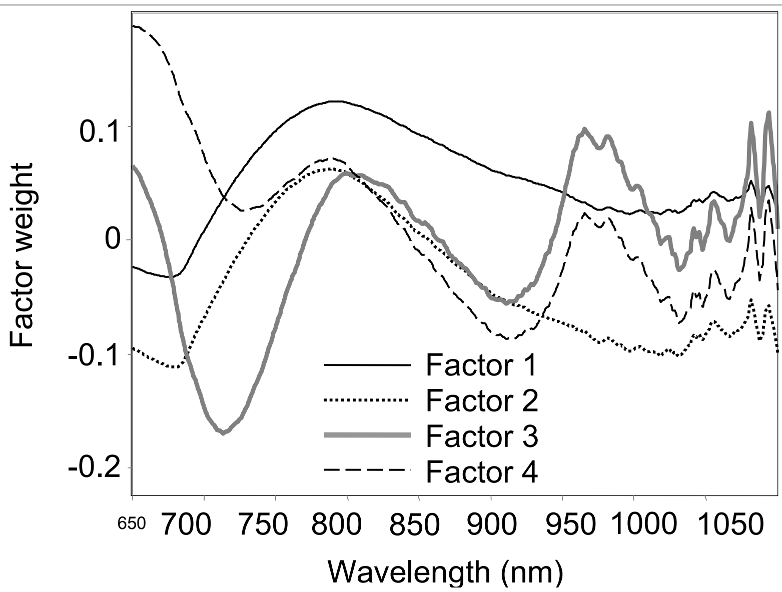

Figure 2. Factor weights with four factors of PLS regression models from the Ocean Optics SD2000 spectrophotometer indicating wavelengths correlated to infestation prediction in blueberries.

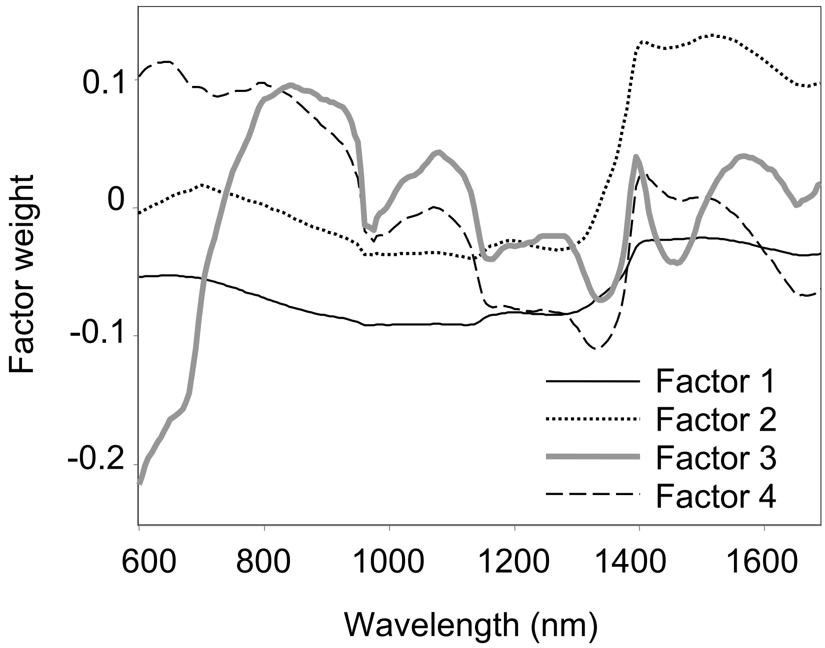

Figure 3. Factor weights with four factors of PLS regression models from the Perten DA7000 spectrophotometer indicating wavelengths correlated to infestation prediction in blueberries.

are presented in Figure 2 and Figure 3, respectively. Factor weights for the MS-257 models were not considered here because of their poor prediction as previously established. The first four factors explain approximately 99% of the spectral variation (data not shown), where the 1st factors explained from 74% to 77% of the spectral variation.

The first two (1st and 2nd) and the second two (3rd and 4th) factors can be grouped in pairs due to similar shape and likely similar chemical origin (Figures 2 and 3). The most important peaks in the factor weights are summarised in Table 4. The 650–750 nm absorption bands in both instruments indicated the importance of colour carried in blueberries mainly by anthocyanins and chlorophyll. Other important bands in the SD2000 models (Figure 2) include 800 nm and 920 nm assigned to –CH third overtone and 980 nm assigned to –OH second overtone.[31] These absorption bands are assigned to mono- and poly-carbohydrates (fructose, glucose, pectin) and water in blueberries.[13] The 982 nm band has also been identified as a key for infestation detection in wheat.[32]

For the DA7000 instrument, the factor weight profiles in Figure 3 (850–920 nm, 1080 nm, 1160nm and 1335 nm) correspond closely to the first, second and third overtones of –CH, –OH and –NH assigned to carbohydrates, water and peptides.[17] The 1420–1480 nm region coincides with the absorption bands of water and peptide bonds. Although increased amino acid concentrations in fruit have been linked to infestation,[33] water is the most likely absorber. The 1700 nm bands have also been attributed to chitin in the insect cuticle (CH or CH_2 groups).[15] The 1140–1370 nm and 1550–1700 nm absorbance bands have been related to infestation in grains and seeds.[17,19]

Interestingly, the first two weight profiles (1st and 2nd) were analogous to a NIR spectrum of a live larva (Figure 4) and the second two profiles (3rd and 4th) to the difference between averaged larva and non-infested blueberry spectra. This suggests

Table 4. Summary of important absorption bands in SD2000 and DA7000 spectra identified by PLS factor weights (Figures 2 and 3). Peak or valley width was measured at half height from zero line.

Peak and valley positions in factor weights ± width (nm)		
Factor number	Ocean Optics SD2000	Perten DA7000
1	677 ± 11, 790 ± 60	600–1370, 1500–1700
2	678 ± 29, 787 ± 36, 1018 ± 83	698 ± 40, 900–1350, 1400–1700
3	650 ± 13, 714 ± 22, 800 ± 20, 912 ± 25, 980 ± 20	609 ± 85, 843 ± 76, 1079 ± 46, 1160 ± 20, 1337 ± 40, 1395 ± 10, 1454 ± 30, 1568 ± 40
4	654 ± 36, 788 ± 30, 911 ± 30, 980 ± 5, 1030 ± 29	650 ± 45, 797 ± 110, 1165 ± 35, 1335 ± 45, 1409 ± 20, 1652 ± 60

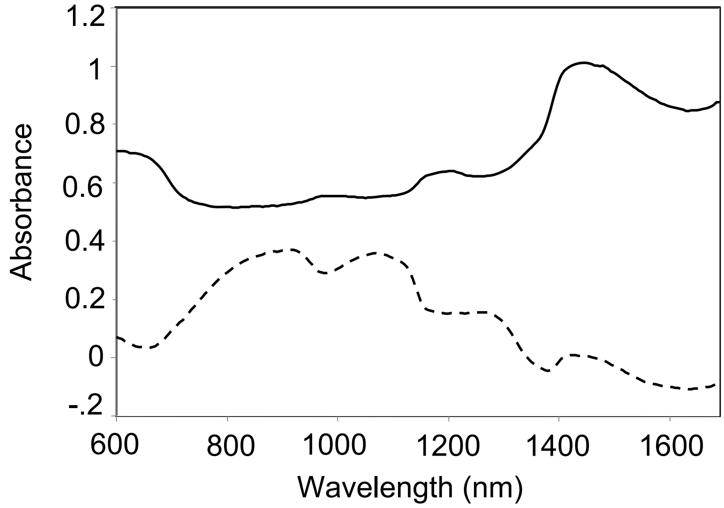

Figure 4. NIR spectra of a live larva (solid line) and a subtraction of a blueberry spectrum from a larva spectrum (dashed line). Spectra were collected with the Perten DA7000 spectrophotometer.

that the origin of the NIR signal is from the larva and the associated chemical changes in the blueberries.

These results and the results from wavelength range comparisons suggest that the wider wavelength range of the DA7000 instrument provides a prediction advantage resulting in slightly better PLS models than the SD2000 instrument.

Conclusions

This study showed that NIR spectroscopy is a feasible technique for rapid classification of insect infested fruit considering that the conventional methods for blueberry infestation detection are slow, destructive and use a small number of random samples. In general, the Perten DA7000 and the Ocean Optics SD2000 instruments were suitable for infestation prediction in wild blueberries. The PLS models from the Oriel MS-257 instrument were inferior which was likely due to the small number of samples and higher spectral noise. The DA7000 had the highest S/N and provided the highest infestation prediction ratio. However, the SD2000 PLS models were only 3–6% less accurate despite lower S/N and shorter wavelength range. Thus, the Ocean Optics SD2000 can be a viable alternative in applications requiring high portability and low cost. However, further optimisation of the PLS prediction models and validation with fruit from different seasons and field origin are required before this technique is adopted in routine analysis. Estimation of detection limits measured by larvae size should also be incorporated in future studies.

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