Feasibility study for non-invasive detection of fruit fly eggs and larvae in intact mangoes using a hand-held near infared instrument

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Introduction

Fruit fly is one of the major pests causing significant damage and economic losses to growers, processors, and exporters of fruit worldwide. Some countries or areas such as Japan, parts of the United States, and Southern Australia, are considered fruit fly free zones while certain others are considered fruit fly habitats. Many fruit fly free zones require fruit imported from habitat zones to be disinfected using techniques such as vapour heat treatment (VHT) or irradiation. The treatments, while effective, can impact food quality. In addition, many people are opposed to food irradiation.

Most fruits, both ripe and unripe, are comprised of water and carbohydrates, either in the form of starch or sugar. On the other hand, the major constituents of insects are proteins and lipids. This suggests that NIR spectroscopy is ideally suited as a method for detecting fruit containing insects by measuring the increase in protein and/or fat content. In this study the feasibility of using NIR spectroscopy as the basis for a real-time detection system for fruit fly eggs and larvae in intact mangoes was investigated.

Materials and methods

Mangoes

Export grade bagged mature green mangoes (*Mangifera indica* cv. Nam Dok Mai and cv. Rad were used. The fruits were kept in an air-conditioned room at approximately 25 ºC for one night, and then were subjected to the forced infestation treatment. None of the mangoes used in this experiment were treated with VHT or irradiation.

Fruit fly infestation

Both control and soon to be infested mangoes were pored using sterile sewing needles. A total of nine pores of 2-mm depth were made within a 1 sq.cm area on the shoulder of each fruit. Forced infestation was accomplished by placing the pored mangoes in a cage holding about 2000 - 2500 Oriental fruit flies for 30 minutes. Control fruits were kept under nets to prevent infestation. After the final NIR measurements, a 3 cm × 3 cm area surrounding the location of the 9 pores was cut aseptically. The fruit portion was kept in an air-circulated sterile box for 5 days for the enumeration of fruit fly larvae (instar 3).

Spectral acquisition

A portable silicon diode array instrument ("FQA-NIR Gun", Shizuoka Shibuya Seiki, Hamamatsu, Japan), measuring spectra in the short wavelength region from 700 nm to 1100 nm and in the interactance mode was used (Figure 1).

Three consecutive NIR measurements were performed. Prior to NIR measurements, mango samples were dipped into a 25°C water bath for 15 minutes. A polyethylene sheet was used to cover the water surface, preventing direct contact between the fruit and the water. NIR measurements were performed at 0, 24, 36 and 48 hours after infestation.

Data analysis

The three spectra of each sample measured were averaged prior to a second derivative pretreatment. Both original [log(1/*R*)] and second derivative [d2log(1/*R*)] spectra were utilised to find the best classification model, using partial least squares discriminant analysis (PLS-DA). The infested samples were assigned a dummy value of "1" and the control ones were assigned a value

Figure 1. NIR measurement by the FQA-NIR Gun.

of "0." Optimisations of wavelength region were performed at 50 nm intervals. Optimisations of the averaging size of the Savitzky-Golay second derivative were also conducted. Validation of the developed equations was performed using full cross validation. All calculations were performed using The Unscrambler software (CAMO, Oslo, Norway).

Results and discussion

NIR spectra

In the second derivative [$d^2 2 \log(1/R)$] spectra of unripe green mangoes three negative peaks were observed at the vicinities of 740 nm, 829 nm and 951 nm. From the shape of the spectra and the similar shifting size of approximately 20 nm, it was assumed that these peaks were water bands typically observed at the vicinities of 760 nm, 844 nm and 970 nm when NIR spectra were acquired with other NIR instruments such as the NIRSystems 6250 or 6500.[1,2]

For non-infested mangoes, no obvious spectral shift was observed at any time point when compared to 0-day spectra. However, for the infested samples, a shift in the location of the peak maxima and an increase in the peak magnitude were observed in the vicinity of 951 nm for time points beyond 24 hours. Thus, it was assumed that the shifts were not related to the presence of fruit fly eggs in the samples, but rather to the presence of larvae which would have hatched at 12-24 hours after infestation.

Classification

Table 1 shows the PLS-DA classification results for the cross validation of infested and control fruits at different periods after infestation for each mango cultivar.

Table 1. PLS-DA classification results for the cross validation of infested and non-infested Nam Dok Mai (NDM) and Rad mangoes.

Samples	Wavelength (nm)	F	Non-infested		Infested	
			Correct	False	Correct	False
NDM-00hr	700–950	6	74	22	59	37
NDM-24hr	700–950	7	93	3	89	7
NDM 36hr	700–950	7	93	3	86	10
NDM-48hr	700–950	7	95	1	90	6
Rad-00hr	700–950	5	64	14	48	30
Rad-24hr	700–950	6	77	1	70	8
Rad 36hr	700–950	7	78	0	74	4
Rad-48hr	700–950	6	78	0	74	4

F: The number of factors used; *SD*: Standard deviation of predicted class values; Second derivative condition: 28 nm averaging for each side, 2nd order polynomial;
Range of fruit fly larvae found in each fruit: NDM (54-441), Rad (51-385)

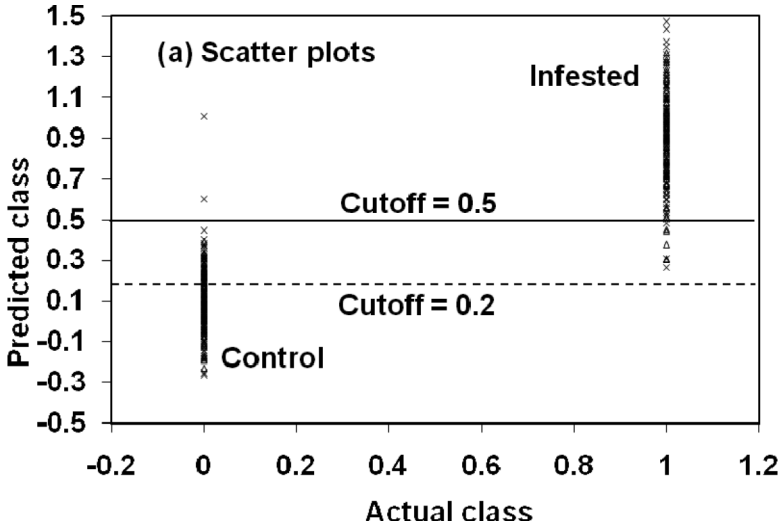

Figure 2. The plots of the best classification models developed for combined model of Nam dok mai and Rad mangoes for predicting the infestation classes using the full cross validation method.

The cut-off value was set at 0.5. Satisfactory calibration results were not obtained from spectra acquired soon after infestation (0 hr), but significant improvement was noted for spectra measured at later time points. The lowest number of miss-classified samples was obtained at the latest time point(s) of 48 hours after infestation for Nam Dok Mai mangoes and of 36 hours and 48 hours after infestation for Rad. The classification plots of the best calibration equations for predicting the infestation classes using the full cross validation method on the combined sample set of the two cultivars are shown in Figure 2.

The plots suggest that if the cut-off value was reduced to 0.2, complete removal of infested mangoes could be performed while more than 50% of the control ones could still be kept and exported without any disinfection treatment.

To understand the structure of the classification models developed, the regression coefficient plots of the PLS-DA equations were investigated. The plots indicate the key peak for classification at 734 nm, corresponding to the unique peak of dried larvae.

Conclusion

NIR spectroscopy in the short wavelength region was shown to be a promising technology for classifying mango fruit infested with fruit flies vs. non-infested samples. However, a certain period of incubation time is required to allow fruit fly larvae to hatch and develop. Better sensitivity, especially when only a small number of fruit flies are present, could be expected by using NIR imaging technology in which the information extracted from fruit is derived from a smaller area, thus reducing the dilution effect.

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