zeConfidential Research Report

Detection of 'Zebra Chip' Disease in 'Russet Burbank' Potatoes using Near Infrared (NIR) Spectroscopy

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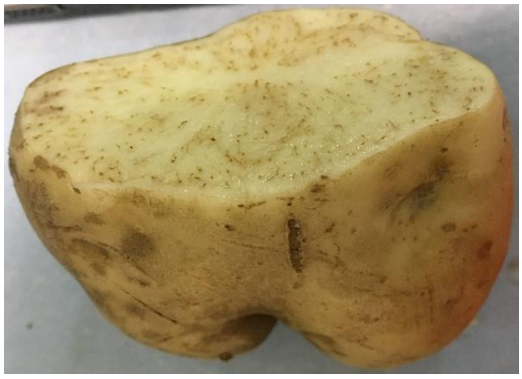

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1. Background

Near infrared (NIR) spectroscopy is a technique that has been used to evaluate the quality of various agricultural and horticultural commodities (Williams et al., 2006; Jha, 2010). For the potato industry, one of the first applications of NIR was to measure moisture content of chips (McDermott, 1988; Shiroma and Rodriguez-Saona, 2009; Ni et al., 2011), with high correlation ($r^2 > 0.90$) and low prediction errors (SEP < 0.30). Another application of NIR spectroscopy is the estimation of starch content and dry matter concentration (DMC) as these are directly related to the final quality of potato products and can influence the criteria for payments to farmers (López et al., 2013). The starch content and DMC were found to be predictable ($r^2 > 0.80$) in previous literature (Dull et al., 1989; Hartmann and Büning-Pfaue, 1998; Scanlon et al., 1999; Haase, 2003, 2006; Pedreschi et al., 2010). Peiris et al. (1999) found that the DMC was greater near the surface of the tuber and hence better correlation were found for the outside section of the tuber (Scanlon et al., 1997). Prediction of protein in various types of potato samples (Evans and Muir, 1999; Fernández-Ahumada et al., 2006) was less successful ($r^2 \approx 0.60 – 0.80$). In addition, sprouting capacity (Jeong et al., 2008) and fat content (Ni et al., 2011) were also estimated by NIR measurements.

For processing potatoes such as crisps, low sugar content is required, as during frying sugars react with amino compounds which are also present in the tuber, contributing to dark colours in the final product and poor quality of processed potatoes (Roe et al., 1990). For instance, the Zebra chip disease caused by the potato-tomato psyllid, liberibacter-infective *Bactericera cockerelli*, has been found in many countries including New Zealand (Liefting et al., 2008). The symptoms of infected potato tubers have been shown to have elevated levels of reducing sugars (Gao et al., 2009) which lead to overall darkening observed in raw tubers and the dark strips found in fried products (Buchman et al., 2011). As a result, prediction of processing quality of potato mainly focuses on the ability to provide information on reducing sugars with respect to Maillard reaction. Analyses of these sugar precursors are often costly. There is a need for an inexpensive and yet rapid prediction tool for quality control purposes (Haase, 2011).

Robust NIR calibration models to predict sugar content in potatoes have not been well established (López et al., 2013). Yet previous attempts to predict reducing sugars content in intact or processed tubers showed that rough estimation of these constituents was possible. Mehrübeoglu and Coté (1997) found that prediction of reducing sugars using NIR reflectance of intact tubers was possible for the cortical layer (R^2 = 0.90) but not accurate enough for estimating processing quality due to the low concentrations of these sugars. Hartmann and Büning-Pfaue (1998) found good correlation between NIR spectral data and the amount of total reducing sugars (R^2 = 0.82; % SEP = 0.06) and fructose (R^2 = 0.89) in ground potato samples, and reasonable correlation for sucrose (R^2 = 0.62) and glucose (R^2 = 0.70). Haase (2011) predicted the quality of ground potato aliquots using NIR reflectance spectroscopy, and found that the best R^2 for reducing sugars, sucrose and total sugar was 0.43, 0.71 and 0.66 respectively and the models were not good enough for screening purposes (SDR < 2.0). In a very recent study (published after our own experimental work), the detection of Zebra chip disease in 'Atlantic' potato tubers was found to be successful using NIR spectrometers in the range of 900 – 2500 nm (Liang et al., 2018), suggesting the potential of this technology to be applied in other potato cultivars.

In summary, there is potential for applying NIR to predict sugar concentrations of processing potatoes. However, the accuracy needs to be improved in order to justify a wider applicability. It may be possible to utilize NIR to identify individual tubers or batches of tubers infected with Zebra chip disease through prediction of reducing sugars. This can be useful for segregation of batches or lines into quality classes in order to perform screening of potatoes for processing. In addition, constituents of potato are not evenly distributed within and between tubers and hence obtaining representative samples of intact tubers is important (Haase, 2011) for developing robust NIR calibration models.

To test the industrial application of NIR, a proof of concept study was conducted. This study was proposed to develop a calibration model to qualitatively predict the incidence of Zebra chip disease in potato tubers. Industrial application of NIR requires segregation of diseased tubers prior to the peeling process, mainly because after peeling, tubers become wet and this wetness on the tuber surface could attenuate NIR signal and hence influence the intensity of light that reach the surface

of tuber causing NIR sampling error. Demonstrating NIR to be successful in segregating diseased tubers prior to peeling process, would improve potato processing and minimize peeling efforts for diseased tubers. Ensuring efficient removal of diseased tubers would reduce processing losses and also provide a potential future opportunity to access export markets such as Japan for intact tubers.

2. Objectives

1. Understand the potato processing system and scope appropriate NIR application stages at a commercial potato processing facility.
2. Collect NIR spectral data for both healthy and diseased tubers to develop a calibration model to segregate the two populations.
3. Collect an independent data set and use for validating the model segregation performance.
4. Make recommendations for industrial application of NIR to segregate potato tubers.

3. Materials and Methods

3.1 Calibration Data Collection

For calibration, NIR spectral data for each of 300 diseased and healthy intact 'Russet Burbank' potato tubers were collected on 26-27th Oct, 2017 at Talley's Group Limited, Ashburton, New Zealand. Samples collected from a batch which was harvested on 21-24th April, 2017 and stored at 8-12°C with 95% RH. Samples were collected at washing stage after removing the field dirt. For classification model development, each tuber was peeled from one side to visually confirm non-existence (healthy, Fig. 1a) and existence (diseased, Fig. 1b) of Zebra chip disease symptom of brown streaks. Upon correct visual categorisation of 300 tubers for each of the two categories, NIR spectral data of each tuber at 3 selected locations (around the equator at relatively flat surfaces) were collected for model calibration. The calibration data set was then used to develop a classification model to qualitatively segregate the tubers into two populations: healthy and diseased.

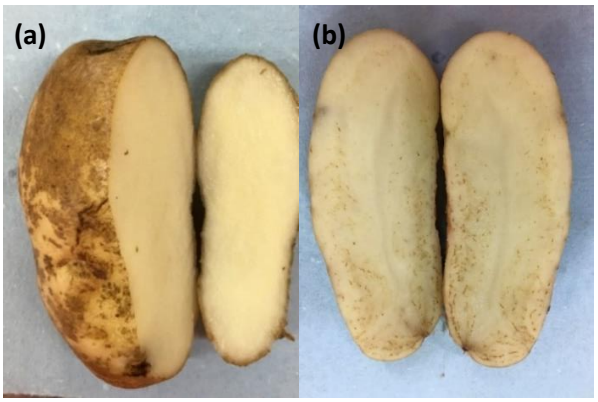

Figure 1: Healthy (a) and diseased (b) 'Russet Burbank' potato tubers were visually assessed to confirm the correct categorisation.

3.2 Validation Data Collection

For validation, NIR spectral data for each of 200 diseased and healthy intact tubers were collected on 2nd and 3rd Nov, 2017 at Talley's Group Limited, Ashburton, New Zealand. Samples were collected from the same batch as for calibration dataset. The developed model using calibration data set was then validated for prediction accuracy for different population of healthy and diseased tubers.

3.3 Vis-NIR Spectral Data Collection

A commercial full-range Vis-NIR spectroscopy system (FieldSpec® Pro, PANalytical., USA) was used for spectra collection. Within the instrument, three types of detectors are installed to cover both the visible and the NIR range of the spectrum including: a silicon detector (350 – 1000 nm); an InGaAs detector that measures shortwave infrared (1000 – 1800 nm); and a second InGaAs detector (1800 – 2500 nm). The optical fibre of the instrument was coupled with a contact probe (Hi-Brite, PANalytical B.V., Boulder, USA) for contact measurements with a spot size of 10 mm in diameter. The contact probe was fitted with a high intensity halogen lamp to produce consistent illumination in a broad electromagnetic spectrum. A diffuse reflectance material (Spectralon®, Labsphere Inc., North Sutton, USA) panel was used as a reflectance standard and to convert raw spectra to reflectance.

4. Data Analysis

4.1 Preprocessing of Vis-NIR Spectral Data

The raw NIR spectral data were preprocessed in RStudio (R Foundation for Statistical Computing, Vienna, Austria). Spectral data were first truncated to 400 – 2450 nm (Fig. 2a) so that fluctuation and noise at both ends were eliminated. Reflectance was then converted to absorbance by a Log transformation (Fig. 2b) which can be related to concentration by Beer's law. Second order derivation using a Savitzky-Golay smoothing algorithm was then applied (Fig. 2c). The purpose was to reveal the hidden information in the spectra as well as to reduce the noise in the data without reducing the number of variables. Lastly, normalisation and scaling transformation were applied (Fig. 2d) so that the final data were standardised and interpreted in terms of variation around the mean rather than the absolute values of the observations.

4.2 Principal Component Analysis (PCA)

The PCA plots project the main information carried by the spectra onto a smaller number of latent variables called principal components (PC). An important function of PCA plots is to help find patterns or groups in a set of sample populations. Samples with similar spectral characteristics form clusters, whereas samples that are different are far away from each other. For this purpose, wavebands for differentiating the spectral characteristics of the population were identified by all 5 PCs (Fig. 3a). These include absorption bands for sucrose (e.g. at 990 nm), water (e.g. at 1450 nm) and starch (e.g. at 1200 and 1780 nm; Fig. 3b). Clusters of good and diseased tubers were visualised using PCs 3, 4 and 5 (Fig. 3c-d). This suggests that the information captured in the spectral data was able to detect the patterns causing the differences between diseased and good tubers, showing the potential to classify tubers based on spectral data.

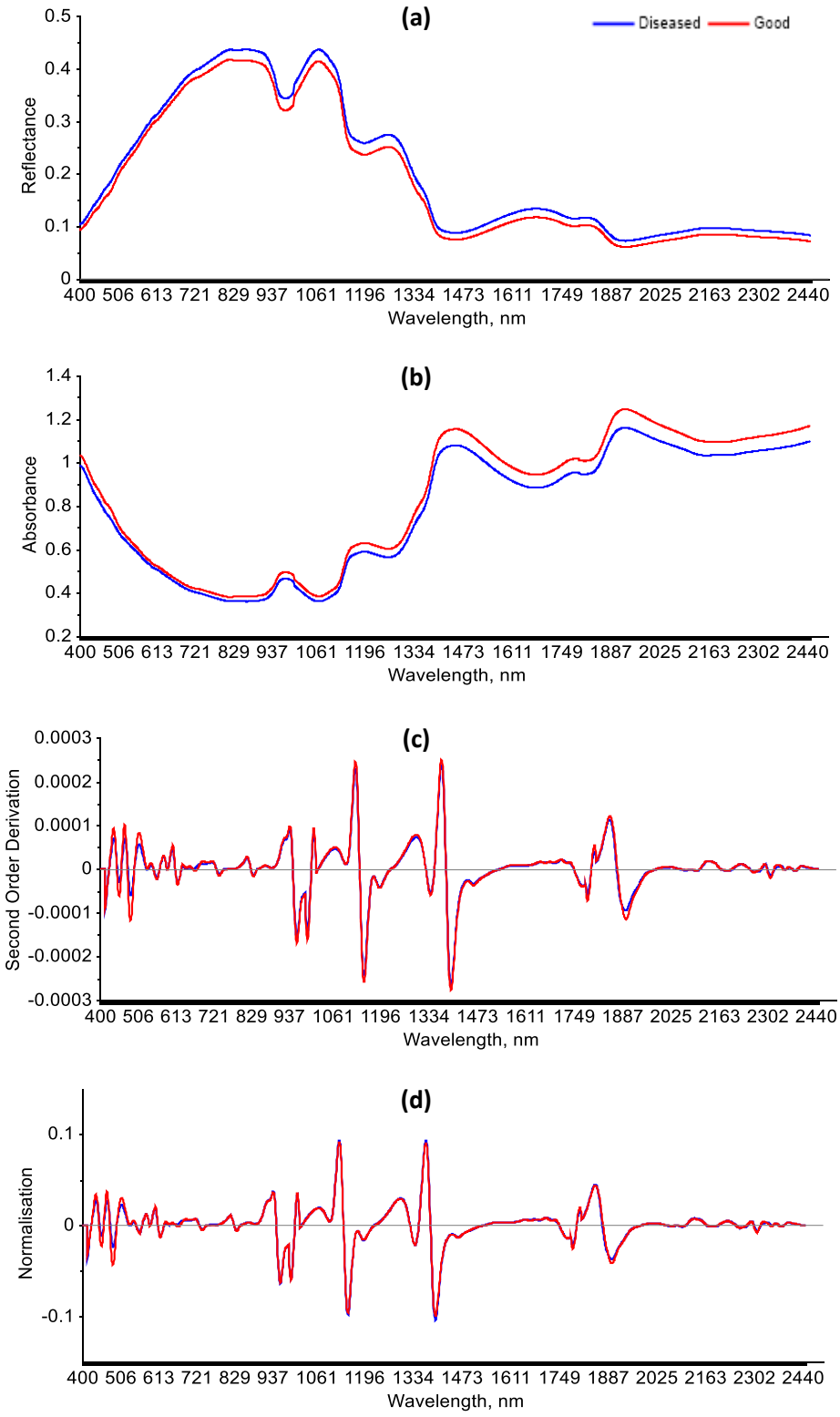

Fig. 2 Pre-processing of Vis-NIR spectral data: (a) reflectance; (b) absorbance; (c) second order derivation and (d) normalisation within the wavelength range of 400 – 2500 nm.

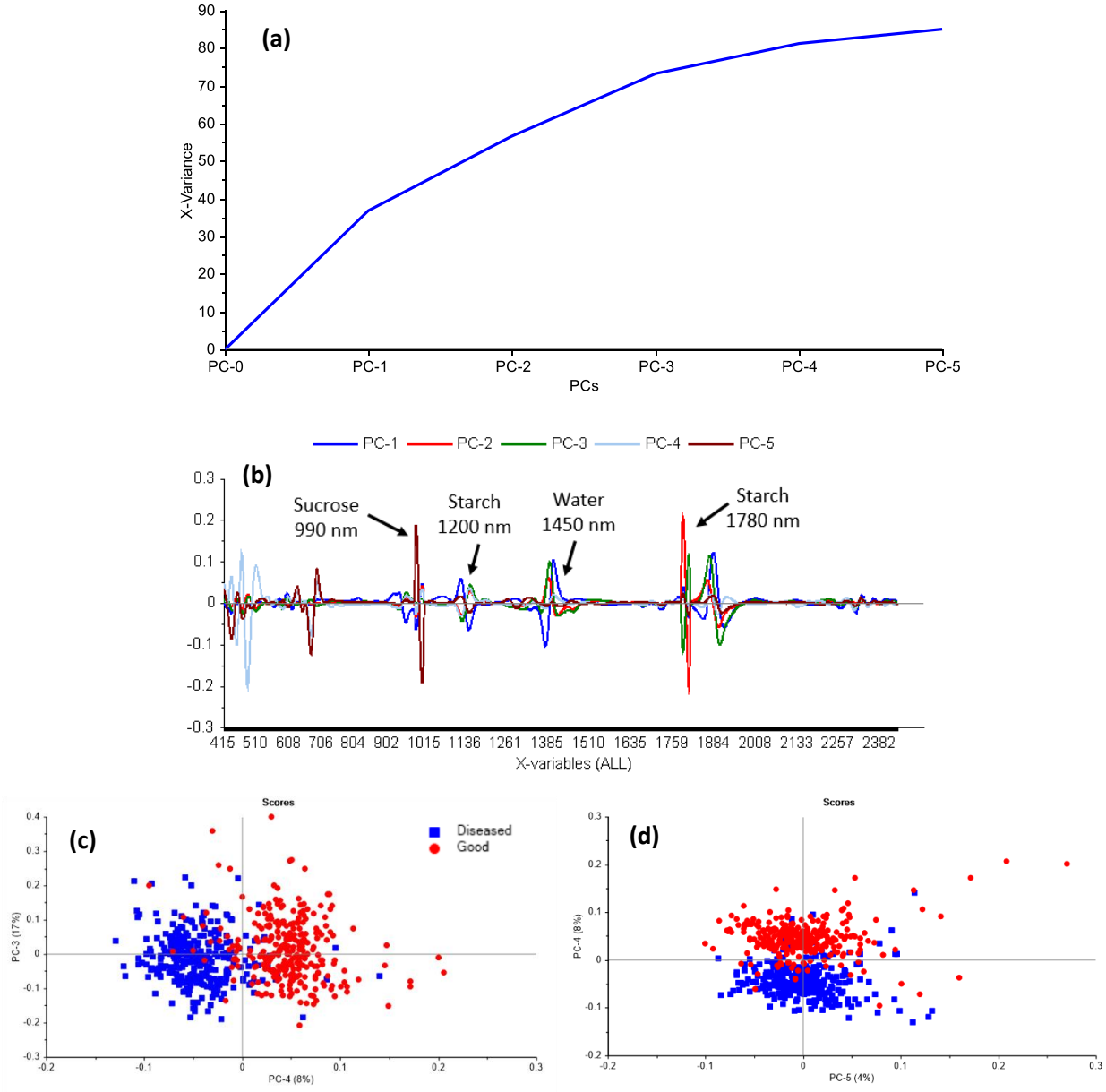

Fig. 3 Principal component (PC) analysis of pre-processed NIR spectral data identifying the cumulative percentage contributions of PCs towards explaining the variance for X-variable (wavebands) when different numbers of PCs are used (a) and important wavebands using loadings of x-variables (wavelengths) for all 5 PCs (b). PCs 3 & 4 (c) and PCs 4 & 5 (d) showing the segregation strength.

4.3 Multivariate Data Analysis

For calibration model development, the data set (collected on 26 – 27th Oct 2017) was randomly divided into two subsets; training (75%) and testing (25%) sets. A calibration model was first developed using the training data set in R-studio using support vector machines (SVM) classification. Internal L-fold (L = 20) cross validation was applied to avoid over-fitting. In this method, samples were divided into L segments. Each segment was removed from the data set and a calibration model was developed based on the remaining samples. The model was then used to predict the segment left out and estimated the prediction error. The process was repeated until every segment had been left out once, and then an average prediction error was estimated. Once the calibration model was developed, the robustness of the model was then validated using the test set collected on 2nd – 3rd Nov.

To assess the model performance, the percentage of accurate classification was calculated for each group. Performance metrics was used to evaluate the four classifiers. True positive (TP) is referred to as correctly classified diseased tubers. True negative (TN) is the correctly classified good tubers. False positive (FP) is the number of classified diseased tubers which are actually good. False negative (FN) is the number of classified good tubers which are in fact diseased. Once the calibration model was developed it was then applied to the test data set collected during model validation trial. Class predictions provided by the model were compared to actual class labels in order to evaluate the robustness of the model.

5. Model Performance

For the purpose of enabling export market of intact NZ potatoes by automatic grading to filter out Zebra chip diseased tubers, the important parameters are the TP rate (correctly classified diseased tubers) and FN rate (diseased tubers wrongly classified as good). Higher TP rate and lower FN rate would indicate that the model has a potential to enable online grading of tubers for the purpose of exporting intact tubers.

The model developed using the calibration data set showed good predictive performance (Table 1). The total accuracy was 93.3% considering both good and diseased classes. Both sensitivity and specificity were > 0.90, showing high predictive accuracy. The FN rate was very low (3%). This indicates that there were very few diseased tubers in the predicted good category. This would reduce the risks of having infected tubers in a good batch and hence contribute to commercial benefit if the predicted good class were to be exported to distant market.

Table 1. Performance metrics used to evaluate performance of the calibration classification model.

Parameter	Definition	Range	**Value**
Accuracy	**Percentage of correct predictions in the entire population**	**0 – 100%**	**93.3%**
Kappa	Indicates the reliability of a classifier on a specific dataset. The closer the value is to 1, the more reliable the classifying algorithm is	0 – 1	0.87
Sensitivity	The ability of the classifier to correctly classify 'good' tubers	0 – 1	0.90
Specificity	The ability of the classifier to correctly classify 'diseased' tubers	0 – 1	0.97
False Negative	**The proportion of diseased tubers which are classified as good tubers. The FN value should be as low as possible**	**0 – 100%**	**3%**
Area Under Curve	A higher AUC value suggests better classification performance. An AUC value between 0.8 – 1.0 indicates good to excellent classification accuracy	0 – 1	0.99

In model validation, the classification model developed using calibration data set continued to show good reliability and high accuracy in the prediction of tuber classes (Table 2). The TP rate was 96% for diseased tubers, highly comparable to the 97% obtained in model calibration. The TN rate reduced from 89% in model calibration to 81% in model validation (Table 2). This would mean that approx. 19% of the good tubers would have been classified as diseased tubers. For online

grading this would require a secondary (re)sorting of the predicted diseased tubers by peeling followed by visual observation, so that good tubers could be recycled from the designated diseased population. The results obtained in the current study are highly comparable to the recent study of Liang et al. (2018), where NIR was used to detect Zebra chip disease in intact 'Atlantic' tubers and achieved 97% accurate classification of diseased tuber in internal cross validation. It is important to note that, unlike Liang et al. (2018), external validation (use of independent validation data set) was performed in the current study and hence the reported predictive accuracy represents more realistic performance of the classification model for prediction of unknown samples.

Table 2. Performance of model calibration and validation using TP (correctly classified diseased tubers), TN (correctly classified good tubers), FP (number of classified diseased tubers which are actually good) and FN (number of classified good tubers which are in fact diseased) rates.

Confusion Matrix		*Predicted*			
		Calibration		**Validation**	
		Diseased (n = 299)	***Good (n = 300)***	***Diseased (n = 200)***	***Good (n = 200)***
Actual	***Diseased***	**97%**	3%	**96%**	4%
	Good	11%	**89%**	19%	**81%**

6. Conclusion and Recommendations

Near Infrared (NIR) spectroscopy was tested to detect Zebra Chip disease in potato tubers. Results showed that in calibration data set 97% of diseased tubers were correctly categorised. The same results were observed when the model was applied on validation data set and 96% of diseased tubers were correctly identified. In both calibration and validation data set, only 3 and 4% (respectively) of diseased tubers were wrongly classified as good. Overall, this study demonstrates that NIR has potential to detect Zebra chip disease tubers in a commercial environment. However, in this study, samples (for both calibration and validation) were collected from the same batch and after storage of around 6 months. Incidence and expression of disease symptoms in freshly

harvested tubers and at different stages of storage may or may not be varying. Therefore, for industrial implication, testing this model on freshly harvested tubers and at different stages of storage would be required to ensure the robustness in the model to segregate the diseased and good tubers throughout the processing season. Moreover, in this work a hand-held full-range Vis-NIR spectroscopy equipment was used for spectral data collection and still there is lot more to understand for industrial application of this technology in segregating diseased tubers. Handling, reduced data acquisition to enable cheap sensor deployment, potentially imaging systems, rapid data processing and segregation actuation will also be required to enable successful deployment of this technology in the industrial context. These challenges, although not small are all engineering challenges which should be able to be overcome in due course.

7. References

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