**SORTING FOR INTERNAL FLESH BROWNING IN APPLE USING VISIBLE-SHORTWAVE NEAR INFRARED SPECTROSCOPY**

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**ABSTRACT**

Visible – short wave near infrared spectroscopy (Vis-SWNIR) has been suggested for detection of internal defects such as internal flesh browning in pome fruits like apple and pear. A 5 point scale visual score was used as a reference assessment method. Fruit spectra were acquired using a MMS1 spectrometer in a partial transmittance configuration and pre-processed using averaging, multiple scatter correction or standard normal variate. Partial least squares regression and classification tools were trialed. Prediction statistics for PLSR models of around \( r^2_p = 0.75 \), RMSEP = 0.21 were achieved. Sorting of defect from acceptable fruit was based on a threshold prediction value of 2.49. This result was compared to that achieved using a straight discriminant analysis involving K Nearest neighbours (KNN), Partial Least Square Discriminant Analysis (PLS-DA), Linear Discriminant Analysis (LDA), Support Vector Machine (SVM) and multinomial logistic regression methods. Prediction results were similar for all methods, except for a poor result with SIMCA. The technique is recommended for commercial sorting of apple fruit with internal browning.

**KEYWORDS:** fruit defects, classification, spectra

**INTRODUCTION**

Certain apple cultivars are prone to development of internal flesh browning after prolonged storage in controlled atmosphere (CA) storage, rendering consignments unacceptable to the market. The syndrome is believed to result from membrane disruption, with oxidation of polyphenols normally localised to the vacuole to the brown compound, quinone, or its insoluble polymer, melanin¹. The disorder is associated with varieties with lower intercellular space content but incidence severity is influenced by both pre and postharvest factors, and their interaction². The incidence of the disorder can therefore be erratic³. Fruit retailers set a specification of <2% of fruit affected by these ‘major defects’ in the given consignment. This market pressure creates demand for a technology capable of detection of the disorder in fruit, allowing for sorting to remove defect fruit. A number of methods has been proposed for detection of internal defects in fruit, including X ray imaging⁴, magnetic resonance imaging (MRI)⁵, near infrared spectroscopy (NIR)⁶ and time resolved spectroscopy (TRS)⁷. Visible-shortwave NIR spectroscopy has potential for detecting and sorting internal browning in apple⁸, with previous reports considering use of indices based on ratio or difference of absorbance at two wavelengths, or based on partial least square (PLS) regression using a visible-SWNIR region. However, this application is suited to a discriminant analysis, with accept/reject grading. In the current study, we compare a PLS based model to a number of discriminant techniques.

**MATERIALS AND METHODS**

**Fruit**

Apple cv. Pink Lady™ grown in Stanthorpe, Queensland, Australia were harvested at commercial maturity in April 2013, stored for 24 weeks in an atmosphere of 1-2% O2 and 4-5% CO2 concentration, transported to Rockhampton, Queensland at 25 °C and then stored for another 8 weeks at 4 °C. Fruit temperature was raised to 25 °C for 6 h before spectra were acquired. Three independent populations of fruit with a total of 227 fruit (71 good and 156 defect) were used. Population 1 (90 fruit; 31 good and 59 defect), was used for calibration model development. Population 2 and 3 (60 fruit; 12 good and 48 defect, and 77 fruit, 28 good and 49 defect, respectively), were used in testing of the developed models. Four spectra were acquired per fruit, at equidistant positions around the fruit equator.
Instrumentation

An in-house instrumentation named Internal Defect Detection, IDD0 (Fig. 1), employing a partial transmission optical geometry with a 300W halogen lamp and a MMS 1 photo-diode array spectrometer (302-1150 nm; with the 500-975 nm region used in models) operated with an integration time of 1000 ms;

![Diagram of IDD0 instrumentation](image)

Reference method

Visual browning score

A panel of scorers was trained in the score assignment for the severity of defect as visible in a transverse equatorial cut of the fruit, using a 5 point scale, where scorers were assisted by a reference pictorial chart (Fig. 2.). The average value of all scorers was used.

![Cut surfaces of apple with diffuse internal browning symptoms](image)

Data analysis

Data analysis was carried out by using Excel (Microsoft, USA), The Unscrambler 10.3 (Camo, Norway) and MAT-Lab R2014a (Mathworks Inc., Natick, USA) with PLS toolbox 7.3 (Eigenvector Research Inc. Wenatchee, USA)

RESULTS AND DISCUSSION

Spectral features and regression results

Fruit absorbance spectra is characterized by features associated with anthocyanin at around 550 nm, chlorophyll at around 665 nm and water at 730, 840 and 950 nm. Average absorbance values were higher for defect relative to sound fruit at wavelengths less than 730 nm for the IDD0 spectra (Fig. 3). The higher absorbance values in this region may represent absorption of light by the polyphenols associated with browning. The strongest correlation
between absorbance at a single wavelength and defect intensity was achieved around 590 nm. PLSR models were developed using 500-975 nm with visual score as a reference parameter ($R^2=0.83$, RMSECV=0.62). Smooth regression coefficients were obtained for the region 500-975 nm. The PLSR model gave strong weighting to absorbance at 670, 710 and 900 nm.

Figure 3. Average absorption spectra of good and defect fruit, and the difference spectra (A), correlation coefficients for the univariate correlation of absorbance at a given wavelength and visual score (B) and the regression coefficients of a PLS model (C) from a mixed population of 1 and 2.

Classification

A PLSR model developed using population 1 was used in prediction of populations 1 and 2, with $r^2_p = 0.66$ and 0.85, and RMSEP = 0.83 and 1.27, respectively. Use a threshold value of 2.49, fruit were classified into good and defect groups, with classification errors detailed in Table 1.

Discrimination of sound fruit from defect based on spectral information was trialed using several classification algorithms, including partial least square discriminant analysis (PLSDA), linear discriminant analysis (LDA), k-nearest neighborhood (k-NN), Soft Independent Modelling of Class Analogy (SIMCA), Support Vector Machine Classification (SVM) and logistic regression (LR). Comparable results were obtained with most of classification algorithms, except for SIMCA, which yielded a poorer result. Discriminant procedures such as SVM, logistic regression and PLS-DA outperformed the PLSR with threshold approach (for prediction set 2)(Table 1).
Table 1. Classification of good and defect fruit in calibration and in prediction of independent sets using absorbance spectra collected at 500-975 nm against a reference assessment of visual score. TPR is true positive rate, TNR is true negative rate. Accuracy (Accu.) is mean of TPR and TNR, false discovery rate (FDR) is \([FP/(TP+FP)]\) Units are in percentage.

<table>
<thead>
<tr>
<th>Algorithms</th>
<th>TPR</th>
<th>TNR</th>
<th>Accu.</th>
<th>FDR</th>
<th>TPR</th>
<th>TNR</th>
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<td>83.0</td>
<td>98.3</td>
<td>90.7</td>
<td>2.0</td>
<td>83.3</td>
<td>99.1</td>
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<td>80.5</td>
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<td>98.3</td>
<td>96.3</td>
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CONCLUSION

Internal diffuse browning in apple developing during controlled atmosphere storage is a problem that has likely been exacerbated by recent breeding programs focussed on improved crispness in the eating experience. In line sorting to remove affected fruit, to meet retailer specifications of <2% affected fruit, is a desirable to avoid rejection of whole consignments. Another possibility is monitoring of a number of 'sentinel' fruit within the controlled atmosphere storage to detect the onset of the disorder. Near infrared spectroscopy using a partial or full transmission geometry over the wavelength range 500-975 nm is recommended for detection of the internal flesh browning using a PLS –DA classification method.

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References


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