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Assessment of internal flesh browning in intact apple using visible-short wave near infrared spectroscopy

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ABSTRACT

Certain cultivars of apple are prone to an internal flesh browning defect following extended controlled atmosphere storage. A number of (destructive) reference methods were assessed for scoring the severity of this defect in a fruit, including visual assessment, image analysis (% cross section area affected), International Commission on Illumination (CIE) chromameter Lab values of a cut surface and juice Abs_{420}, of which visual scoring on a 5 point scale and a colour index based on CIE Lab were recommended. Non-invasive detection of this disorder using three instruments operating in the visible-shortwave near infrared (NIR) but varying in optical geometry (interactance, partial transmission and full transmission) was attempted. Quantitative prediction of defect level was best assessed using visible-shortwave NIRS in a transmission optical geometry, with a typical partial least squares (PLS) regression model with correlation coefficient of determination, $R^2_p = 0.83$ and root mean square of errors of prediction = 0.63 (5 point defect score scale). The binary classification approaches of linear discriminant analysis, PLS discriminant analysis, support vector machine approach and logistic regression were trialled for separation of acceptable fruit, with the best result achieved using the PLS discriminant analysis method, followed by linear discriminant analysis and support vector machine classification. Classification accuracy [(True Positive + True Negative)/(Positive + Negative)] on an independent validation population of >95% and a false discovery rate [False Positive/(True Positive + False Positive)]of <2% was achieved.

1. Introduction

Several types of internal browning are recognised in apple (*Malus domestica* Borkh.) and other pome fruit, including radial, diffuse, bulge or internal breakdown (Bergman et al., 2012; James and Jobling, 2009). The browning symptom is considered to result from membrane disruption, with consequent oxidation of polyphenols otherwise localised to the vacuole of the brown compound, quinone, or its insoluble polymer, melanin (Hatoum et al., 2014a). The diffuse browning disorder developing during controlled atmosphere storage is suggested to be associated with varieties bred for increased crispness which also have lower intercellular space content (Herremans et al., 2014), although incidence severity is influenced by both pre and postharvest factors and their interaction (Castro et al., 2007; Franck et al., 2007; Castro et al., 2008; Benkeblia et al., 2011; Bergman et al., 2012; Wang and Sugar, 2013; Hatoum et al., 2014b). The incidence of the disorder can therefore be erratic (James and Jobling, 2009). Pre-harvest factors include nutrition (chiefly calcium and nitrogen), irrigation, harvest maturity, growing degree days and days after full bloom (Moggia et al., 2015). The main postharvest factor associated with diffuse internal browning is high carbon dioxide concentration, (Ferguson et al., 1999; Hatoum et al., 2014b), with probability of incidence increasing for fruit in controlled atmosphere storage beyond six months (Castro et al., 2007).

Internal browning is considered a 'major defect' by retailers, with consignments subject to rejection if more than 2% of fruit display the disorder (e.g. Woolworths, 2015). This market pressure creates demand for a technology capable of detection of the disorder in fruit, allowing for sorting to remove defect fruit. A range of technologies have potential for detection of this disorder, including visible-short wave near infrared spectroscopy (SWNIRS), acoustic, X-ray or magnetic resonance imaging (MRI). For example, Gonzalez et al. (2001) reported the use of MRI in detection of internal browning in 'Fuji' apples, with difference in longitudinal (T1) and transverse (T2) relaxation time and proton density between the normal, moderate and severe browning fruit. Indeed, Chayaprasert and Stroshine (2005) reported use of MRI for

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detection of browning in intact apple in an online sorting conveyor belt achieving classification accuracy [(TP + TN)/(P + N)] of 88% (where TP is true positive identifications of defect fruit, TN is true negative, P is all positives, N is all negatives). However, conveyor speed was less than 150 mm/s, too slow for commercial application (typically 1000 mm/s). Visible SWNIRS shows more promise for adoption into online sorting.

Norris (1958) reported in (Aulenbach et al., 1972) first reported on the measurement of transmittance of intact agricultural products for detection of internal defects. Birth and Olsen (1964) reported on the use of the difference in optical density at 760 and 810 nm of intact apple fruit as an index for water core, and this concept was expanded to the detection of both water core and internal browning by Francis et al. (1965). The topic was then left unreported for 30 years, until the work of Upchurch et al. (1997), Clark et al. (2003) McGlone et al. (2005) and then others. Francis et al. (1965) reported that the difference of absorbance at 840 and 740 nm was a useful index of the internal defect detection with 91% accuracy for separating normal fruit from defect in a population involving 50 fruit. Similarly, Upchurch et al. (1997) reported use of a ratio between transmittance at 720 and 810 nm as a classifier for discrimination between defective and good apples (the ratio decreased as the browning intensity increased, correlation coefficient of determination (R^2) = 0.71). Error rates of 6.3% good apples misclassified as defect (false negative) and 12% defect fruit misclassified as good (false positive) were reported, with the presence of bruises contributing to instances of false negatives. Clark et al. (2003) reported use of partial least squares regression using absorbance data (697–861 nm)for estimation of the degree of internal browning in Braeburn apple. Model statistics of correlation coefficient of determination for prediction [(R^2_p) = 0.91, root mean sum of squares of errors of prediction (RMSEP) = 7.9%] were reported when using the average of spectra obtained from opposite sides of the fruit. Increased absorbance in the visible range is presumably by phenolics associated with the browning symptoms.

More recently, Li (2013) reported use of a PLS model based on FTNIR reflectance spectra (950–2300 nm) for determination of extent of defect (using a 'chemical method' reference assessment, presumably absorbance of extracted juice) in Fuji apple (PLS R^2 increasing from 0.68 to 0.82 with removal of 'outliers', and a MLR based on 7 wavelengths achieving a R^2 of 0.88 reported, but SD not indicated). The use of a reflectance geometry and use of longer wavelengths is counter intuitive in terms of collecting information from within the fruit, and these models are unlikely to be robust in use with new populations of fruit.

Given the reported positive results, the integration of SWNIR technology into on-line sorting is logical. McGlone et al. (2005) reported detection of brownheart disorder of Braeburn apples of moving fruit at speed of five fruit per second, using SWNIR transmittance spectra over the range 650–950 nm range. Use of a 'large aperture spectrometer' was recommended for online measurement, with a partial least square regression (PLSR) model result of R^2_p = 0.9, and RMSEP = 4%.

Prediction of the level of disorder in fruit is, however, unnecessary as in practice separation to only two classes is required – acceptable and non-acceptable. Han et al. (2006) reported use of a discriminant technique for detection of brown core in another member of the pome fruit family, pear (*Pyrus communis* L.), with spectra collected over the range 651–1282 nm with a transmission optical geometry. Using the absorbance difference between 713 and 743 nm as a classifier, 5.3% of good pears were incorrectly classified as defect, while only 4.3% of defect fruit were classified as good. Fu et al. (2007) collected transmittance spectra (400–1028 nm) and also reported that defect fruit demonstrated a higher absorbance below 750 nm and a lower absorbance above 750 nm. In calibration, a discrimination accuracy of 89% was noted, while in validation, accuracy decreased to 81%. Torres et al. (2015) reported use of PCA and PLS-DA methods with reflectance spectra (392–442, 542–592 and 642–692 nm) to discriminate apple fruit with high levels of diffuse browning (in which the defect is present close to the skin) from sound fruit, but not fruit with moderate browning from sound fruit, nor fruit with radial browning (in which the defect is present deeper in the mesocarp). Other discrimination techniques are also relevant to this application,e.g. soft independent modelling of class analogy (SIMCA), partial least square discriminant analysis (PLS-DA), k-nearest neighbourhood (k-NN) and linear discriminant analysis (LDA) (Moscetti et al., 2015; Pérez-Marín et al., 2011).

Recent publications have focussed on the application of novel techniques. Li (2011) reported assessment of internal browning in Fuji apples using Fourier Transform (FT) NIR in an interactance optical geometry, with the best PLSR model (R^2 = 0.87) achieved at wavelength ranges of 950–1440 nm, 1480–1890 nm and 1960–2300 nm. Vanoli et al. (2014) reported the use of time resolved reflectance spectroscopy, with estimation of absorption and scattering coefficients at 780 nm used for separation of apple fruit with internal browning. They reported correct classification of 90% of good fruit and 71% of defect fruits.

Thus a number of reports indicate that non-invasive sorting of fruit on internal browning is possible using light transmission. Published reports generally recommend a transmission geometry and the use of wavelengths in the vis-SWNIR region, but recommendations vary s on the best wavelength range and algorithm (PLSR, simple ratio, discriminant analysis etc.). One study has considered defect detection of moving fruit. The comparison of published studies is difficult, as results depend on instrumentation, population distribution and the reference method used to assess level of defect. For example, the 'reference' method used in assessment of the internal browning defect varies between published studies, from visual assessment to image analysis of the cut fruit surface or measure of browning of extracted juice. Further, all reports mentioned were based on relatively small sample sizes, basically limited to a single population of fruit divided into training and validation sets, and thus fail to consider the range in variation of fruit optical properties occurring between populations (of different growing conditions etc.). Perhaps it should not be a surprise then that commercial adoption of non-invasive technology for detection and sorting of this disorder is not widespread, an indication that application is not as straightforward as some of the scientific literature suggests. There are some commercial providers of instrumentation (e.g. as advertised by Compac, www.compacsort.com Greefa, www.greefa.nl, MAF www.maf.com, and www.sorter.eu, among others), but there are no reports in the scientific literature on use of this equipment in context of detection of apple fruit with internal diffuse browning.

A specific issue not considered in previous reports is that of the effect of sample temperature. Fruit consist of approximately 80% water. The IR/NIR spectrum of water is affected by temperature due to an effect on the extent of hydrogen bonding, and this can impact prediction of soluble solids content (SSC) and dry matter (DM) in intact fruit using SWNIRS with the simplest accommodating measure being the inclusion of samples of a range of temperatures into the training sets (Acharya et al., 2014). Given that the spectral information relevant to internal browning may be restricted to the visible region, spectral based measures of internal browning may be free from interference associated with temperature change, however, this issue should be explicitly considered.

In the current exercise, results for detection of internal browning in apple are compared for spectra collected with instruments varying in optical geometry, with consideration of