CLASSIFICATION of browning on Regal Seedless, using near-infrared spectroscopy

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Consumer preferences for table grapes are influenced by characteristics like taste and flavour, as well as visual appearance. This includes the berry colour and any form of deterioration, such as bruises, spots, skin breaks, rot or decay that may appear on the fruit. The quality of table grapes does not improve postharvest, since it's a non-climacteric fruit. Maintaining the quality table grapes had at harvest is therefore of the utmost importance and must be done throughout the value chain. After harvesting and packaging, the transport and storage of table grapes can take from three to 22 days from SA to the intended market and consumer. It is during this period that postharvest defects like berry crack, gray mold rot, sulphur dioxide (SO2) damage and browning can develop.

Although browning is such a significant problem in the production of white table grapes worldwide, there is very limited published research on the browning phenomenon. However, several different phenotypes of berry browning on table grapes have been described. Some of these phenotypes, such as chocolate browning, show a brown discolouration that originates mostly from the stylar-end of the berry, while in severe instances the whole berry may appear brown. Friction browning on the other hand is expressed as circular spots close to the pedicel area and associated with rubbing of berries against each other. These complex and huge differences in the characteristics of grape berry browning clearly pose challenges for implementation of fast non-destructive monitoring methods for its detection. Fourier transform near infrared (FT-NIR) spectroscopy, however, has shown to be quite a versatile technology to determine a range of different parameters on the same sample.

Since its release to the South African table grape industry in 1997, the white seedless cultivar Regal Seedless has grown in commercial significance in the international market. In its naturally occurring form, this cultivar bears large medium-loose bunches with large berries and has good storage ability. However, just like Waltham Cross, Victoria and Thompson Seedless, it also develops browning during the cold storage period. This cold storage period can last up to six weeks, depending on the export destination.

Scanning whole intact bunches with NIR light, cold storage and evaluation for browning

Regal Seedless grapes were harvested from two different vineyards during January 2016, located respectively in the Wellington region (33°37'03,5" S, 18°58'05,3" E) and the Hex River Valley region (33°27'53,9"S, 19°39'43,7"E) of the Western Cape, SA. All harvested boxes

contained about eight to 10 intact whole bunches (Figure 1A). Contactless scanning of each bunch was done using Bruker's MATRIX-F spectrometer, immediately after harvest and then again each week after cold storage (Figure 1B). That is at harvest (W0), and every week after cold storage (W1-W6) (Figure 1C). Evaluation of the W0 box was done immediately and evaluation of boxes of W1-W6 was done after the second scan. The data for week 1 and week 2 (W1 and W2) were put together and similarly those for W3 and 4, and W5 and 6. After each bunch had been scanned the loose berries in the carry bag were classified as loose berries. These berries were not evaluated for browning or any of the other defects. All the berries that were still attached to the bunch were removed with scissors. They were then each evaluated for one type of defect only, i.e. either berry crack, SO2, chocolate browning, or friction browning, whichever one appeared the most prominently. A contingency table was set up when a specific defect was present on the bunch. The defect was then assigned a value of 1, and when it was absent it was assigned a value of 0.

Figure 1: A, B, C

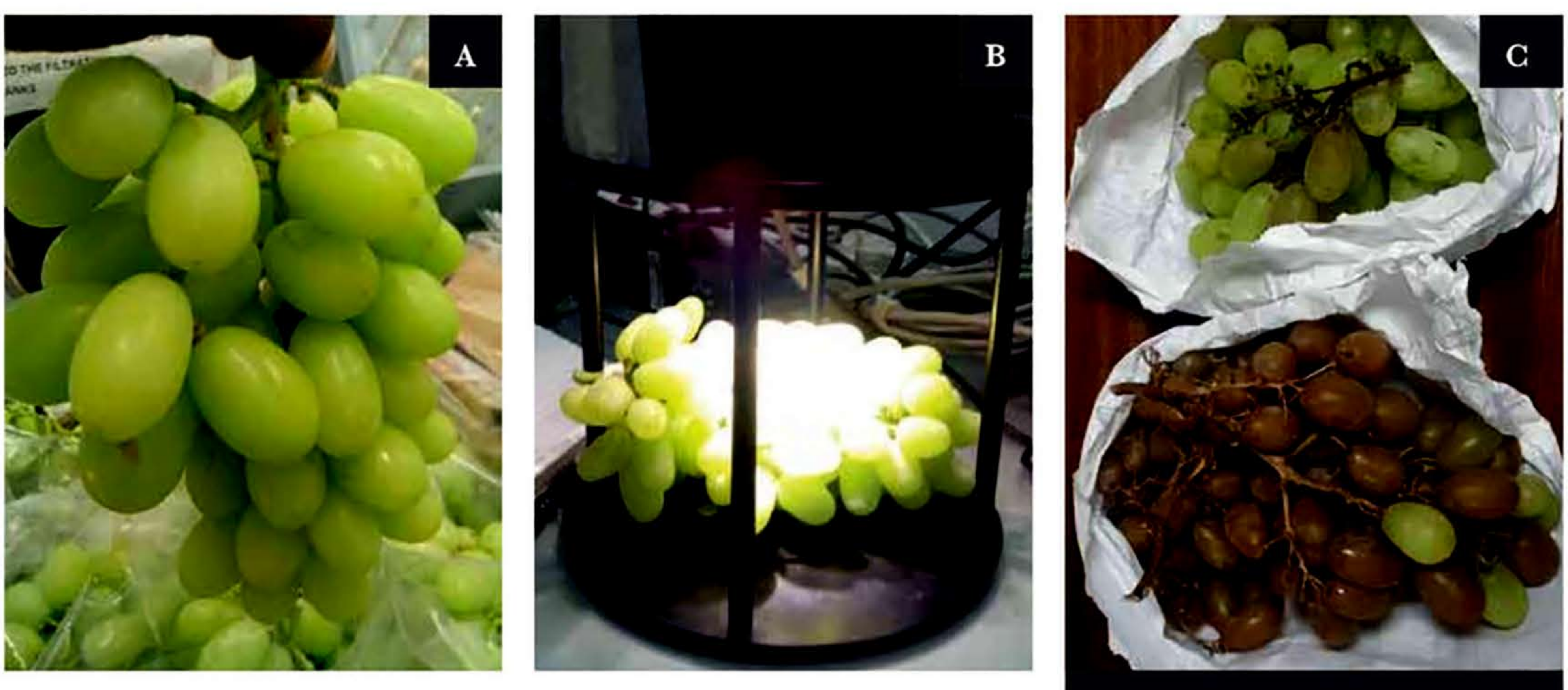

Figure 1: A. Intact whole Regal Seedless bunch taken from the box just after harvest, for scanning before cold storage and evaluation **B.** Contactless scanning of whole intact bunch with the Bruker's MATRIX-F spectrometer. **C.** Whole intact Regal Seedless bunches after several weeks of cold storage, displaying various stages of browning with the bunch on the bottom clearly illustrating how berries act as individual fruit, where some berries had turned brown and others remained green.

How partial least squares-discriminant analysis was used to classify browning

Partial least squares discriminant analysis (PLS-DA) is a derived version of the standard PLS regression procedure that uses class variables, instead of numeric variables. In PLS, the dummy variable Y is used as an answer variable, and the answer would for example be 16, 17 or 18°Brix if sugar was being predicted. In PLS-DA however, the Y variable is set to 1 if the sample is one of either class, and 0 if not. In our case, the defects were then scored as 0 = no defect and 1 = defect present. The cut-off value was set at 0.5, above which the sample is predicted as 1 and below which it is predicted as 0. The models were built using the spectral information that was obtained before and after cold storage and matching the values 1 if the defect was present, and 0 if the defect was not present to each spectrum of each sample. In addition, the classification error rate (CER) for calibration and cross-validation were also used to evaluate models. All calculations were performed by PLS-Toolbox for MATLAB (version 8.6.1, Eigenvector Research Inc., USA).

How accurately could browning of regal seedless be classified?

In order to determine how accurately the chocolate and friction browning of the different cold storage weeks were classified the CER was used. In general, an error in classification means classifying a sample as belonging to one class when it belongs to another class and the error rate refers to the percentage of misclassified samples out of the total samples in the validation data. In Figure 2 this was illustrated by some of the black circles (Class 1 = defect present) being where the majority of the clear circles (Class 0 = no defect) are, and vice versa.

The CER of chocolate browning for W3 and W4 (Figure 2A) was higher (25%) than

that of W5 and W6 (Figure 2B), which was 22% for both class 0 and class 1. This means that the chocolate browning could be classified with a higher accuracy of 78% for W5 and W6, than the 75% for W3 and W4. This might be attributed to the longer times that the samples of W5 and W6 were in cold storage, and the defects developed better and were therefore more pronounced on the bunches and could easily be detected by the infrared light. The incidence could also have been more (more chocolate brown berries) in W5 and W6 than in W3 and 4.

For friction browning the CER for class 1 (26%) was lower than that of class 0 (46%), and almost similar to that of class 0 and class1 of chocolate browning for W3 and W4 (25%) (Figure 2C). This means that berries that had friction browning could be classified with a higher accuracy (74%) than those that did not have any (54%). The reason for this might be because friction browning is mostly concentrated at the pedicel part of the berries, that the light did not always fall completely on those parts and that they were obscured by the other berries. It is also possible that the spectra could be picking up other phenotypes of browning that were also present on those bunches and not only the friction browning, hence the misclassification of mostly clear (class 0) bunches than class 1 bunches.

Figure 2: A, B, C

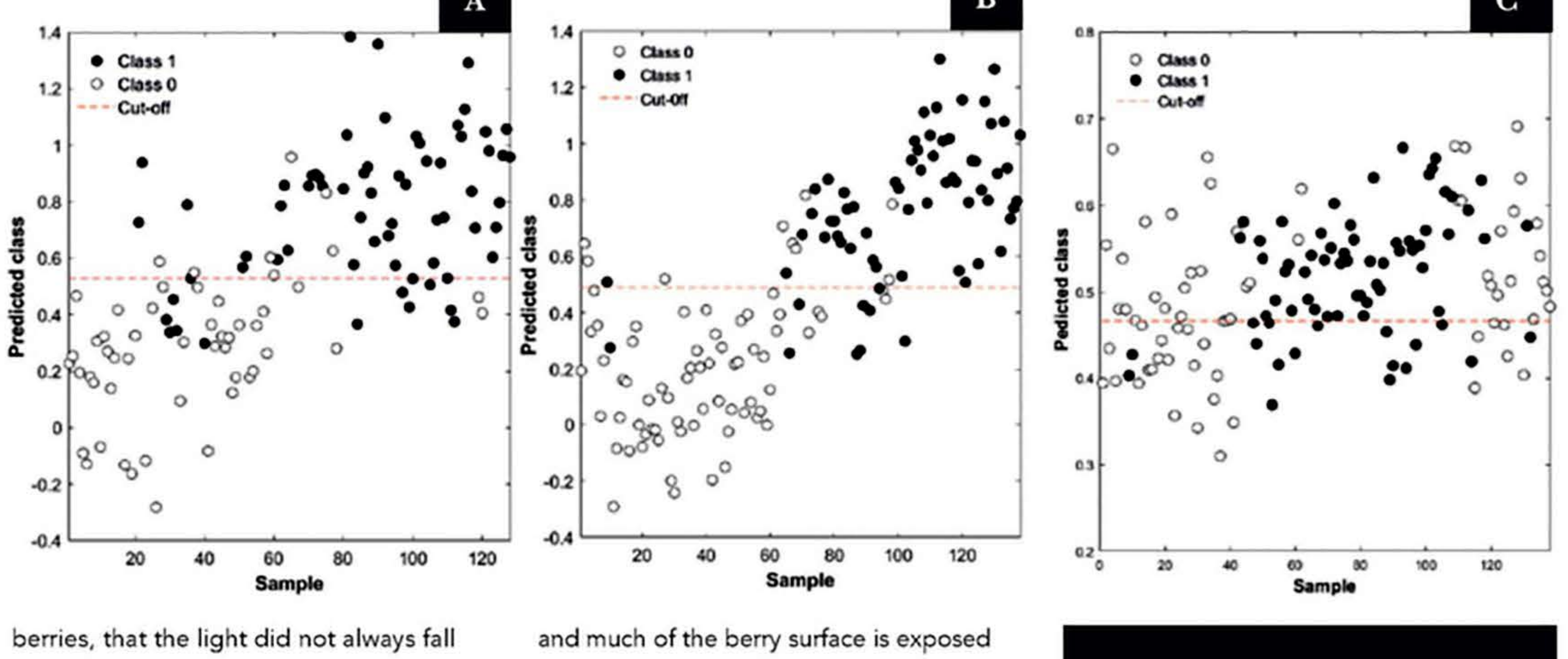

Figure 2: Absence (Class 0, open circle) or presence (Class 1, closed circle) for chocolate browning performed by PLS-DA model, based on NIR spectral data during **A** Weeks 3 and 4, and **B** Weeks 5 and 6. **C** is friction browning performed by PLS-DA model, based on NIR spectral data during Weeks 3 and 4.

Enlargement sprays are usually applied when berries are 4-5 mm in diameter. This enlargement of the berries can cause bunches to be compact, which leads to friction browning. The physical removal of berries and/or laterals on a bunch so that the bunch is not too compact are usually done when the berries are 8 -10 mm in diameter to ensure a looser bunch. Therefore, it is important that proper bunch thinning is done, so that the bunch is less compact and much of the berry surface is exposed when a technique such as this is employed to classify defects. This way, the maximum amount of information on the berries and the bunch can be collected. It should also be noted that a grape bunch is not a uniform sample with many edges, berries are sticking out in all directions, increasing the signal-to-noise ratio during spectral data collection. The information that is thus collected during the scans is of all these different parts of the bunch (berries and stems).

The correct classification of produce has always been a very important objective for any industry, and even more so for the table grape industry that deals with such a highly perishable product. Therefore, all stops should be pulled out to ensure that the fruit reaches the consumer in a good state. A technique such as the one developed here will go a long way in ensuring that fruit that has the potential to develop any defect, is detected early enough and the fruit is classified correctly for the different export markets. This is especially pertinent when it comes to browning, which is not always visually visible at harvest and/or at packaging. Other data analysis methods like machine learning will be explored further.