

Hyperspectral imaging to non-destructively predict citrus chilling injury

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Summary

Queensland lemons are exported at low (i.e. $<3^{\circ}\text{C}$) temperature to meet market access protocols. While the fruit generally tolerate these conditions, skin damage consistent with chilling injury (CI) is occasionally encountered and results in significant economic and reputational loss. The robustness of fruit to withstand export handling is inherently variable and difficult to predict. This study evaluated the potential of hyperspectral imaging to detect and predict CI in fruit before it was visible to the human eye.

Seeded and seedless Eureka lemons were sourced from a north Queensland orchard. They were treated with either 2.6 or 18.5 parts per million (ppm) ethylene gas at 20.4 or 28.9°C for 5 days to hasten the loss of green skin colour, as per commercial degreening practice. The degreened fruit were stored at 1.9°C for 5 weeks to simulate seafreight to export markets, followed by 10 days at 20°C to assess CI under typical retail display conditions.

Spectral data was collected within 2-3 h after harvest, degreening or cold storage. Three near infrared (NIR) devices were used; Bruker Matrix-F for spot assessment; Pika NIR 320 and Pika XC2 for hyperspectral imaging, covering wavelengths from 830-2500 nm, 900-1700 nm and 400-1000 nm, respectively. The supervised classification method of linear discriminate analysis was applied to the spectra collected from the Bruker Matrix-F, whereas partial least squares discriminant analysis (PLS-DA) was used to analyse spectra and images obtained from the Pika XC2 and NIR 320 instruments.

The results showed that there is potential for NIR hyperspectral imaging to predict CI in seeded and seedless Eureka lemons, when assessed after degreening and particularly after cold shipment. Spectra collected after degreening and cold storage demonstrated reasonable levels (i.e. 70%) of correct classification. The highest correct classification was obtained for scans taken after storage and is likely due to CI having been induced in the fruit. Spectra collected 2-3 h after harvest using the Bruker Matrix-F device were not analysed due to fruit damage from the technique itself.

Future research is needed to explore wavelength selections to refine, verify and develop models against an independent prediction set that combines the two varieties. The maximum level of spatial and/or spectral binning for the hyperspectral images that results in minimal loss of information should also be investigated. Once models are tested and validated successfully at a laboratory level, there would be scope for RD&E investment in a model that could be integrated into handheld or in-line NIR sensors to assist industry in grading out sensitive fruit batches that lack robustness to withstand export.

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Background

Australia produced 479,000 tonnes of citrus fruit in 2018/19, corresponding to a value of \$973 million (ABS 2020). Greater than 50% of Australia's production is exported to about 20 international markets (www.qgso.qld.gov.au, 2020). Principal markets are China, Thailand and Indonesia. Queensland is a significant exporter of mandarins and lemons with 41 millions tonnes exported with a value \$97 million (www.qgso.qld.gov.au, 2020) It is expected that export volumes will increase over the coming years with strong demand for consistent premium quality fruit by customers and consumers.

Lemons from sub-tropical Queensland production regions such as the Burnett are often picked with a green skin and then degreened with ethylene gas to obtain marketable yellow skin colour. Current recommendations are that lemons should be degreened with no more than 5 parts per million (ppm) ethylene at 20-24°C for 3-5 days (Duong *et al.* 2019). However, monitoring across several commercial degreening rooms in Queensland's Central Burnett has previously shown that lemons are sometimes degreened at relatively high temperatures and ethylene concentrations. Exposure to higher than recommended degreening conditions can accelerate the loss of green skin colour but heighten the sensitivity of fruit to developing quality defects such as chilling injury (CI), skin browning and disease (Duong *et al.* 2019; **Image 1**).



Image 1. A photo showing chilling injury symptoms (sunken, dark lesions) on a Eureka lemon fruit.

The export of lemons from Queensland requires adherence to strict phytosanitary protocols due to the potential presence of Queensland fruit fly. These protocols vary between countries, but typically involve prolonged cold storage in-transit of 14-21 days at 1-3.1°C (Department of Agriculture and Water Resources 2017). While the fruit generally tolerate exposure to these conditions, up to 15% of lemon shipments in 2016 and 2017 were downgraded due to skin damage that is consistent with CI (Greg Parr, pers.comm.). CI is typically characterised by pitting and sunken lesions on the surface of the fruit following exposure to low, non-freezing temperatures. We have previously reported that fruit chilling sensitivity varies from farm-to-farm and across and between seasons and varieties (Duong *et al.* 2019). Presently, growers cannot predict at harvest or following degreening which fruit will be better suited to export versus domestic marketing where relatively warmer transport temperatures are employed.

Hyperspectral imaging is a technique of capturing and processing an image at very large wavelengths based on spectroscopy. It is used for determining chemical components or quality attributes of food and agricultural products (Qin and Lu 2008). This technology has previously been shown to detect changes in fruit chemical composition associated with CI and skin defects in cucumber (Cen *et al.* 2016), green bell peppers (Babellahi *et al.* 2020) and apples (EIMasry *et al.* 2009) before the symptoms are visible.

Project Objective

The objectives of this research were to:

- 1) Determine the potential of near infrared (NIR) spot assessment and hyperspectral imaging to detect CI in Eureka seeded and seedless lemons before visible symptoms are evident;

2) Define what stage of postharvest handling that detection of CI is possible (i.e. immediately after harvest, degreening or cold storage/shipment).

Materials and Methodology

Fruit source

Seeded and seedless Eureka lemon fruit, which were uniform in colour, shape and size and free of damage were picked at commercial maturity by farm staff from an orchard at Widem Farming near Dimbulah in north Queensland (17°7'7" S, 145°9'35" E) on 27 February 2020. Previous studies and grower experience have indicated differences in response to CI between these two varieties. For each cultivar, a total of 130 fruit were collected for the study, of which 120 lemons were used for hyperspectral image collection at harvest, after degreening and again after cold storage. The remaining ten fruit were utilised for initial assessments such as fruit diameter, weight and juice content. The fruit were transported to a laboratory in Cairns in an air-conditioned car within 1 hours. They were initially assessed (see below) and then exposed to simulated degreening and shipment conditions.

Degreening treatment

Following initial spectra and image collection, the fruit were transported to the laboratory at Redden St for degreening treatment. The fruit of each cultivar was divided in two groups. The first group was degreened at 20.4°C plus 2.6 ppm ethylene for 5 days as per commercial degreening practice. The second group was degreened at 28.9°C and 18.5 ppm ethylene with a purpose to stress fruit to enhance the potential for development of CI. Ethylene concentration and temperature were recorded using ethylene (MSR Electronics GmbH) and temperature loggers (FriggaTech Ltd.), respectively.

Storage

Following image scanning after the degreening treatment, the fruit were stored at 1.9°C for 5 weeks to simulate different export shipment scenarios and to obtain different degrees of CI damage on lemons. Each fruit was rescanned by the three devices after removal from cold storage, before being kept at 20°C for 10 days to evaluate CI symptoms under typical retail display conditions.

Hyperspectral imaging (HSI) systems

On arrival at the laboratory located at James Cook University (JCU) Smithfield Campus in Cairns, the fruit were numbered and the area for image capture and examining CI expression was marked. A spot assessment instrument and two hyperspectral instruments were used to capture NIR spectra from the lemon fruit.

Diffuse reflectance spectra on a spot diameter of approximately 30 mm were collected over the range 830-2500 nm using a Matrix-F spectrometer (Bruker Optics, Ettlingen Germany) equipped with an external fibre-coupled emission head utilising 4 x 20w tungsten halogen light source. Spectra were collected from 30 fruit of each variety within 2-3 hours following harvest. These fruit appeared to become damaged as a result of excessive time-heat exposure from the Bruker Matrix-F light source and all fruit suffered from oleocellosis or oil spotting (bruising or green spot as result from oil rupture). These fruit are excluded from all analyses. Due to the heat damage occurring on the fruit as a result of excessive light exposure, spectral averaging was changed from 32 to 16 scans to reduce heat exposure. A further 90 fruit of each variety were scanned on opposing sides of the fruit following both degreening and after 5 weeks storage. This provided 180 spectra per variety at two assessment times (treatments).

Ninety fruit of each variety were captured using two NIR hyperspectral imaging systems. The hyperspectral imaging systems utilised included a Pika Vis-NIR XC2 spectrometer (Resonon Inc., USA) covering a spectral region of 400-1000 nm and a Pika NIR-320 covering the 900-1700 nm spectral range. All images and spectra collected from the three devices encompassed the same marked areas

of each fruit. The imaging measurements were performed 2-3h after harvest, degreening treatment and cold removal. Prior to scanning, the fruit were held at room temperature of 24°C for a couple of hours to reduce spectral difference associated with temperature.

Chilling injury

The area of fruit showing CI was assessed for each variety and the CI severity was expressed as the area (cm²) affected on each fruit. Each opposing side of the fruit were classed as having CI or not which was related to the NIR spectra for development of classification models for predicting class association.

Spectral and imaging data processing

The supervised classification method of linear discriminate analysis (LDA) was applied to the spectra collected from the Bruker Matrix-F. The LDA models were run with and without a standard deviation (SD) weighting process, in combination with linear, quadratic and mahalanobis methods. Due to the spectra having more wavelengths than samples (fruit sides), a principal components analysis (PCA) was applied prior to fitting the LDA. A maximum of 10 principal components (PC) was used for all models. Different pre-processing methods were trialled but no wavelength selection was performed. This would suggest the models presented in this report are effectively a 'worse-case' scenario as model refinement through wavelength selection has the potential to improve the models. Each variety is considered separately and models are developed for the individual assessment times (treatments).

Partial least squares discriminant analysis (PLS-DA) was used to analyse spectra and images obtained from the Pika XC2 and NIR 320 instruments. After images were loaded into Matlab, the freeware HYPER-Tools (version 2.0) was used to analyse the images. Due to a restriction for access to a high performance capacity computer during the Covid-19 pandemic, only a small sample showing presence and absence of CI was analysed.

Results

Initial assessment of lemons and chilling injury

Initial at harvest fruit quality including weight, juice content, diameter and total soluble solid for each variety are shown in **Table 1**. There was no significant difference among the two varieties in these quality parameters at harvest.

Table 1. Fruit quality parameters for seeded and seedless Eureka lemon assessed at harvest.

Variety	Fruit weight (g)	Juice content (%)	Fruit diameter (mm)	TSS (°Brix)
Seeded	114.29 (6.05)	39.16 (3.08)	58.36 (1.26)	7.76 (0.05)
Seedless	108.77 (5.15)	42.49 (2.37)	57.56 (1.13)	7.87 (0.07)

TSS stands for total soluble solid

Values in brackets are the standard error (n=10)

Degreening lemons at 28.9°C instead of 20.4°C and with 18.5 ppm ethylene versus 2.6 ppm resulted in greater CI incidence and severity (Table 2). In addition, there was no significant difference in CI severity between two varieties.

Table 2. Chilling injury incidence and severity of seeded and seedless Eureka lemons assessed at 20°C after degreening under two conditions for 5 days, followed by a 5-week storage at 1.9°C.

Variety	CI incidence (%)		CI affected area (cm ²)	
	Low temp + low C ₂ H ₄	High temp + High C ₂ H ₄	Low temp + low C ₂ H ₄	High temp + High C ₂ H ₄
Seeded	28.3	73.3	0.05	0.32
Seedless	48.3	60	0.38	0.27

Low temp + low C₂H₄ equals to 20.4°C + 2.6 ppm

High temp + High C₂H₄ equals to 28.9°C + 18.5 ppm

CI affected area is an average among affected fruit. Lsd = 0.29

NIR spot assessment using Bruker Matrix-F

Initial investigations focused on scans taken after 5 weeks storage and it could be expected these will have a higher probability of being able to separate the fruit into those with and without CI. These scans were taken after the fruit was stored at 1.9°C for 5 weeks in conditions that would induce the CI. The NIR spectral data collected after degreening are on fruit that had not been exposed to conditions that could potentially induce CI

After cold storage

For seeded Eureka fruit, 126 spectra (fruit sides) had no CI recorded. Two spectra were anomalies and removed from the dataset. The remaining 54 spectra (fruit sides) had CI ranging from 0.1 cm² up to 4.5 cm². For spectra collected after 5 weeks storage, the classification model correctly classified 75.0% with no CI (93/124) and 85.2% with CI (46/54) (**Table 3**) Eight spectra that were classified as having no CI, had CI ranging from 0.2 cm² up to 0.8 cm². All fruit with CI of > 0.8 cm² were correctly classified.

For seedless Eureka, 110 spectra developed no CI. The remaining 70 spectra had CI ranging from 0.1 cm² up to 5 cm². For spectra taken after 5 weeks storage, the selected classification model correctly classified 67.3% with no CI (74/110) and 77.1% with CI (54/70) (**Table 3**). The 16 CI spectra for seedless fruit that were incorrectly classified as having no CI, had CI ranging from 0.1 cm² to 0.8 cm²

Table 3. Classification of seeded and seedless fruit with or without CI after cold storage based on spectral analysis.

Raw spectra, mahalanobis, SD weight	Seeded fruit			Seedless fruit		
	Known CI Class		Overall	Known CI Class		Overall
	No CI	CI		No CI	CI	
Predicted Correctly	93	46		74	54	
Predicted Incorrectly	31	8		36	16	
% Correct	75	85.2	78.1	67.3	77.1	71.1

sd stands for standard deviation

After degreening

The spectra for seedless Eureka were recorded over a 2-day period and there was clearly different levels of absorbance between the two sets of scans (**Figure 1**). This shift in absorbance intensity is due to a different white background being used each day. The choice of pre-processing treatment has the potential to overcome this difference in absorbance intensity.

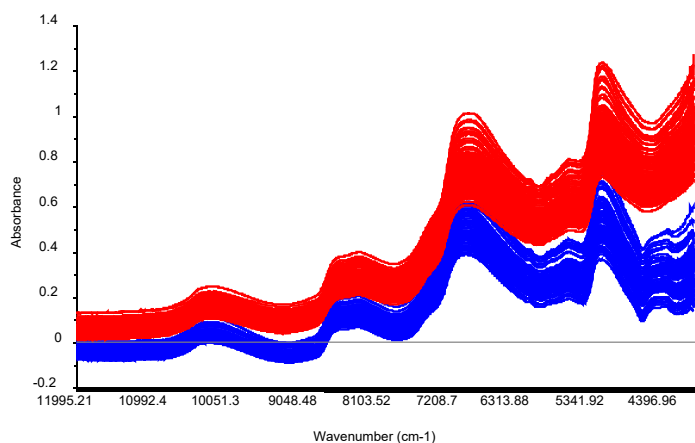


Figure 1. Raw spectra collected on the Bruker Matrix-F after degreening treatment of Eureka lemons. The red spectra were the first set of spectra scanned and the blue spectra were scanned the following day.

Even though the two sets of spectra show differences in the levels of absorbance, the two taken after degreening were combined. For seeded fruit, the selected model correctly classified 73.8% with no CI (93/126) and 83.3% with CI (45/54) (**Table 4**). The 9 spectra with CI that were classified as having no CI had CI ranging from 0.1 cm² to 0.5 cm². For seedless lemons, the selected model correctly classified 70.0% with no CI (77/110) and 75.7% with CI (53/70). The 17 scans from seedless fruit with CI that were classified as having no CI have CI ranging from 0.1 cm² to 2.5 cm². This range in CI for the spectra incorrectly classified is higher than for the spectra collected after 5 weeks storage.

Table 4. Classification of seeded and seedless fruit with or without CI after degreening based on spectral analysis.

SG smooth (7pt, poly 2) + 2nd derivative (15pt, poly 2), mahalanobis	Seeded fruit		Seedless fruit			
	Known CI Class		Overall	Known CI Class		Overall
	No	Yes		No	Yes	
Predicted Correctly	93	45		77	53	
Predicted Incorrectly	33	9		33	17	
% Correct	73.8	83.3	76.7	70	75.7	72.2

SG stands for Savitzky-Golay

NIR hyperspectral data from Pika 320 (900-1700 nm)

For all hyperspectral data sets, six lemons with CI and six lemons without CI were analysed. Two calibration sets were constructed to develop the PLS-DA classification model. The first calibration set included pixels from all 12 lemons. As it is not known where the CI occurred on the lemons, the pixels chosen for the CI calibration set, may not be accurate. The assumption that the chemical changes caused by CI were equally distributed on fruit is a limitation of these models.

The second calibration set used pixels from three lemons with and without CI. Remaining lemons were considered as independent prediction samples. This is a more conservative model, and will provide a better indication of model robustness. The small number of lemons contributing to the calibration set was a limitation of the models. The pixels not used in forming the calibration sets are predicted by the resulting PLS-DA classification models. This process was repeated for both varieties and for all three times of scanning. As results were similar for both varieties and calibration set methods, only results for the seedless Eureka variety are presented based on the more conservative calibration method.

Pika NIR320 after cold storage

Six lemon scans selected from the seedless Eureka variety that showed different levels of CI (3.5, 3.0, 3.5, 4.0, 5.0 and 5.0 cm²) were compared against fruit with no CI. The PLS-DA classification model using the pixels highlighted red in **Figure 2** was based on 6 latent variables. Three fruit in each category are used as an independent prediction set. This model was used to predict all pixels, where pixels are classified as having CI or not. The resulting predictions are shown on the bottom row of **Figure 3**, the percentage of pixels identified as having no CI are 100%, 94.7%, 99.4%, 88.5%, 76.6% and 99.8%. The three independent lemons in the prediction set had 100%, 94.7% and 99.8% of pixels identified as no CI. The percentage of CI pixels predicted in each fruit in the top row, from left to right are 59.4%, 63.9%, 53.1%, 48.6%, 90.7% and 96.8% (**Figure 3**). The fourth lemon from the left on the top row had a majority of pixels representing no CI. The lemon second from the right on the top row is an independent prediction sample with a CI of 5.0 cm² and 90.7% of pixels were classed as CI.

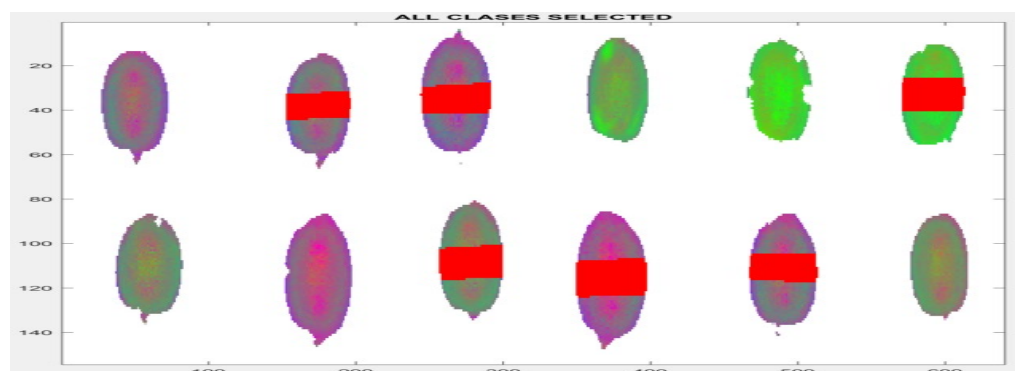


Figure 2. Calibration set for seedless Eureka fruit after cold storage. Top row indicates CI fruit and bottom row illustrates no CI fruit

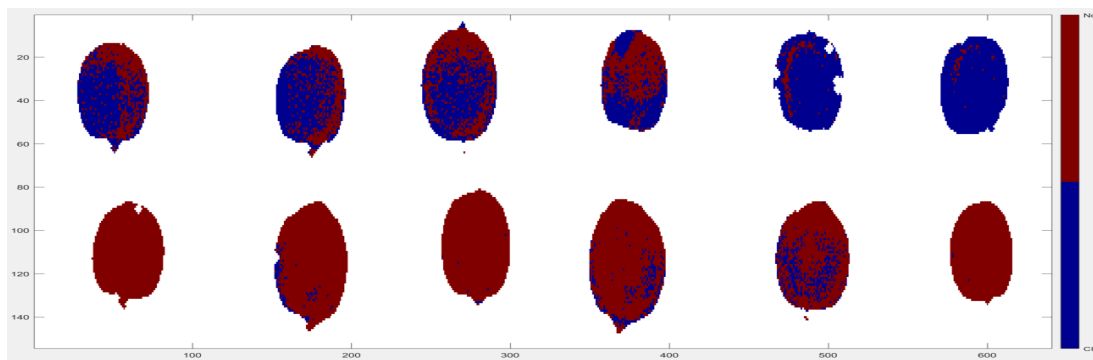


Figure 3. PLS-DA predictions for seedless Eureka fruit after cold storage. Top row have CI, bottom row have no CI. Brown pixels predict no CI, blue pixels predict CI.

Pika NIR320 after degreening

The same approach was applied to seedless Eureka fruit after degreening. All six lemons with no CI were classified into the correct group. Five of these fruit had more than 50% of pixels identified as CI (image not shown). The percentage of CI for these lemons as predicted CI pixels was 78.1%, 50.5%, 68.7%, 35.3%, 94.7% and 92.1%. The lemon with 35.3% of pixels classed as CI had 4 cm² of visible defect after storage and was an independent sample in the prediction set. Other independent samples with CI in the prediction set were predicted to have 78.1% and 94.7% of pixels with CI.

Pika NIR320 at harvest

The same method was employed for seedless Eureka fruit at harvest. The results indicate that the percentage of pixels classed with no CI was 75.8%, 77.2%, 97.5%, 78.0%, 92.7% and 37.1% (image not shown). The lemon with only 37.1% of pixels predicted as no CI was an independent prediction sample. This sample was also poorly predicted in the previous model. For the lemons with CI, the percentage of pixels classed as having CI is 20.6%, 68.5%, 60.8%, 28.9%, 7.7% and 61.1%. The independent prediction samples were those with the lowest percentage of pixels classed as representing CI, 20.6%, 28.9% and 7.7%.

NIR hyperspectral data from Pika XC2 (400-1000 nm)

Results from the Pika NIR320 determined that partial least squares discriminant analysis (PLS-DA) was most suitable and this classification technique is applied to images from the XC2 instrument. The same method was applied to the seedless Eureka fruit scanned at harvest, after degreening and following 5 weeks cold storage. The predictions from the classification model developed after storage, incorrectly classified one lemon in each category. A lemon with 5.0 cm² CI was predicted to have only 9.1% of CI pixels, while a lemon with no CI was predicted to have 84% of CI pixels. Both of these lemons were in the independent prediction set. The other 2 independent lemons with no CI were predicted to have 76.2% and 99.8% no CI. The same independent lemon with no CI was again incorrectly classified to have 91.3% CI pixels when scanned after degreening. Results were similar for images after harvest, with at least one fruit from each category having a majority of pixels incorrectly classified

Conclusions/Significance/Recommendations

The results from this study suggest that all of the models built using spectra collected from the spot assessment device (Bruker Matrix-F) have reasonable levels of accuracy (around 70%) for predicting chilling injury in lemons. Between 15 -17% of the sample examined, spectra data incorrectly classifies fruit as being free of CI when minor symptom developed. However, the models developed from spectra collected after 5 weeks after degreening, misclassified fruit with lower levels of CI, compared to the models using spectra collected after cold storage. This could possibly be explained by the CI having already been induced in the fruit scanned after storage. Although the CI may not be visible at this stage, the chemical changes within the fruit are most likely to have been initiated.

The results from the two hyperspectral devices (Pika 320 and Pika XC2) also suggest there is potential for NIR hyperspectral imaging to predict CI in seeded and seedless Eureka lemon varieties. A higher correct classification was obtained for scans taken after storage, which is likely due to CI having been induced in the fruit. Not all lemons were correctly classified after harvest and following degreening.

All of the classification models presented could possibly be improved through wavelength selection and analysing a larger number of fruit. Having more fruit contributing to the calibration set would introduce more sample variability into the model. It is also not known if the pixels selected in the CI calibration set were those affected by CI, it was assumed for these fruit that the whole fruit showed a level of expression. Photographs of fruit with CI to show the visible areas would aid in selecting the correct areas for the calibration set and determining the accuracy of the predictions. The minimum level of CI that can be detected also needs to be investigated. In these analyses, fruit with higher levels of CI were used for the purpose of determining the feasibility of detecting CI.

Alternative classification models such as support vector machines (SVM) and K-nearest neighbour clustering could also be investigated. At the present time, these approaches are not feasible with the computing power and software available as a result of limited access due to COVID-19 and working from home protocols. To improve our confidence in presenting more accurate classification of CI, the remaining images and spectra obtained from Pika 320 and Pika XC2 still need to be analysed.

Aside from wavelength selection and classification method, other areas which could be investigated for the hyperspectral models include the maximum level of spatial and/or spectral binning without losing information. By increasing the level of binning the size of the hypercube is reduced allowing more images to be loaded.

Based on all results collected from three devices, it is suggested that although a lower correct classification was obtained for scans taken after degreening than those taken after cold storage, a hyperspectral imaging technique could still help growers to determine the risk that fruit will develop CI if they are exposed to cold temperatures along the supply chain.

Key Messages

Hyperspectral imaging shows potential to help growers predict and remove fruit that are likely to develop CI whether they were scanned after degreening or cold storage. A predictive tool such as this would help differentiate fruit suited to domestic versus export chains and provide a means to objectively deal with quality disputes.

Where to next

As mentioned previously, the fruit responded differently to degreening temperature and ethylene. When the fruit were exposed to a higher temperature and ethylene concentration than the recommended level, they tend to develop CI following cold storage. Thus, in order to more accurately predict which fruit will likely express CI after cold storage, the fruit should be firstly scanned at degreening and then ideally scanned again after cold shipment.

Future investigations could consider wavelength selections to refine, verify and develop models with an independent prediction set that combine the two varieties. Aside from the wavelength selection and classification method, other areas which could be investigated for the hyperspectral models, include the maximum level of spatial and/or spectral binning possible without losing information. By increasing the level of binning the size of the hypercube is reduced allowing more images to be loaded. The minimum level of CI that can be detected also needs to be investigated. In these analyses, fruit with higher levels of CI were used for the purpose of determining the feasibility of detecting CI.

Once models can be tested and validated successfully at a laboratory level, there is scope to develop a model that could potentially be integrated into handheld or in-line NIR sensors to assist industry in grading out sensitive fruit whether at harvest, following degreening or at cold removal. This technique shows potential for not only predicting export robustness in lemons but also for other chilling sensitive citrus fruit such oranges and mandarins.

Budget Summary

Items	Budget (\$)
Travel	1,981.00
Hard drives	207.27
Casual wage	1200.00
NIR device rent	2500.00
Software license	1100.00
Purchase of ethylene sensor	2500.00
Data analysis	2000.00
Total	11,488.27

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