# Non-destructive evaluation of external and internal table grape quality

By

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# SUMMARY

Determining the correct harvest maturity parameters of table grapes is an essential step before harvesting. The chemical analysis of table grapes to determine harvest and quality parameters such as total soluble solids (TSS), titratable acidity (TA) and pH, is very time-consuming, expensive, and destructive. Developing faster and more cost-effective methods to obtain the information can benefit the table grape industry by reducing losses suffered at the postharvest stage. There are multitudes of factors that can influence table grape postharvest quality leading to huge losses. These losses are exacerbated even further by the long list of postharvest external and internal defects that can occur, including browning in all its various manifestations. The application of cutting-edge technologies such as Fourier Transform Near-Infrared (FT-NIR) spectroscopy that can accurately assess the external and internal quality of fruit is, therefore, essential. This particularly concerns the identification of defects or assessment of the risks of defects that are likely to develop during post storage. The aim of this application would thus be to evaluate these new technologies to monitor table grape quality non-destructively, before, during, and/or after harvest.

This study, therefore, focussed on the development and optimisation of faster, cost-effective, and fit-for-purpose methods to monitor harvest maturity and quality of table grapes in the vineyard before harvesting and during packaging and cold storage. Harvest of three different cultivars, namely, Thompson Seedless, Regal Seedless and Prime, happened over two seasons (2016 and 2017) from six different commercial vineyards. Five of these vineyards were in the Western Cape (two in the Hex River Valley, three in Wellington) and one in the Northern Cape (Kakamas), South Africa. Harvest occurred twice at each vineyard, at optimum ripeness and two weeks later (after the optimum harvest date). The incidence and intensity of browning on each berry on a bunch were evaluated for different defects and browning phenotypes. Quantitative harvest maturity and indicative quality parameters such as TSS, TA and pH, as well as the sensory-related parameters – sugar:acid ratio (TSS:TA ratio) and BrimA, were investigated by scanning whole table grape bunches contactless with Bruker's MATRIX-F spectrometer in the laboratory. Partial Least Squares (PLS) regression was used to build prediction models for each parameter. Two different infrared spectrometers, namely the Bruker Multipurpose Analyser Fourier Transform Near-Infrared (MPA FT-NIR) and MicroNIR Pro 1700 were also used to determine TSS on whole table grape berries. The MicroNIR Pro 1700 was utilised in the vineyard and the laboratory and the MPA only in the laboratory. The same spectral dataset used to build the quantitative models was used to build classification models for two browning phenotypes, namely chocolate browning and friction browning. Partial Least Squares Discriminant Analysis (PLS-DA) and Artificial Neural Networks (ANN) were used for the classification tasks.

Key results showed that the incidence and intensity of different defects and browning phenotypes such as sulphur dioxide ($SO_2$) damage were prevalent on all three white seedless table grape cultivars. The incidences of fungal infection, sunburn and abrasion damage were high on Regal Seedless and Thompson Seedless in 2016. Contact browning, mottled browning and friction browning and bruising damage had higher incidences in 2017 than in 2016. Overall, the intensity of defects was very high in 2016 except on Regal Seedless from Hex River Valley. Prime from Kakamas and Wellington had the highest intensity of defects in 2017, which appeared on the grapes after 7 weeks of cold storage.

Prediction models were successfully developed for TSS, TA, TSS:TA, pH, and BrimA minus acids on intact table grape bunches using FT-NIR spectroscopy in a contactless measurement mode, and applying spectral pre-processing techniques for regression analysis with PLS. The combination of Savitzky-Golay first derivative coupled with multiplicative scatter correction on the original spectra delivered the best models. Statistical indicators used to evaluate the models were the number of latent variables (LV) used to build the model, the prediction correlation coefficient ($R^2_p$) and root mean square error of prediction (RMSE). For the respective parameters TSS, TA, TSS:TA ratio, pH, and BrimA, the number of LV used when the models were build according to a random split of the calibration and validation set were 6, 4, 5, 5 and 10, the R2p = 0.81, 0.43, 0.66, 0.27, and 0.71, and the RMSEP = 1.30 °Brix, 1.09 g/L, 7.08, 0.14, and 1.80. When 2016 was used as the calibration set and 2017 as the validation set in model building the number of LV used were 9, 5, 5, 4 and the R2p = 0.44, 0.06, 0.17, 0.05, and 0.05 and the RMSEP = 3.22 °Brix, 2.41 g/L, 14.53, 0.21, and 8.03 for for the respective parameters.

Determining TSS of whole table grape berries in the vineyard before and after harvesting using handheld and benchtop spectrometers on intact table grape berries showed that spectra taken in the laboratory with the MicroNIR were more homogenous than those taken in the vineyard with the same spectrometer, over the two years investigated. The results obtained with the MPA were not as good as those obtained with the MicroNIR in the laboratory were. The model constructed with the combined data of 2016 and 2017 taken in the laboratory with the MicroNIR had the best statistics in terms of $R^2_p$ (0.74) and $RPD_p$ (1.97). The model constructed with the 2017 data obtained in the laboratory with the MicroNIR had the lowest prediction error (RMSEP = 1.13°Brix).

Good models were obtained using PLS-DA and ANN to classify bunches as either clear or as having chocolate browning and friction browning based on the spectra obtained from intact table grape bunches with the MATRIX-F spectrometer. The classification error rate (CER), specificity and sensitivity were used to evaluate the models constructed using PLS-DA and the kappa score was used for ANN. The CER for chocolate browning (25%) was better than that of friction browning (46%) after Weeks 3 and 4 in cold storage for both class 0 (absence of browning) and class 1 (presence of browning). Both the specificity and sensitivity of class 0 and

class 1 of friction browning were not as good as for chocolate browning. With ANN, the testing kappa score to classify table grape bunches as clear or having chocolate browning or friction browning showed that chocolate browning could be classified with the strong agreement after Weeks 3 and 4 and Weeks 5 and 6 and that friction browning could be classified with moderate agreement after three and four weeks in cold storage. Classification of chocolate browning and friction browning phenotypes was done using PLS-DA and ANN and the result showed that both types of browning can be classified with moderate agreement.

The implications of the results of this study for the table grape industry are that the industry can move beyond just assessing methods and techniques in the laboratory towards implementation in the vineyard and the packhouse. Much quicker decisions regarding grape quality and destination of export can now be made using a combination of the MicroNIR handheld and MATRIX-F instruments for onsite quality measurement and the models to predict internal (e.g. TSS) and external (browning) quality attributes.

# OPSOMMING

Die bepaling van die korrekte oesrypheidsparameters van tafeldruiwe is 'n noodsaaklike stap voor oes. Chemiese ontleding van tafeldruiwe om oes- en kwaliteitsparameters te bepaal, soos totale oplosbare vaste stowwe (TOVS), titreerbare suur (TS) en pH, is baie tydrowend, duur en vernietigend. Die ontwikkeling van vinniger en kostedoeltreffender maniere om die inligting te bekom, kan die tafeldruifbedryf bevoordeel deur verliese wat in die na-oesstadium gely word, te verminder. Dit sluit die menigte faktore in wat die gehalte van tafeldruiwe ná oes kan beïnvloed en tot verliese lui. Hierdie verliese word nog verder vererger deur die lang lys van verskillende na-oes-verwante gebreke wat kan voorkom, insluitend verbruining in al sy verskillende manifestasies. Die toepassing van toonaangewende tegnologieë soos Fourier-transform-naby-infrarooi (FT-NIR) spektroskopie wat die eksterne en interne kwaliteit van vrugte akkuraat kan beoordeel, is dus noodsaaklik. Dit is veral die identifisering van gebreke, of die beoordeling van die risiko's van gebreke, wat waarskynlik tydens die opberging kan ontstaan. Die doel van hierdie toepassing was dus om hierdie nuwe tegnologieë te evalueer om die kwaliteit van tafeldruiwe nie-vernietigend te monitor, voor, tydens en/of ná oes.

Hierdie studie het dus gefokus op die ontwikkeling en optimalisering van vinniger, koste-effektiewe en geskikte doeleindes om oesrypheid en kwaliteit van tafeldruiwe in die wingerd te monitor voor oes en tydens verpakking en koelopberging. Druiwe-oes van drie verskillende kultivars (Thompson Seedless, Regal Seedless en Prime) het gedurende twee jare (2016 en 2017) uit ses verskillende kommersiële wingerde plaasgevind. Vyf van hierdie wingerde was in die Wes-Kaap (twee in die Hexriviervallei, drie in Wellington) en een in die Noord-Kaap (Kakamas), Suid-Afrika. Die oes het twee keer by elke wingerd plaasgevind, dit wil sê op die beste rypheid en twee weke later ná die optimale oesdatum. Die voorkoms en intensiteit van verbruining op elke korrel op 'n tros is op verskillende defekte en verbruiningsfenotipes geëvalueer. Kwantitatiewe oesrypheid en kwaliteitsindikatiewe parameters, naamlik TOVS, TS en pH, sowel as sensoriese verwante parameters suiker:suur-verhouding (TOVS:TS-verhouding) en BrimA is ondersoek deur heel tafeldruiftrosse sonder kontak met die Bruker se MATRIX-F-spektrometer in die laboratorium te skandeer. Gedeeltelike minste kwadrate (GMK) regressie is gebruik om modelle vir die parameters te bou. Twee verskillende infrarooi-spektrometers naamlik (a) die Bruker Multipurpose Analyzer Fourier Transform Near-Infrared (MPA FT-NIR) en (b) MicroNIR Pro 1700 is ook gebruik om TOVS op heel tafeldruifkorrels te bepaal. Die MicroNIR Pro 1700 is in die wingerd en in die laboratorium gebruik en die MPA slegs in die laboratorium. Met behulp van dieselfde spektrale datastel as die een wat gebruik word om die kwantitatiewe modelle op te stel, is klassifikasiemodelle vir twee verskillende verbruiningsfenotipes (sjokoladeverbruining en wrywingverbruining) gebou. Hierdie keer is gedeeltelike minste-kwadrate-diskriminant-analise (GMK-DA) en kunsmatige neurale netwerke (KNN) gebruik.

Die belangrike resultate het getoon dat die voorkoms en intensiteit van verskillende defekte en verbruiningsfenotipes soos swaeldioksied ($SO_2$)-skade op al drie wit pitlose tafeldruifkultivars voorgekom het. Die voorkoms van swaminfeksie, sonbrand en skaafskuur was hoog op Regal Seedless en Thompson Seedless in 2016. Kontak-, gevlekte- en wrywing verbruining sowel as kneusplekke het in 2017 'n hoër voorkoms as in 2016 gehad. Oor die algemeen was die intensiteit van defekte baie hoog in 2016 behalwe op Regal Seedless vanaf die Hexriviervallei. Prime van Kakamas en Wellington het in 2017 die hoogste intensiteit van gebreke gehad wat ná 7 weke se koelopberging op die druiwe verskyn het.

Die suksesvolle ontwikkeling van modelle vir TOVS, TS, TOVS:TS verhouding, pH en BrimA op heel tafeldruiftrosse met behulp van FT-NIR-spektroskopie is bewys as inderdaad moontlik – veral as GMK met verskillende spektrale voorverwerkingstegnieke gepaard gaan. Statistiese aanwysers wat gebruik is om die modelle te evalueer, was die aantal latente veranderlikes (LV) wat gebruik is om die model te bou, die voorspellingskorrelasiekoëffisiënt ($R^2_p$) en wortelgemiddelde vierkante voorspellingsfout (WGVVF). Die kombinasie van die eerste afgeleide Savitzky-Golay tesame met die vermenigvuldigende verstrooiingskorreksie op die oorspronklike spektra het die beste modelle gelewer. Statistiese aanwysers wat gebruik is om die modelle te evalueer, was die aantal latente veranderlikes (LV) wat gebruik is om die model te bou, die voorspellingskorrelasiekoëffisiënt (R2p) en wortelgemiddelde vierkante voorspellingsfout (RMSE). Vir die onderskeie parameters TSS, TA, TSS: TA-verhouding, pH en BrimA, was die aantal LV wat gebruik is toe die modelle volgens 'n ewekansige verdeling van die kalibrasie- en valideringstel gebou is, 6, 4, 5, 5 en 10, die R2p = 0,81, 0,43, 0,66, 0,27 en 0,71, en die RMSEP = 1,30 ° Brix, 1,09 g / l, 7,08, 0,14 en 1,80. Toe 2016 as die kalibrasiestel gebruik is en 2017 as die validasieset in modelbou, was die aantal gebruikte LV 9, 5, 5, 4 en die R2p = 0,44, 0,06, 0,17, 0,05 en 0,05 en die RMSEP = 3,22 ° Brix, 2,41 g / l, 14,53, 0,21 en 8,03 vir die onderskeie parameters. Die bepaling van TOVS van heel tafeldruifkorrels in die wingerd voor en ná oes oor twee jaar met behulp van hand- en tafelbladspektrometers het getoon dat spektra wat in die laboratorium met die MicroNIR geneem is meer homogeen was as dié wat in die wingerd met dieselfde spektrometer geneem is. Die resultate wat met die MPA behaal is, was nie so goed soos met die MicroNIR in die laboratorium nie. Die model wat saamgestel is met die gekombineerde data van 2016 en 2017 wat in die laboratorium met die MicroNIR geneem is, het die beste statistieke gehad in terme van die $R^2_p$ (0.74) en die $RPD_p$ (1.97). Die model wat opgestel is met die 2017 data wat in die laboratorium met die MicroNIR verkry is, het die laagste voorspellingsfout (RMSEP = 1.13°Brix) gehad.

Goeie modelle is verkry met behulp van GMK-DA en KNN om trosse as skoon te klassifiseer, of as sjokoladeverbruining en wrywingsverbruining gebaseer op die spektra van die heel tafeldruiftrosse wat met die MATRIX-F-spektrometer geneem is. Die klassifikasiesyfer (KS), spesifisiteit en sensitiwiteit is gebruik om die modelle wat met behulp van GMK-DA saamgestel is, te evalueer en die kappa-telling is vir KNN gebruik. Die KS vir

sjokoladeverbruining (25%) was beter as dié van wrywingsverbruining (46%) vir week 3 en week 4 vir beide klas 0 (afwesigheid van verbruining) en klas 1 (teenwoordigheid van verbruining). Beide die spesifisiteit en sensitiwiteit van klas 0 en klas 1 vir wrywingverbruining was nie so goed soos vir sjokoladeverbruining nie. Met KNN het die toetskappa-telling om tafeldruiftrosse as skoon of sjokoladeverbruining of wrywingsverbruining te klassifiseer, getoon dat sjokoladeverbruining tydens Week 3 en Week 4 en Week 5 en Week 6 met 'n matige ooreenstemming geklassifiseer kan word en dat wrywingsverbruining met matige ooreenstemming tydens Week 3 en Week 4 geklassifiseer kan word.

Die implikasies van hierdie resultate vir die tafeldruifbedryf is van so 'n aard dat die bedryf nou verder kan gaan as om net metodes en tegnieke in die laboratorium te beoordeel, maar kan beweeg na implementering in die wingerd en die pakhuis. Die neem van baie vinniger besluite rakende die kwaliteit van die druiwe, dit wil sê in watter klas druiwe geplaas kan word en na watter uitvoermark druiwe gestuur kan word, is nou moontlik. Veel vinniger besluite rakende druiwekwaliteit en bestemming van uitvoer kan nou geneem word met behulp van 'n kombinasie van die MicroNIR-hand- en MATRIX-F-instrumente vir kwaliteitsmeting *in situ* en die modelle om interne (bv. TOVS) en eksterne (verbruining) kwaliteitseienskappe te voorspel.

This thesis is dedicated to

My unborn children

Angelica my darling daughter and Agamemnon my dearest son
Knowledge of whence Almighty God will bring you forth to me
I do not yet possess
Be it from the womb of a pain-inflicted mother
Or the loins of a drug-infested father
Is of no concern to me
Just as long as you are safely within arms
Then in a toilet or rubbish heap somewhere

Milita you fair and smart and Tiberius you brave and strong
Every drop of water, power I now save
I pray that Mother Nature will one day
In Her bounty give it back to you

Saint René mon amour and Saint Remey mijn zoete kind
Oh the joy you will bring into my life
Once filled with so much pain
Now transcended to more than just a faint glimmer in the past

De Maupassant and De Montpellier
Daniels is the surname you will bear
Love or loathe it I don’t care
Just as long as you are there
To call me father of daddy dear

# BIOGRAPHICAL SKETCH

Andries Daniels was born in Kimberley, South Africa on 29 November 1981. He attended Venus Primary School, then Homevale Senior Secondary School No. 2 and matriculated at Adamantia High School in 1999. Andries obtained a BScAgric degree in Viticulture and Oenology in 2005 and an Honours BScAgric degree in Viticulture in 2008 at the Stellenbosch University. In 2009, he enrolled for an MScAgric in Viticulture at the Stellenbosch University that he obtained in 2013. Andries works as a Junior Researcher in the Table Grape Breeding and Evaluation part of the Crop Development Division of the Agricultural Research Council that he joined in 2006. In 2015, he enrolled for a PhD in Viticulture at Stellenbosch University.

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# PREFACE

This dissertation is presented as a compilation of seven chapters.

| | |
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| **Chapter 1** | **General Introduction and project aims** |
| **Chapter 2** | **Literature review:** Grape expectations: a critical evaluation of present-day strategies for the control and monitoring of table grape quality |
| **Chapter 3** | **Incidence and intensity of table grape defects during cold storage** |
| **Chapter 4** | **Measuring maturity and sensory parameters contactless on intact table grape bunches using NIR spectroscopy** |
| **Chapter 5** | **Evaluation of handheld NIR spectrometers to quantify quality attributes of whole berries** |
| **Chapter 6** | **Classification of browning on intact table grape bunches using near infrared spectroscopy coupled with partial least squares discriminant analysis and artificial neural networks** |
| **Chapter 7** | **General discussion and conclusion** |

# List of publications

## 1. Papers published in international journals

1.1 Daniels AJ, Nieuwoudt HH, Opara UL. (2018). Novel approach for measuring sugar and acidity non-destructively in whole table grape bunches. *Acta Hortic*. **1201**, 17-324. DOI 10.17660/ActaHortic.2018.1201.43

1.2 Daniels AJ, Nieuwoudt HH, Poblete-Echeverría C. and Opara UL. (2019). Measuring maturity and sensory parameters contactless on intact table grape bunches using NIR spectroscopy. *Frontiers in Plant Science* https://doi.org/10.3389/fpls.2019.01517

1.3 Daniels AJ, Nieuwoudt HH, Poblete-Echeverría C. and Opara UL. (2020). New technologies to maintain quality and reduce postharvest losses of table grapes. *Acta Hortic.* **1275**, 113 – 120. DOI: 10.17660/ActaHortic.2020.1275.16

1.4 Poblete-Echeverría C., Daniels AJ, Nieuwoudt HH, Opara UL. (2020). Artificial neural network as alternative method for prediction of sugar and acidity using near-infrared spectroscopy in table grapes. *Acta Hortic*. **1292,** 321-328**.** DOI: 10.17660/ ActaHortic.2020.1292.42

## 2. Manuscripts in preparation for submission:

2.1. Grape expectations: a critical evaluation of present-day strategies for the control and monitoring of table grape quality
To be submitted to *South African Journal of Enology and Viticulture*

2.2. Incidence and intensity of table grape defects during cold storage
To be submitted to *South African Journal of Enology and Viticulture*

2.3. Evaluation of handheld NIR spectrometers for quantitative purposes on whole berries
To be submitted to *Foods*

2.4. Classification of browning on intact table grape bunches using near infrared spectroscopy coupled with partial least squares discriminant analysis and artificial neural networks
To be submitted to *Postharvest Biology and Technology*

## 3. Conference presentations

3.1. Daniels AJ, Nieuwoudt HH, Opara UL. (2016). Steps towards a multi-evaluation of table grape quality: New vs Old. SASEV 38th International Conference. 23 August 2016. Lord Charles Hotel, Somerset West.

5 Daniels AJ, Nieuwoudt HH, Poblete-Echeverria, C. Opara UL. (2017). approach for measuring sugar and acidity non-destructively in whole table grape bunches. VII International Conference on Managing Quality in Chains (MQUIC) 5 September 2017, Jannasch Hall, Stellenbosch University.

3.2. Daniels AJ, Nieuwoudt HH, Poblete-Echeverría C. and Opara UL. (2018). New technologies to maintain quality and reduce postharvest losses of table grapes. XXX International Horticultural Congress – 12 – 16 August 2018, Istanbul Congress Center, Hall 7, Istanbul, Turkey.

3.3. Daniels AJ, Botha, N. Nieuwoudt HH, Opara UL. (2018). Brown or not brown – using artificial neural networks (ANN) to classify whole white seedless table grape bunches scanned nondestructively with near infrared (NIR) spectroscopy. 41st SASEV-WINETECH Conference. 2- 4 October 2018. Lord Charles Hotel, Somerset West.

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# Chapter 1: Introduction and project aims

## 1.1 INTRODUCTION

The South African Table Grape Industry (SATI) is the tenth largest producers of table grapes (*Vitis vinifera* L.) for fresh consumption in the world (Phaleng and Tshitiza, 2019). The prospects for future improvement in profitability look promising thus far, but this will require farmers to carefully take into consideration investments in infrastructure, cultivar selection and export market selection options as well as the fine balance that exists between volume and quality. Since the annual growth rate is estimated to be less than 1% in both hectares and volumes, it is expected that producers will move towards yields that are more conservative (BFAP, 2019). To curb these challenges, one of the promising and cost-effective strategies is for the industry to maintain its profitability through the constant delivery of superior quality products (Barrientos and Visser, 2012). Quality is a broad term in any commodity and in table grapes the external and internal quality are major aspects, which are dictated by the consumer. The producer, by law, needs to conform to the minimum criteria of these two aspects, as stipulated by the South African Agricultural Product Standards Act No. 119 of 1990.

There are still, however, a lot of challenges when it comes to maintaining the postharvest quality of table grapes, as can be seen in the studies conducted to help improve it (Champa *et al.*, 2014; Sabir and Sabir, 2017a, 2017b, 2019). This challenge can also clearly be seen in the number of 4.5 kg cartons that were rejected (682 269 in the 2011/2012 season and 539 346 in the 2012/2013 season) by the Perishable Products Exports Control Board (PPECB). The losses were more than 1% of the total volume of 4.5 kg cartons inspected (51 263 110 in 2011/2012 and 50 138 109 in 2012/2013) (PPECB, 2014). The two main reasons were due to physiology and pathology (Louw, 2017). Concerning physiology, the rejections included small berries and insufficient total soluble solids (TSS) and concerning pathology, grey mould. The problem of small berries can be managed through the improved pre-harvest practice of removing small berries a week after the application of gibberellic acid ($GA_3$) or other berry growth hormones to the grapes. Removal of small berries missed in the vineyard occurs after harvest in the packing shed before grapes are packed. Packing of small berries can even be excluded through using an online-computerised horizontal conveyor combination system such as Vizier's grape sizing system (Smit *et al*., 2011). Insufficient TSS can be managed through a more accurate sampling of berries to test with a handheld refractometer before harvest in the vineyard. Although TSS can still be measured in the packhouse, managing it postharvest is not possible, since grapes that were harvested with insufficient TSS cannot be exported and have to be discarded. Management of grey mould caused by the fungus *Botrytis cinerea* (Gaber *et al*., 2013) on the other hand has a plethora of methods that have been described in numerous publications

(Chervin *et al.,* 2005; Lurie *et al.,* 2006; Romanazzi *et al.,* 2007). These have been used for many years to minimise its detrimental effect on foreign income that is not received due to its occurrence. Other postharvest defects like browning, however, are not so easily manageable before export because not all phenotypes are visually observable at harvest and are only expressed on the grapes later during cold storage.

Technology that can accurately address the occurrence of these shortcomings and defects before the postharvest stage are thus necessary, especially given the complex levels of table grapes for export. Technologies such as near-infrared (NIR) spectroscopy can be used on their own or in conjunction with a system like the Vizier online grading system to reduce the effect of these problems on the table grape industry. These technologies help with the sorting and grading of fruit. Firmness, skin and flesh colour, and dry matter content of pickling cucumbers (Kavdir *et al*., 2007) and soluble solid content (SSC) and firmness of bell pepper (Penchaiya *et al*., 2009) have been determined with great success using NIR spectroscopy. The lycopene content on intact tomato fruit (Clément *et al*., 2008) and TSS on whole grape berries (Baiano *et al*., 2012) have also been determined using NIR spectroscopy.

## 1.2 PROBLEM STATEMENT AND RESEARCH QUESTIONS

The scope of this study has two focus points: one to address the complex levels for table grape quality and one to address techniques to measure these different quality aspects.

### 1.2.1 Complex levels for export of table grapes

The cultivation and production of table grapes for export is a very costly, labour-intensive and high-risk endeavour. There are many intricate processes that they have to go through during the separate parts/stages of the production chain. The stages are (i) the developmental stage in the vineyard, which includes ripening; (ii) harvesting; (iii) transport to the packhouse; (iv) sorting and packaging in the packhouse; and (iv) cold storage. Depending on the number of grapes harvested on a specific day and the capacity of a packhouse to pack all those grapes on that day, cold storage may also occur before packaging. Many packhouses also make use of forced-air cooling to cool down grapes that were harvested during the hotter period of the day. It can, however, also be used after fruit has been packaged (Ngcobo *et al*., 2013). The next stage during the logistics of table grapes for export, after being packed in multi-layered packages in the packhouse (Ngcobo *et al*., 2012a; Ngcobo *et al*., 2012b), comes with the transport of the grapes from the packhouse of the farm to the harbour in cold trucks. This is followed by the unloading of the cargo from the cold trucks onto the ships in refrigerated containers. This is preceded by an inspection of random boxes by the PPECB to see if the assignment meets the minimum criteria for export. Cold storage at the appropriate conditions (temperature and relative humidity) should be maintained during transport to and on arrival at the export destination. This

long and complicated journey of export table grapes comes to an end with transport to the supermarket chain stores that ordered the grapes. Here grapes will first be unloaded in a cold room before being put on the shelves, before the consumer finally gets to see, taste (if allowed) and buy the product. This can take anywhere from 14 to 22 days, depending on the country exported to (Burger *et al*., 2005). How does the producer, therefore, ensure that the quality of the table grapes harvested in the vineyard and packaged in the packhouse is still the best possible product when it is delivered to the consumer?

### 1.2.2 Different quality aspects of table grapes

Table grapes are non-climacteric fruit (Coombe and Hale, 1973) and will not ripen and neither will the quality improve after being harvested. Furthermore, there is a multitude of factors that can influence table grape postharvest quality (Freiboth *et al*., 2013). This includes internal and external attributes. The internal attributes are the chemical parameters such as TSS and titratable acidity (TA) which are quantitative since their concentrations can be measured in exact numbers. External attributes are the physical parameters such as bunch and berry colour, shape and quality since they can be described. In a study by Zahedipour *et al.* (2019) comparing the quality characteristics and physiological reactions of organic and conventionally cultivated table grapes during cold storage they found that grape berries from the organic vineyard were sweeter and softer and had more desirable colour parameters, higher antioxidant capacity and phytochemical compounds content than berries harvested from the conventional growing vineyard.

#### 1.2.2.1 Quantitative aspects of table grape quality

When it comes to the chemical maturity and quality determining parameters of table grapes, TSS and TA, or the sugar:acid (TSS:TA) ratio, remain the most important ones (Mascarenhas *et al*., 2012; Wei *et al*., 2002). The producer determining the correct ripening or harvesting stage at which the table grapes are, ultimately influences the price consumers are willing to pay when buying grapes (Chervin *et al*., 2012), whilst everything possible is done to ensure that the grapes are indeed at the correct TSS or TSS:TA ratio (PPECB, 2013). It is, however, still a daunting task if one takes into consideration that the TSS and TA levels can not only differ amongst the grapes of the same cultivar in a block but between rows, between sections in a row, between vines in a section, between bunches on a vine and even between berries on the same bunch (Šuklje *et al.*, 2012). What makes this even more difficult is the different factors such as vine vigour (Wei *et al*., 2002) and crop load and irrigation (Howell *et al*., 2012) that can also affect these parameters. Thus the possibility of harvesting and ultimately packing grapes for export with different TSS, TA or TSS:TA ratios (some below and some above the intended), no matter how small, should not be overlooked.

### 1.2.2.2 Qualitative aspects of table grape quality

Similar to any other freshly consumed fruit type or vegetables, table grapes are reliant on their physical appearance (size, colour and shape) and taste (flavour, firmness, sweetness, tartness or balance thereof) as quality parameters pre- and postharvest (Lin and Zhao, 2007). In most markets, locally and especially internationally, the main visual/physical quality parameters are berry size (Abu-Zahra, 2013), weight (Cubero *et al*., 2012) and colour (Kamiloğlu, 2011). Many studies relating to quality parameters of table grapes either always include one (Wei *et al*., 2002) or both of these as quality determining parameters (Kamiloğlu, 2011). Both can also either be manipulated chemically through the application of growth regulators like $GA_3$ and Naphthalene acetic acid (NAA) (Abu-Zahra, 2013) or manually/viticulturally through irrigation and crop load (Howell *et al*., 2012), although in most instances such practices might not always be as successful or positive as initially intended, as was seen in the studies conducted by Howell *et al*. (2012) and Raban *et al*. (2013). The latter especially in terms of another quality aspect of grapes, which is the rachis that is inclined to turn brown during cold storage. Ngcobo *et al.* (2012c) emphasised this complex structure of whole table grape bunches (consisting of the rachis and the grape berries) in their study looking at the moisture loss characteristics of fresh table grapes packed in different film liners during cold storage. Another important physical characteristic of table grapes that is always looked at is the texture of the berry or firmness (Mascarenhas *et al*., 2012). This is because consumers almost always equate firmness to the freshness of the produce that they buy (Iwatani *et al*., 2011). Some of the main defects that affect the appearance and ultimate quality of table grapes are browning of the berries and rachis of bunches, grey mould, sulphur dioxide ($SO_2$) damage and berry crack or split.

*Browning*

Browning is a very complex phenomenon that can occur both on the outside (external browning) and inside (internal browning) of both white and red cultivars, although it is more prevalent on white cultivars (Moelich, 2010). Numerous studies have been undertaken to elucidate the exact factors that lead to the occurrence of this phenomenon and how exactly it progresses up until it is perceived visually. What makes it even more problematic is the many different phenotypes that it can express and even these are caused by different factors (Fourie, 2009). It is only recently that advances have been made to try and predict whether it will occur on whole white seedless berries using NIR spectroscopy (Daniels, 2013), but not on whole bunches yet.

*Decay*

Decay on table grapes, also known as rot or grey mould, is mainly caused by the fungus *Botrytis cinerea* and is traditionally controlled by $SO_2$ fumigation and storage at −0.5°C. Due to

many reasons, however, amongst other $SO_2$ damage that can occur on the grapes, this is not the ideal treatment (Gabler *et al*., 2010). Throughout the years, many different ways of applying the $SO_2$ treatments have been investigated (Lichter *et al*., 2008). Alternative treatments have also been tried either through different packaging strategies (Sabir *et al*., 2010) or application of different compounds; these include application of aloe vera in vitro (Castillo *et al*., 2010), zinc oxide nanoparticles (He *et al*., 2011), ethanol and calcium chloride (Chervin *et al*., 2009), fumigation with high concentrations of ozone gas (Gabler *et al*., 2010), a combination of chitosan and ethanol (Romanazzi *et al*., 2007), and grapefruit seed extract and chitosan (Xu *et al*., 2007). Some of these are more effective than others but still do not provide the means to eliminate the occurrence of this postharvest defect.

*Sulphur dioxide ($SO_2$) damage*

The control of postharvest decay of table grapes with $SO_2$ fumigation, especially other decay-causing fungi, such as *Alternaria* spp. and *Penicillium* spp., is still successful in some instances. Negative issues, however, such as bleaching of especially non-white cultivars (Marois *et al*., 1986) and the taste of grapes after such treatments still exist (Feliziani *et al*., 2014). This especially if the cultivar is more likely to such damage due to the structure of its epidermis layer (Fernández-Trujillo *et al*., 2008). This can even be exacerbated if the grapes are also prone to berry crack either around the pedicel or on the berry itself.

*Berry crack*

Rot, berry crack or split and berry abscission are the main reasons why economically important cultivars such as Thompson Seedless have such a very short period during which they can maintain their quality (Burger *et al*., 2005). Berry split can occur due to many reasons, like rainfall (Howell *et al*., 2012), external forces being exerted onto the fruit during packing and/or handling (Burger *et al*., 2005) as well as viticultural practices like the application of plant growth regulators to the grapes before harvest and $SO_2$ after harvest and during cold storage (Zoffoli *et al*., 2009). Either way, the effect that it has on the postharvest quality of table grapes especially because of an increase in the severity of grey mould when it occurs, is detrimental to the table grape industry.

### 1.2.3 Near-infrared (NIR) spectroscopy

The basic concepts, equipment and use of near-infrared spectroscopy to determine a plethora of attributes of different varieties of fruit and vegetables have been extensively documented in reviews such as those of Nicolaï *et al*. (2007), Jha et al. (2010) and Magwaza *et al.* (2012).

Infrared spectroscopy (near, mid and attenuated total reflectance) has only recently been used here in South Africa (Daniels, 2013) to investigate the possibility of predicting quality parameters in table grape juice and table grape berries in terms of browning. This technology has, however, been used extensively for many years in South Africa (Magwaza *et al*., 2012, 2013) as well as in other countries (Clark *et al*., 2003; Fu *et al*., 2007) in all the different fruit industries, including the wine industry (Barnaba *et al*., 2013). NIR spectroscopy has been used for non-destructive measurement of vitamin C, total polyphenol and sugar content in apples (Pissard *et al*., 2012)**;** titratable acidity, malic acid, and citric acid in bayberry fruit (Xie *et al*., 2011); jujube quality (Wang *et al*., 2011); b-carotene content in mango (Rungpichayapichet *et al*., 2015; Rungpichayapichet *et al*., 2016); pH and firmness of star fruit (Omar and Matjafri, 2013); passion fruit (de Oliveira *et al*., 2014); colour-related external quality parameters as well as firmness, soluble solids content and titratable acidity of strawberries (Sánchez *et al*., 2012); on wine grapes (González-Caballero *et al*., 2010; Kemps *et al*., 2010; Barnaba *et al*., 2013) and even on table grapes (Baiano *et al*., 2012, Parpinello *et al*., 2013). When it comes to table grapes, however, there is still a largely uncharted area of its use especially in terms of determining pre-and postharvest quality parameters non-destructively in the vineyard already or once grapes are in the packing shed.

The following research questions have been formulated:

- What are all the parameters that need to be measured in the vineyard? TSS alone or TSS together with TA and TSS:TA ratio as well?
- How can the available quality measurement technologies assist in harvesting berries at the right maturity?
- Can postharvest defects be detected and predicted in the vineyard before harvest?
- What are all the parameters that need to be measured in the packhouse? TSS alone or TSS together with TA, TSS:TA ratio, pH and BrimA—another sensory-based parameter calculated as Brix minus Acid (TA) (Jordan, 2001), perhaps?
- Can measurements in the vineyard be corroborated in the packhouse?
- Can defects such as the potential to develop browning be detected in the vineyard and the packhouse already?
- Can the quantitative (TSS together with TA, TSS:TA ratio, pH and BrimA) and qualitative (browning, decay, $SO_2$ damage and berry crack) quality parameters be determined fast and accurately?
- Can South African table grapes be divided into those that would be marketed locally based on their high potential for developing defects and those that should be exported?

## 1.3 PROJECT AIMS AND OBJECTIVES

The overall aim of this study was to non-destructively evaluate the external and internal quality of table grapes. To achieve this, the study included the following aims and objectives.

### 1.3.1 Aims

#### 1.3.1.1 Berry logistics

To use the same handheld device to measure whole single berries before harvest in the vineyard and again after harvest in the laboratory. To measure the same whole single table grape berries after measurement in the laboratory with the handheld device with a benchtop instrument. Compare accuracies of spectra obtained for berries in the vineyard to those obtained in the laboratory with the same handheld device. Compare those spectra also to the ones obtained of the same berries on the benchtop instrument.

#### 1.3.1.2 Berry quality

Determine berry quality by measuring the TSS of each berry. Evaluate each berry for defects.

### 1.3.2 Objectives

#### 1.3.2.1 Calibration and classification models

Construct calibration models for TSS, TA, TSS:TA ratio, pH and BrimA and build classification models for classifying bunches according to those that turned brown during cold storage and those that did not.

#### 1.3.2.2 Single berry quality prediction

Predict the quality of single berries based on TSS models constructed on the same berries measured in the vineyard and the laboratory with the same handheld instrument, and also using a benchtop instrument in the one year.

#### 1.3.2.3 Whole bunch quality prediction

Predict whole bunch quality in terms of quantitative parameters TSS, TA, TSS:TA ratio, pH and BrimA, as well as in terms of the incidence and intensity of defects that occurred on them during different weeks at cold storage. Build classification models for different browning defects.

## 1.4 EXPERIMENTAL DESIGN SUMMARY

The entire experimental design for this study is illustrated in Figure 1.1 In total, eight different experiments were conducted. The order in which the experiments took place was as follows: Single berries still attached to the bunch and bunches still attached to the vines were scanned in the vineyard with the MicroNIR handheld spectrometer (1). Bunches were harvested and packed in the vineyard and then transported to the laboratory. The same berries that were scanned in the vineyard were scanned again in the laboratory, first with the MicroNIR spectrometer (2) and then with the solid probe of Brukers MPA spectrometer (3). The TSS of each of those berries was then determined with a handheld refractometer (4). In the laboratory, whole bunches were scanned with the MATRIX-F spectrometer (5). One box was evaluated immediately for defects after scanning (6) and the other boxes were put into cold storage, scanned again after one, two, three, four, five and six weeks respectively at cold storage and then evaluated for defects (7). Twenty berries that were in the focus area of light from the MATRIX-F spectrometer were removed (ten from each side of the bunch that was scanned). These berries were crushed and the TSS, TA and pH were determined from them (8). The collected spectra in experiments 1, 2, 3 and 5 were paired with the reference values obtained in 4, 6,7 and 8 and regression models were built (prediction and classification) using either partial least squares (PLS) regression, partial least squares discriminant analysis (PLS-DA) and artificial neural networks (ANN).


**Figure 1.1** A summary of the project's experimental design: The first bracket indicates the materials used in the experiments and the second bracket indicates the methods or different steps followed during the experiments.

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# Chapter 2: Grape expectations: a critical evaluation of present-day strategies for the control and monitoring of table grape quality


## ABSTRACT

Table grape quality is a very complex concept since it is quantitative and qualitative in nature. Quantitative attributes are those measured in the vineyard and the packhouse such as total soluble solids, titratable acidity, and pH of grapes, while qualitative characteristics are the physical aspects such as the shape, colour and size of bunches. Both these determine the consumers' preference and buying decision, thus the quality should be perfect when they reach them. Since table grapes are non-climacteric fruit, maintenance of quality after harvest is key throughout the value chain. This chain is also very complex since it not only involves the multiple packaging levels but also the cold storage and transport period during which defects such as rot, sulphur dioxide damage, berry crack and browning usually occur. This review not only takes a critical look at the multifaceted nature of table grape quality, but also highlights the measures that should be put in place to prevent delivering products of a poor quality and any of the defects occuring postharvest. There is an exploration of a modern day technologies such as near infrared spectroscopy, hyperspectral imaging, computer vision systems and image analysis techniques to try to circumvent some of the problems experienced with table grape quality to see how these assist in their applications for determining quantitative and qualitative aspects in fruit.


## 2.1 INTRODUCTION

South Africa is the tenth largest producer of table grapes in the world and the fourth-largest exporter after Chile, Peru and the United States of America. China is the largest producer but also the leading consumer of table grapes (Phaleng and Tshitiza, 2019). In 2019, there was an export of 59.4 million 4.5 kg cartons garnering an income of almost R12 billion in export value (BFAP, 2020), highlighting the importance of table grapes to the South Africa economy. South Africa has five main regions of table grape production. These are the Northern provinces, Orange River, Olifants River, Berg River and Hex River regions. Crimson Seedless is the most planted and exported cultivar followed by Prime. In 2020 Crimson Seedless was 19% of total hectares of vines planted and Prime 8%. Crimson Seedless comprised 20% of the 4.5 kg equaivalent cartons exported during the 2019/2020 season and Prime 9%. Crimson Seedless is an attractive pink late-ripening cultivar that has elongated berries with crispy excellent flavour and Prime is a white seedless cultivar with a good berry size and a crisp new season taste.

Prime is also the earliest South African cultivar to be harvested. White seedless grape harvest starts the earliest from the second week of October in the Northern provinces, followed by the Orange River, Olifants River, Berg River and Hex River regions. Harvest then peaks during January and then gradually declines up until the end of April (Ferreira, 2020).

South Africa has been supplying the Northern hemisphere the longest and unfailingly with table grapes with the bulk of the exports going to the European Union (50%) and United Kingdom (25%). The next volumes of cartons exported are to Canada (7%), the Far East (5%), the Middle East (5%), South East Asia (5%), Russian Federation (2%), Africa (1%) and United States of America (1%) as well as the Indian Ocean Islands and other regions (Ferreira, 2020). South Africa holds the 7$^{th}$ position with a 6.2% share in the world's imports, the industry is, therefore, under constant pressure to supply produce of exceptional quality, not only to retain a competitive advantage in the international market but also to meet the demands and preferences of a heterogeneous international consumer base that is continually changing.

Consumers perceive quality through visual and organoleptic means. These attributes initially interest the consumer and, therefore, have a big influence on buying choices. Any aspect that affects the taste and/or appearance of the grapes negatively will finally decrease the product's market value, the consumer's confidence in the cultivar and make the fruit unmarketable (Cavallo *et al*., 2019). The best conceivable quality of any product is at harvest time and the challenge is to bring a product to the consumer with a similar level of freshness that it had at harvest (Kyriacou and Rouphael, 2018). Table grapes (*Vitis vinifera* L.) are non-climacteric fruit, which unlike climacteric fruit will not continue to ripen after harvest (Fuentes et al. (2019). Quality will not improve after harvest and maintaining the level achieved in the vineyard during all the multiple postharvest stages that grapes have to go through is essential.

What complicates things even further is the selling of table grapes as bunches, although they consist of berries that function as individual fruit. Grape bunches are packed into individual plastic carry bags or punnets, in turn, packed into boxes lined with perforated low-density polyethene (LDPE) liner bags (Figure 2.1). Maintaining quality is on multiple levels and it is clear that there is also a multi-packaging aspect to it. This multi-packaging aspect and its effect on table grape quality were investigated in detail by Ngcobo *et al*. (2013), but the others such as the multi-levels (vineyard vs packhouse and berry vs bunch), multi-criteria (appearance vs taste) and multi-parameters (sugar and acidity vs defects) have not yet been fully explored. This begs the question as to how science can help the table grape industry in monitoring table grape quality qualitatively (visual appearance) and quantitatively (chemical constituents) successfully throughout the whole value chain. It is very difficult to single out every stage or level as these follow so closely upon each other and link intricately. Whatever treatment is applied to the vine, e.g. irrigation, fertilisation, girdling or crop load treatment, will affect the bunch. Similarly, whatever treatment is applied to a bunch, e.g. the application of plant growth hormones and

regulators such as abscisic acid (ABA) and/or gibberellic acid ($GA_3$) will affect the berries and resultant quality (Rusjan, 2010).

The main aim of this literature review was to combine available literature and identify research gaps in present-day practices and systems in use for the control of table grape quality through the whole production value chain from harvesting in the vineyard, through packaging and cold storage of bunches, up to retail shelves in export markets where consumers make purchase decisions. This required a balance of knowledge of the biology of table grape quality, production, packaging, distribution and consumer perceptions. This review will, therefore, focus on harvest, packaging, cold storage, but exclude the consumer aspects of monitoring table grape quality non-destructively using present-day technologies such as near-infrared spectroscopy (NIR), hyperspectral imaging (HSI), computer vision systems and image processing.


**Figure 2.1** Prime bunch a few hours after harvest, taken out of the individual carry bag (previously packaged in). The lined box with an LDPE liner bag shows bunches still in individual carry bags. This bunch shows the desired visual appearance of the fruit. These are an even colour, shape and size of the bunch and berries, blemish- and damage-free berry skins, firm berry attachments, fresh and green stems. The bunch is also not compact (Ahmed *et al*., 2018).

## 2.2 SECTION I: LITERATURE REVIEW METHODOLOGY

For this review the focus was to look at different aspects of table grape quality, the problems experienced during the different stages of production, harvest, packaging, cold storage and transport, show how these problems have been dealt with in the past and how recent technologies such as near infrared spectroscopy, hyperspectral imaging, computer vision systems and image processing techniques can be applied to resolve them. The intention with the literature searches was to focus only on what has been published in the last decade (post 2010). This was relatively easy for near infrared spectroscopy, hyperspectral imaging, computer vision systems and image processing techniques since a lot of work has been done and published in the last decade where these technologies were used on different fruit and vegetables, including table grapes, to determine a wide range of parameters, especially firmness, total soluble solid (TSS) or soluble solid content (SSC) as well as titatrable acidity, pH and colour. When it came to searching for table grape quality aspects, however, there was a limit on new work or literature been published that did not reference previous work done on these aspect pre-dating 2010. These limiting parameters for example related to a concept such as that of consumers acceptance of grapes being dependant on many factors such as colour, flavour, size, bloom, and texture, are already highlighted in the first sentence of the introduction of an article by Nelson et al. in 1963 on the chemical and sensory variability in table grapes. In this publication the taste aspect of flavour is already also described and subsequent publications always refer to this original article just like with the new concept BrimA that was added, initially suggested by Jordan et al. in 2001 as an alternative to the TSS:TA ratio. Thus where-ever possible, only articles published after 2010 were used.

The methodology that was, therefore, followed was to do the literature search using various databases like CAB Abstracts, Scopus, Google Scholar, Science Direct and Web of Science. Search words/phrases that were used included “biology of fruit quality”, “table grape quality”, “machine learning” and “fruit quality”, “fruit grading image processing”, and the search strings in the databases were "machine learning" OR "near-infrared spectroscopy" AND "fruit quality" AND "table grapes". Various other searches relating to the defects on defects and alternatives to treatments available to prevent them was also done on these databases.

## 2.3 SECTION II: CHALLENGES WITH MONITORING TABLE GRAPE QUALITY

### 2.3.1 Consumer aspects of table grape quality

#### 2.3.1.1 Visual appearance

Table grapes are not exported in their natural state and need to be prepared in the vineyard to meet the consumer’s preferences. Aspects concerning the visual appearance of the fruit like berry shape and size, bunch shape and size (Figure 2.1) and berry colour (Fahmi et al., 2012).

Taste includes the total soluble solids (TSS), titratable acidity, sugar:acid (TSS:TA) ratio, skin and flesh firmness (Rolle et al., 2011; Mascarenhas et al., 2012) and flavour which is the sum of interactions between taste and odour (smell) (Wu et al., 2018).

*Berry*

Bunches consist of berries each attached with short stems (pedicels) to a central axis called a rachis. Each berry behaves like an individual fruit. This is illustrated in Figure 2.2 where some berries turned brown and some did not, and *vice versa*. Table grape berries can contain seeds (seeded) or not (seedless) and seedless varieties have become more popular than the seeded varieties in the past few decades (Faci et al., 2014). Table grape berries have different shapes (elliptical, oval, oblong) and sizes (medium-large, large) as well as different colours by which they can be described (light green, dark violet and golden yellow for example concerning white varieties). These play a very important role in the physical/external quality of table grapes whereas the texture (crispness) and flavour (sweetness, acidity and juiciness) of the berry play a crucial role in the chemical/internal/taste of the grapes (Wang et al., 2017). A lot of research has focussed on the aspect of colour as an important attribute when it comes to the quality of products (Pathare *et al*., 2013) including that of table grape berries of red and black varieties. Berry colour results from the synthesis and accumulation of anthocyanins in berry skin (Xie *et al*., 2015). Segade *et al*. (2013) looked at the characterization of Italia table grapes on chromatic attributes but found, bowever, that the colour parameters for the Italia cultivar were different to those found in other white table grapes. There is thus a real need to develop strategies to monitor and help distinguish between healthy bunches and ones showing any signs of a defect like browning at harvest and during packaging and cold storage.

**Figure 2.2** Two bunches illustrating berries acting as individual fruit where some berries turned completely brown and others remained green after cold storage under the same circumstances (packaging materials, temperature and duration of cold storage)

*Bunch*

Harvested table grape bunches are packed and exported as either individual bunches in a punnet or a box with other bunches. Since berries behave like individual fruit, meaning that during the growth stages of the grapes, each berry follows its own double sigmoidal growth curve synonymous with grape developmental stages. Bunches, therefore, will also differ from each other in terms of quality aspects. The accumulation of anthocyanin and development of colour in each berry does not necessarily take place in the same relation, at the same time, or at the same pace as in the other bunches (Ferrara et al., 2015). Thus, all the berries on a bunch, all bunches on a vine and all the vines in a block may not be at the same maturity level at harvest (Šuklje *et al*., 2012). Not every berry on a bunch and within a block is monitored for harvest readiness so the presumption is that all are at the same maturity level. The Perishable Products Exports Control Board (PPECB) conducts a random sampling of packed table grape boxes and punnets at the harbour before export. Grapes found to be at the incorrect TSS, TA and/or TSS:TA ratio can lead to whole consignments rejected for export and even returned once they have reached the intended market. There is thus a huge need for assistance in the vineyard to measure as many as possible berries as fast as possible and non-destructively.

#### 2.3.1.2 Taste

Consumers' awareness regarding the sensory quality of products has significantly increased and their acceptance of products such as table grapes having the right quality is reliant on qualitative properties like flavour and taste. The flavour of any food is the sum of interactions between taste and odor (smell) and taste for fruits such as table grapes, refers to sugars and acids that activate taste receptors (Wu et al., 2018). Four main descriptors are commonly used to illustrate the flavour of table grape varieties belonging to *Vitis vinifera*, L namely, 'Muscat', 'neutral', 'herbaceous', and 'fruity' (Maoz et al., 2020) and there are four possible tastes in grapes namley acidness (tart, or sour), sweetness, saltiness, and bitterness (Muñoz-Robredo et al., 2011).

*Total soluble solids (TSS), Titratable acidity (TA), sugar:acid (TSS:TA) ratio, pH and BrimA*

Total soluble solids (TSS) and titratable acidity (TA) are commonly measured to determine the basic taste of the berry, whereas the composition of volatile compounds, such as the level of total free monoterpenes in the skin, methoxypyrazines, $C_6$ aldehydes and alcohols can be measured to determine what gives the berries their unique flavour (Maoz et al., 2020). TSS, also known as soluble solids content (SSC) and in grapes is the number of sugars (glucose and fructose) measured in °Brix. The measurement of organic acid compositions occurs as TA (expressed as g/L tartaric acid) and pH (Shiraishi *et al*., 2010; Fahmi *et al*., 2012). The juice pH is a measure of the hydrogen ion concentration in the berry and generally relates to juice acidity. The sugar:acid (TSS:TA) ratio is also regurlarly determined with TSS and TA as a classical parameter of consumer acceptability of table grapes (Rolle et al., 2013). However, since TSS:TA ratio is determined by dividing the % TSS by % TA, expressed to the first decimal (Wongkhot et al., 2012), the same ratio may be derived from different concentrations of TSS and TA, leading to different taste perceptions for the same ratio (Jamshidi et al., 2012; Magwaza and Opara, 2015). A value of 14, for example, indicates a sample with 14 parts soluble solid and one part acid. The taste of the grapes is sweeter the higher the value or ratio and vice-versa (Wongkhot et al., 2012). Since the presence of acids have a sweetness-reducing effect, the problem with the low reliability of this index increases because TSS is the sum of sugars, acids and other minor components. Therefore, having acids as part of the numerator (TSS) and the denominator in the TSS:TA ratio might also be part of the problem (Wongkhot et al., 2012; Magwaza and Opara, 2015).

A new index in which the TSS reading is modified to account for this sweetness-reducing effect was developed by Jordan (2001) and called BrimA (pronounced bree-mah) as alternative to TSS:TA ratio. BrimA (an abbreviation for Brix minus Acid) measures the balance between Brix (sweetness) and acidity (sourness) (Wongkhot et al., 2012; Magwaza and Opara, 2015)

BrimA is calculated as TSS – *k* x TA. The constant *k* shows that the tongue is more sensitive to acid than it is to sugar. Due to different fruit containing different ratios of acids and sugars the *k* value range from 2 to 10. A *k* value of 5 is suggested for table grapes and was accordingly used in this study (Jordan *et al*., 2001). Other than Wongkhot et al. (2012) who investigated the use of BrimA as an index for ripening stages of mango fruit and Fawole and Opara (2013) who also included it as a parameter in their study to determine changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. Ruby) fruit at five maturity stages, BrimA has mostly been included as a parameter on oranges (Jamshidi et al., 2012; McDonald et al., 2013). In all these studies BrimA was found to be a good or even better taste index parameter than the TSS:TA ratio.

*Colour, gloss, odour, firmness and flavour*

The importance of sensory quality variables such as colour, gloss, odour, firmness and flavour are highlighted by their inclusion in many studies. Pastor et al. (2011) also included them in their study when they investigated the quality and safety of table grapes coated with hydroxypropylmethylcellulose (HPMC) edible coatings containing an ethanolic extract of propolis. Propolis is a sticky substance collected by bees from secretions by plants and mixed with wax and bee enzymes (Sforcina and Bankova, 2011). Pastore et al. (2011) wanted to take advantage of the valuable health properties of propolis to see if quality and shelf life could improve during cold storage if applied to the table grape cultivar Muscatel. Their results for the sensory analysis showed that grapes coated wwith HPMC had a gloss more significant than the uncoated grapes but no significant differences occurred in firmness. They, however, detected a significant difference in odour and flavour increase between the samples not coated with HPMC than those coated with HPMC containing propolis. This was because of the influence that the taste of propolis had on the overall flavour and odour of the grapes they summasied.

The effects of chitosan from *Cunninghamella elegans*, a non-ligninolytic Zygomycete, (Aydin et al., 2017) on the virulence of post-harvest pathogenic fungi (*Botrytis cinerea* and *Penicillium expansum*) on the table grape cultivar Isabella (*Vitis labrusca* L.) was studied by de Oliveira et al. (2014). *Vitis labrusca* is native grape species in the United States that have a characteristic fruity, floral ('foxy') aroma, distinctly different from the European grape cultivars (*Vitis vinifera* L.) predominantly planted worldwide (Köse 2014). De Oliveira et al. (2014) used the grapes appearance, colour, flavour, texture, aftertaste and overall assessment as sensory quality variables in their study. For all the assessed sensory parameters they found that the scores for the grapes submitted to the different treatments always corresponded to "liked slightly" or "liked moderately" indicating that the treatments had some effect on sensory quality.

Unlike Pastore et al. (2011) and de Oliveira et al. (2014) who evaluated sensory quality of grapes after application of coatings to enhance storage life, Ma et al. (2016) focused on

evaluation of sensory quality and consumers' satisfaction of table grape based on home storage performance. The reasoning they used behind this was that the preservation and storage of the fruit at home storage might have the worse quality than that in the commercial cold warehouses since temperature fluctuates more (opening and closing of refrigerators) and does not remain stable as during experimental and/or commercial cold storage. They examined internal sensory quality attributes such as texture, taste and odour and appearance indices such as colour and cleanliness (berry bloom) of the grapes. Their key results showed that during home storage, all the internal attributes decreased rapidly as time went on, and cleanliness and colour appeared to be deteriorating in a lower speed. All three of the above studies made use of tasting panels to evaluate the parameters, but only Pastore et al. (2011) made use of instruments as well to measure mechanical properties such as texture using a compression test with a texture analyser and colour using a spectrocolorimeter.

Rolle et al. (2011) again did not make use of a tasting panel, but only instrumental analysis to compare the texture, colour, and chemical characteristics of ten white table-grape varieties. They found an explanation for the different sensory characteristics with the different tests conducted and that there was notable differences among the varieties in each of the considered parameters approving their significance in the description of the cultivar and in the assessment of possible consumer acceptability. When Baiano et al. (2012) used hyperspectral imaging for prediction of physico-chemical and sensory characteristics of table grapes, the spectra information were highly correlated with the physical-chemical data (pH, SSC, TA and SSC:TA ratio), but was not correlated with the sensory data which made predicting consumer liking difficult. However, these researchers argued that since physical-chemical parameters of table grapes does seem to affect their observed quality, it was realistic to expect that SSC, TA and pH would correlate well with sensory attributes such as sweetness and acidity and with the Overall Liking Scores (OLS). They, therefore, tested simple correlation among the couples TA-acidity, pH-acidity, SSC-sweetness, SSC-OLS, TA-OLS, pH-OLS, and SSC:TA-OLS to investigate the extent to which sensory measures could be explained by physical and chemical composition. They found that all the chemical-physical characteristics correlated poorly to the sensory attributes and that especially OLS only correlated partly.

It is clear from the above that a whole range of parameters demands monitoring for table grape sensory quality and taking all of this into consideration, while each method used on its own seems to deliver accurate results, all of them should be used and tested in connection with the other in one study in order to create the best possible models for their future prediction.

### 2.3.2 Production and harvest of table grapes (vineyard)

The vineyard is the first level where quality is determined and when the correct cultivation processes are followed, these will have a positive influence on the development of the desired

profile of the grapes (appearance and taste) including everything done on a berry, bunch, vine, section and block-level. Kamiloğlu, (2011) conducted a study to determine the influence of different cultural practices on vine yield and fruit quality (bunch and berry characteristics, juice quality, total anthocyanins and individual anthocyanins) for the table grape cultivar 'Horoz Karası'. Their study consisted of the comparison of seven different cultural practices in addition to the control vines and highlighted how different cultural practices done separately and in different combinations with each other can ultimately affect grape quality and composition. Practices such as girdling, irrigation manipulations, fertilization treatments, as well as crop load management are done in the vineyard to ensure good quality grapes.

The importance of girdling as a horticultural practice lies in that it allows for an increase in yields or ripening that is controlled. Little, however is known about how it affects metabolic processes. Tyagi et al. (2020) looked at girdling of table grapes at fruit set to see if the phenylpropanoid pathway could be diverted towards accumulation of proanthocyanidins and change the volatile composition of Superior Seedless and Sable Seedless table grape cultivars. They found that girdling had the same positive effect on the increase of berry size for the two cultivars, but differed in terms of TSS. On whether metabolic pathways could be diverted in a manner that may have significant effect on the taste and flavour of grapes, their study indicated that it was indeed possible.

Irrigation focuses on the postharvest quality of table grapes when water-saving strategies are applied. Conesa *et al*. (2015). examined the effects of deficit irrigation strategies on some physical quality attributes in 'Crimson Seedless' table grapes at different stages (at harvest, after 28 days of cold storage at 0 °C and after an additional shelf-life period of 3 days at 15 °C) over three years. They found that it was possible to decrease irrigation of table grapes without adversely affecting the physical quality of the berries. Fertilisation treatments are usually applied to the soil to help with different growth aspects of the vine and resultant grape quality. But in cases where organic farming is practiced, alternatives to nitrogen fertilizers are applied and their effects investigated.

Tarricone et al. (2020) looked at how different cover crops affected the yield, cluster components, crop quality, and organic matter status of Scarlotta Seedless table grapes under plastic film covering in an organic table grape experiment located in a typical area of the mediterranean environment in Southern Italy. They found that cover crops did not affect cluster weight, berry weight, and juice composition, but did have an influence on the detachment force of the berry.

Crop load management aims to establish an increase in the cluster weight and yield of the plant overall. Benavente *et al*. (2014) conducted a study over two consecutive seasons to determine if crop load adjustment and cluster tipping in relation to cluster shape can allow an increase in cluster weight and yield per plant in 'Thompson Seedless' table grapes. Three different cluster shapes (cylindrical, spherical or conical) was thus evaluated. A cluster-tipping

trial combining crop load with cluster shape and berry number was also additionally carried out. The results showed how different cluster shapes as well as different number of berries per cluster had different effects on crop load. Once more highlighting how different vineyard management practices can eventually influence grape quality.

Removing underdeveloped or excess berries, berry-clusters or even flower buds or individual bunches is known as grape thinning. This is usually done before or after the application of $GA_3$. This cultural practice can be performed to reduce crop load or as was done in the case of Roberto et al. (2017), to reduce compactness of 'Black Star' table grapes. Cluster stinning applied for reduction in compactness can lead to berries reaching maximum size including other important features like avoiding deformation of berries, lack of colour and sweetness on the inside of bunches (Preszler et al., 2010) and due to the higher source/drain ratio, improving the quality of the remaining fruits (Pastore et al., 2011).

Visual subjective assessment of table grape quality have always occured in the vineyard, but sensors are now available to monitor grape maturation by specifically monitoring anthocyanin accumulation (Ghozlen *et al*., 2010). Liu et al. (2017) have already looked at a completely automatic system for grapevine yield estimation allowing farmers to adjust their management practices for improved outputs by investigating a computer vision system. They presented a novel shoot detection framework that included image processing, feature extraction, unsupervised feature selection and unsupervised learning as a final classification step. A procedure for converting shoot counts from videos to yield estimates was then introduced. Strategies to monitor the development of quality in an entire vineyard block before harvest are, however, also needed.

### 2.3.3 Packaging and distribution (packhouse)

Table grapes can be packed in the vineyard already (Crisosto *et al*., 2001; Crososto *et al*., 2002) but most producers prefer to do it in a packhouse. The most important task in the packhouse is to weigh and grade the grapes according to the different classes based on their bunch and berry sizes. After an initial screening for damaged or small berries missed in the vineyard, removal of any that are still present occurs. Not much is possible if the harvest of poor-quality grapes happened in the vineyard already. If harvested grapes do not meet the appropriate size requirements, exclusion of these can happen through using an online-computerised horizontal conveyor combination system such as Vizier's grape-sizing system (Smit *et al*., 2011). Not many packhouses, however, are equipped with this instrument and there is still no way yet that other quantitative and qualitative aspects of the grapes can be determined fast and simultaneously at this stage. The need to determine the taste of grapes and whether the development of any defects after they have been packed and placed into cold storage non-destructively is, therefore, still prevalent. Irrespective of the slow adaptation of

technology such as NIR spectroscopy in-line and online (Magwaza *et al*., 2013), the value of its application in all stages and levels of the table grape value chain, especially in the packhouse where the final decision over export quality is made, is still critical. Brosnan and Sun (2002) reviewed the inspection, grading of agricultural and food products by computer vision systems and improving quality inspection (Brosnan and Sun, 2004). Bhargava and Bansal (2018) also conducted a review of fruit and vegetable quality evaluation using computer vision. All these highlighted the important role that computers can play in the elimination of the challenges faced in classifying products fast, accurately and safely in the packhouse.

Due to the many defects that can occur on table grapes because of rapid water-loss once harvested (Crisosto *et al*., 2001), the table grape industry mostly makes use of multi-scale packaging to maintain the postharvest quality of grapes (Ngcobo *et al*., 2012b; Ngcobo *et al*., 2013). Following an initial inspection for any defects or small and/or damaged berries, there is a weighing of each bunch and placement into an individual plastic carry bag. After placement into the individual plastic carry bags, bunches are packaged into a closed-top corrugated fibreboard carton in the packhouse.

Enclosing of the entire carton content is in a 2-mm LDPE liner bag that contains a corrugated cardboard sheet at the bottom to reduce abrasion damage. By using plastic liners, maintenance of relative humidity can be ensured, which prevents the loss of moisture from berries (Delele *et al*., 2013). Ngcobo *et al*. (2012a) and Ngcobo *et al*. (2012c) found there are definitive differences between the type of packaging liners that are used. When Ngcobo *et al*. (2012a) looked at the effects of different carton liners on the cooling rate and quality attributes such as weight loss, stem dehydration and browning, $SO_2$ injury, decay, berry firmness and colour of the table grape cultivar ‘Regal Seedless’, they found that the non-perforated versus the perforated influenced the relaive humidity during cold storage and during a 7 day shelf life period differently, resulting in different effects of the quality attributes measured. When Ngcobo *et al*. (2012c) looked at the moisture loss characteristics (transpiration coefficients) of the different parts of grapes (stems versus berries) packed in different packaging liner films during cold storage, they found that the different parts had a different reaction to the different types of packaging used.

An Uvasys® sulphur dioxide ($SO_2$) generator sheet (http://www.uvasys.com/) is used to cover the grapes to control decay (Junior *et al*., 2019). However, due to the high probability of $SO_2$ damage developing, various studies have undertaken to investigate modified atmosphere packaging (MAP) as another method either on its own (Costa et al., 2011; Silva-Sanzana *et al*., 2016) or in combination with other treatments (Utsun *et al*., 2012). Costa et al. (2011) investigated the influence that passive and active MAP can have on packaged table grape quality. Passive MAP refers to grapes packaged in air and active MAP to grapes packaged in under three different initial headspace gas compositions. They used three films made up of oriented polypropylene and characterized by a different thickness (20, 40 and 80 lm,

respectively) and grape samples were also stored without packaging gas control. Compared to the unpackaged product, they found that all selected packaging films significantly prevented product decay and that the thickest polymeric matrix sealed in air delivered the best results. When Silva-Sanzana *et al*. (2016) investigated te effect of MAP on rachis quality of 'Red Globe' table grape cultivar, they found that MAP helped to reduce the green colour loss on rachises stored for 90 days of storage at 0 °C compared with a conventional storage even after a shelf life period, without negatively affecting the quality of the berries when used in combination with other treatments as was the case with Utsun *et al*. (2012). These researchers looked at how perforated polyethylene (PPE) or ZOEpac MAP bags, with or without different grades of ethanol vapor-generating sachets or an $SO_2$-generating pad influenced the chemical composition (sugar, organic acid, and anthocyanin contents) and antioxidant capacity of 'Red Globe' table grapes during cold storage. The different quality attributes was affected differently by the different combinations of treatments used.

Different packaging strategies and different combination of the ones available, therefore, also influences the quality of grapes differently just like different cultural practices in the vineyard does.

#### 2.3.3.1 Pallet

Placing packed boxes onto pallets enables loading onto cold trucks and then cold storage units at the harbour. A delay in transport and temperature variations will always play a part in the postharvest quality of table grapes. Maintenance of postharvest quality during this time is critical. The greatest challenges, possibly, in keeping table grapes fresh are the delay between harvest and until the fruit reaches the consumer, as well as the temperature variations experienced during all that time. Immediately after harvest and during handling and transportation table grapes start to lose water (Candir *et al*., 2012). Serious challenges are posed by this during the long storage and transport period that table grapes have to withstand when the export market is far from the country of origin. To maintain high quality throughout all these different stages, the appropriate postharvest strategies like the right cold storage and controlled atmosphere (CA) conditions (which is 2% oxygen ($O_2$) with or without 5% $CO_2$) have to be followed (Balic *et al*., 2012). Adequate cooling usually at 1 °C commercial storage conditions (Feliziani et al., 2014) is, therefore, the most critical phase in the postharvest handling procedure to maintain quality. Displayed grapes in the supermarkets are at higher temperatures, mostly above 15 °C. Any defects expressed here is then a clear indication that not everything possible occurred to either prevent or detect it successfully before export of the grapes. Certainly, non-destructive technologies and/or strategies such as NIR spectroscopy, HSI, computer vision systems and image processing exist that could help to detect the presence or the possibility of defects occurring before the grapes are loaded onto the pallets

thereby preventing the export of grapes that will eventually lead to the whole consignment being rejected once the intended destination/supermarket is reached

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Walsh et al. (2020) reviewed NIR spectroscopy on how it can support decision making during postharvest. First by looking at the direct assessment of chemical or physical attributes related to postharvest quality at the time of assessment and then at the forward prediction of a postharvest attribute of the fruit or vegetable for e.g. when it has already been packaged and put on a pallet. Visible (VIS)/NIR transmittance spectroscopy was for instance assessed by Sun et al. (2016) to simultaneously measure brown core and SSC in pear on-line. They obtained very good results with classification accuracy of brown core pears being 98.3% and the percentages of SSC predictive precision up to 99%.

Munera et al. (2017) used VIS/NIR hyperspectral imaging together with colour analysis to non-destructively assess the internal quality of intact persimmon. These researchers found good correlations in some colour parameters with the data obtained by the imaging system improving previous results. Calvo et al. (2016) proved the versatility of computer vision systems by looking at a practical framework for automatic food products classification using three different basic food products: Hass Avocado, Manila Mango and Corn Tortillas. The reason for their decision on these three was that in economical terms all these products are very important for the overwhelming volume of their production and marketing and that each product has specific traits that involve different ways of handling the quality inspection similar to the different logistical stages of table grapes.

Kicherer et al. (2013) looked at developing an automated image interpretation tool to acquire berry morphology traits, especially the number of berries per cluster and the mean berry diameter. These researchers found all placed berries could be counted 100 % correctly by their berry analysis tool (BAT) and that manual ratings compared with BAT ratings showed strong correlation for mean berry diameter/image and for cluster volume. This could prove to be quite a powerful tool if adjusted for estimation of these same parameters for table grapes already packed and placed on pallets.

### 2.3.4 Cold storage

#### 2.3.4.1 Grey mould, sulphur dioxide ($SO_2$) damage, berry crack and browning

Regardless of the risk that long transport periods and temperature fluctuations during that time pose to fruit quality, consumers still prefer that the grapes look as good and fresh as the day it was harvested. Grapes should, therefore, have no possible skin disruptions, bruises, spots, rots, decay, and other deterioration (Costescu, 2013). To meet all these high expectations

export table grape cultivars must not only have a decent storage life but a very decent shelf life as well. This is a difficult task as grapes are very perishable commodities (Sortino et al., 2017) and prone to different postharvest quality defects like berry crack, grey mould, $SO_2$ damage and browning. Berry crack is when the berry splits open along either the side of the berry or around the stem and sometimes at both positions (Yu et al., 2020). Grey mould is the type of rot on table grape berries caused by the fungus *Botrytis cinerea* during storage for long periods (Jiang et al., 2015). The cause of $SO_2$ damage is fumigation of grapes directly with the gas or the use of $SO_2$-generating pads to control postharvest diseases, especially grey mould on table grapes (Sortino et al., 2017). Treatment with $SO_2$, however, can also lead to stems prematurely turning brown (Candir et al., 2011) and the berry skin bleaching (Bal et al., 2017). $SO_2$ also causes hairline cracks on table grape berries (Abdolahi et al., 2010). These cracks are not visible to the naked eye and very fine in comparison to normal berry cracks. A technology like image processing would be perfect to help and resolve this issue.

Grey mould *and $SO_2$ damage*

Grey mould is traditionally controlled by $SO_2$ fumigation and storage at -0.5 °C but due to many reasons, amongst others $SO_2$ damage that can occur on the grapes, this is not the ideal treatment (Gabler *et al*., 2010). Throughout the years, many different ways of applying the $SO_2$ treatments have been investigated to reduce the detrimental effects that it has on table grape quality (Xiao et al., 2019). Alternative treatments have, therefore, been tried throughout the years.

Al-Qurashi and Awad (2013) evaluated the effects of pre-harvest calcium chloride (CC) (at 1 or 2 %) and ethanol (at 10 or 20 %) spray at 30 and 7 days before harvest on the quality of the table grape cultivar 'El-Bayadi' during cold storage at 0 °C ± 1 plus 1 day of shelf life at 20 °C. They found that berry decay percentage significantly decreased during storage when pre-harvest spray of CC and ethanol at both low and high concentrations was applied in comparison to the control. Their results showed that decay was also decreased by a combination between CC and ethanol compared to the control but was less effective than each one alone and that there were thus no significant differences between low and high concentration of CC and ethanol. The overall quality characteristics of berries in their study such as firmness, TSS, acidity, TSS:TA ratio, pH, vitamin C, total phenols and soluble tannins were also not negatively affected by both CC and ethanol spray treatments. Because both CC and ethanol spray also did not cause either foliar damage on the vines or significant changes in berry quality they could safely suggest the pre-harvest spray of 1 % CC or 20 % ethanol as practical alternatives to synthetic fungicides and $SO_2$ to decrease postharvest decay and improve quality of 'El-Bayadi' table grapes.

Shin et al. (2014) looked at the effect of Thymol and Linalool (plant essential oils) fumigation on table grape postharvest diseases such as grey mould. They found that fumigation with 30 µg/mL Thymol and 120 µg/mL linalool significantly inhibited mycelial growth and conidia germination of *Botrytis cinerea* and, therefore, the occurrence rate of grey mould rot in several table grape cultivars. They saw that fumigation with especially 30 µg/mL thymol had no influence on the sugar content and hardness of the grapes.

Sabir et al. (2019) again conducted a study to evaluate the effects of different concentrations of chitosan coating to maintain the quality of the detached berries of the table grape cultivar 'Alphonse Lavallée' during storage at 1 °C, as a healthy alternative to the chemical postharvest $SO_2$ treatment. They looked at the weight loss and visual quality (skin colour and rupture force of the berries) and did chemical analyses on the berry must for changes in SSC, TA, pH and maturity index (MI) as well as changes for bioactive components (antioxidant activity and total phenol). They found that chitosan coating at all doses significantly retarded the loss in berry weight, extended the skin rupture force and total phenol content. Their results indicated that chitosan was also effective at all concentrations to delay the MI (used to express postharvest senescence) and changes in berry colour. They, however, noticed that SSC underwent a slight but insignificant increase through the storage, that TA content of berries progressively decreased during storage with the highest decrease in the control, though the differences were insignificant and the pH also showed a general remarkable decrease along with the storage time.

Their investigations overall implied that storage life of detached table grape berries treated with chitosan can be increased by about one week compared to non-coated berries. All these different studies worked very well as can be seen from their findings, and compared very well to each other in that they all successfully inhibited the occurrence of grey mould. The most recent one by Sabir et al. (2019) compared very well to a similar study by Hashim et al. (2019 in which chitosan was also applied in combination with ecofriendly nanomaterials for controlling grey mould of table grapes and maintaining postharvest quality. Hashim et al. (2019) found that only one application of chitosan or silica nanoparticles, at veraison stage, was able to reduce gray mold of table grapes.

*Berry crack*

Apart from grey mould, berry abscission and berry crack or berry split are some of the main reasons why economic losses are suffred due to the impact these have on the on fruit yield and/or quality (Chang et al., 2019). The mechanics and mechanism of berry split are very complex. Splitting can be simplified as being initiated when the internal pressure of a spherical fruit such as table grapes with radius r and skin thickness $t \ll r$ (the thin shell sphere) surpasses the splitting resistance (Chang et al., 2019). Strain of the cuticular membrane (CM) is an

important factor in formation of microscopic cracks (microcracks) in the CM of fleshy fruit such as table grapes. Microcracks damage the barrier function of the CM resulting in an increased incidence of fruit rot, uncontrolled water transport, and, possibly, cracking.

Howell et al. (2012) carried out a field trial in a drip irrigated vineyard near Paarl in the Berg River Valley region of South Africa. They compared three fertigation strategies on the table grape cultivar 'Dan-ben-Hannah' grafted onto Ramsey as a rootstock. They applied fertilisers in the following manner (i) two weeks after bud break, fruit set and post-harvest termed LF, (ii) weekly from two weeks after bud break until ten weeks after harvest, except during berry ripening termed WF, and (iii) in daily irrigation pulses termed DF. They found that berry crack following rainfall was notably more pronounced in the case of the LF and WF grapevines, and eventually contributed to the low export percentages compared with the DF treatments. They, however, did not rule out the possibility that larger bunches with larger berries could also have contributed to the higher number of export cartons from the DF treatments and not just less berry crack. They noticed that the number of bunches with berry crack increased with the pressure at which the berry skins burst and that these differences were significant, since Dan-ben-Hannah berry skins are generally regarded as being strong compared to other cultivars such as Crimson Seedless. Their results, therefore, suggests that the grapes of the DF grapevines had thinner or more elastic berry skins, which allowed them to adapt to rapid expansion when rainfall occurred. From this they concluded that the ability of the berry skin to stretch at each development stage must be sufficient to accommodate the rapid expansion brought about by wet or humid atmospheric conditions so that splitting is avoided. They also noted a seasonal difference in berry crack because although it also occurred in the 2003/04 season, damage to the grapes was not as extensive as in the 2002/03 season.

*Browning*

When the pulp and/or berry skin of fruit turns to a brown colour it is known as browning. Enzymatic browning consist of the main oxidative reactions which involve two oxidoreductases enzymes: polyphenoloxidase (PPO) and peroxydase (POD). PPO catalyzes two reactions; a hydroxylation of monophenols to diphenols is the first, which is relatively slow and results in products with no colour. The oxidation of diphenols to quinines is the second, which is rapid and gives products with colour. The substrates involved in these reactions are located in the vacuoles while enzymes are in the cytoplasm; the reactions can take place only if they are mixed and in the presence of oxygen. So, all phenomena (cutting, shock, loss of firmness) lead to the starting of browning reactions which induce losses or changes of flavour, odour and nutritional value. To avoid this phenomenon various methods have been developed. The role of these methods is either to inactivate PPO or to avoid contact between the enzyme and its substrate, either by adding antioxidants or by maintaining the structural integrity of the food.

There are however, many other possible influences on browning reactions. These influences include cultivar and seasonal variations, relative amounts of individual phenolic compounds in grapes, and phenolic distribution in the flesh and skin. This list of factors/parameters is extended even further to include biological factors (presence of microorganisms), physical factors (pH, etc.), or chemical factors (interference of inhibitors or positive effectors) responsible for accelerating or slowing the process (Loannou and Ghoul, 2013).

What makes browning such a very complex phenomenon is that it can occur both on the outside (external browning) and inside (internal browning) of fruit. Internal browning of fruit especially is understood very poorly especially as to what the background causes may be to it during cold storage (Gabriëls *et al*., 2020). Numerous studies have ensued to elucidate the exact factors that lead to the occurrence of this phenomenon and how exactly it progresses up until its visual perception (Moelich, 2010; Weksler *et al*., 2015). It, however, will be of more value to know whether detection can happen at harvest already before grapes are packed and exported. It is only recently that advances were made to predict whether it would occur on whole white seedless berries using NIR spectroscopy (Daniels, 2013). Gaps in detecting and monitoring it on whole bunches, however, still exist.

The complexity of the challenges related to monitoring table grape quality from the vineyard to the packhouse and during cold storage is clear. Any action taken during any of these stages will ultimately influence the eventual quality of the grapes that the consumers receive. It is thus the sole responsibility of the producer to ensure that the correct procedures is followed and quality maintained throughout. Given, however, that there is nothing producers can do further to ensure this once grapes have been packed into boxes and onto pallets, there is a real need for the application of modern-day strategies that can help to monitor fruit quality, non-destructively throughout the different stages.

## 2.4 SECTION III: APPLICATION OF PRESENT-DAY STRATEGIES TO MONITOR FRUIT QUALITY

The quality of fruit, in general, and for decades, has been through visual and destructive methods at the various stages of production and processing (Pathmanaban *et al*., 2019). With the aim of monitoring table grape quality non-destructively throughout the value chain and supplying a high-quality product consistently, there are several non-destructive technologies such as NIR spectroscopy, HSI, computer vision systems and image processing techniques available (Donis-González *et al*., 2020).

Analytical infrared spectra are centred on the absorption or reflection of the electromagnetic radiation detected among 1 and 1000 µm and divided in three forms of IR: near IR (NIR) in the 0.76–2.5 µm (760–2500 nm) region, mid IR (MIR) in the 2.5–25 µm (2500-25000) region, and

far IR (FIR) beyond 25 µm (25000 nm) (Figure 2.3) (Balan *et al*., 2019). NIR spectroscopy can be used to obtain information that can support the development of useful qualitative and/or quantitative attributes of a sample by probing a it with electromagnetic radiation in the wavelength range from 750 to 2500 nm (Pasquini, 2018). HSI systems operate in the NIR region as well, it serves as an extension of both spectroscopy and imaging techniques (Elmasry *et al*., 2012).

Bhargava and Bansal (2018) describe the technology of computer vision as the outcome of human vision in quality inspection of fruits and vegetables by distinguishing an image electronically, interpreting and recognising the characteristics and information, which is then supplied for the quality grading and sorting to the machines. Many features like texture, shape, colour, size and defects can be graded and inspected automatically, using a computer vision system, but this, however, can be a difficult undertaking since some defects due to texture and colour are undistinguishable to the skin (Bhargava and Bansal, 2018). Image processing through feature extraction (Cavallo *et al*., 2019) can assist with the last problem.

NIR spectroscopy and HSI predominantly makes use of the classical chemometric techniques such as partial least squares (PLS) regression (Giovenzana *et al*., 2014; Mo *et al*., 2017) and partial least squares discriminant analysis (PLS-DA) (Folch-Fortuny *et al*., 2016) to extract important chemical information from samples. The machine learning algorithms such as artificial neural networks (ANN), super vector machines (SVM) and least-squared support vector machines (LS-SVM) are used by computer vision and image processing technology to extract information from images for classification and prediction of fruit's quality (Annmariya *et al*., 2020). These algorithms are also sometimes used for NIR and HSI. The use of these techniques can either be alone, meaning just ANN (Siripatrawan *et al*., 2011; Binetti *et al*., 2017) and LS-SVM (Chauchard *et al*., 2004; Fan *et al*., 2017) or together meaning PLS, PLS-DA, ANN, SVM and LS-SVM (Balabin and Smirnov, 2011; Sun *et al*., 2017; Poblete-Echeverria *et al*., 2020) in one study. PLS and PLS-DA are, however, also used for machine vision and image processing analysis and are not only limited to ANN, SVM, LS-SVM and other similar algorithms.

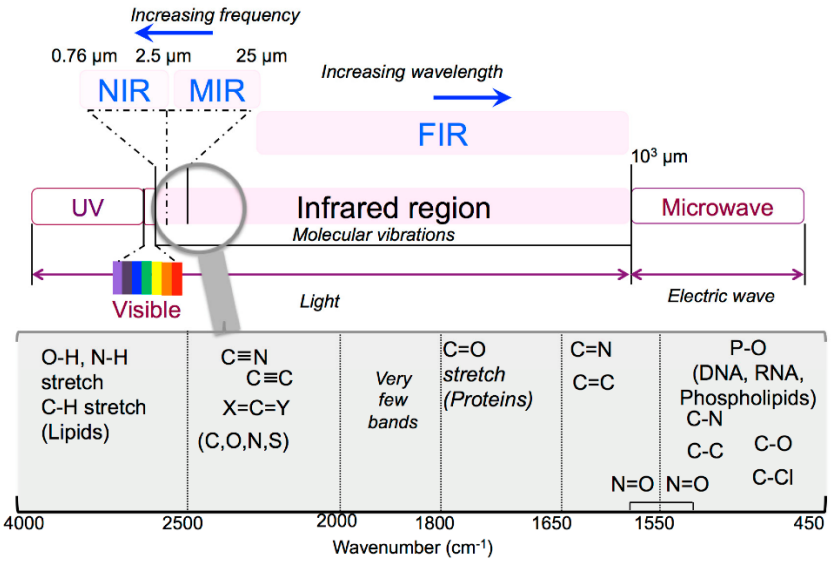


**Figure 2.3** Diagrammatic illustration of the three different regions (near-, mid- and far-infrared) of the infrared region of the electromagnetic spectrum, with their relation to the region of the visible spectrum. Also shown are the different wavelengths and wavenumbers over which they span as well as their transitions, intensity and characteristic molecules in which they cause vibrations (reprinted with permission from Balan *et al*., 2019).

### 2.4.1 NIR spectroscopy

The swiftness with which NIR spectroscopy has evolved has caused it to be widely accepted and it has become the measurement technique of choice in many industries (Siesler, 2007). These include the pharmaceutical (Blanco *et al*., 1998; Abrahamson *et al*., 2005; Saerens *et al*., 2012), medical (Ferrari and Quaresima, 2012; Balan *et al*., 2019), mining (Kaihara *et al*., 2002; Dalm *et al*., 2014; Shankar, 2015) and agricultural (Slaughter *et al*., 2001; Magwaza *et al*., 2012; Morellos *et al*., 2016) industries. NIR spectroscopy's ease of measuring multiple chemical and physicochemical parameters simultaneously without the generation of any chemical waste and, therefore, pollution is another of its advantages (Escuredo et al., 2021).

In the agricultural sector, TSS and firmness of fruit are the two parameters that have been measured the most, either on their own or in combination with others such as TA, TSS:TA ratio and/or pH and colour. To assist with monitoring table grape quality in the vineyard, great success has been achieved with NIR handheld or portable instruments. Kanchanomai *et al*. (2020) used a portable NIR spectrometer in interaction mode to determine SSC, pH, firmness, and seedlessness of table grapes in the vineyard and the laboratory.

The interaction of the NIR radiation with the sample can be promoted in several ways. Transmittance, diffuse reflectance, diffuse transmittance, interactance, and transflectance are terms employed to refer to different geometric arrangements of the probing radiation beam, sample, and detection system used to gather analytical spectral information about the sample. Among these measurement modes, those based on diffuse reflectance predominate. In this type of measurement, the NIR radiation is cast on ground or intact solid samples. The interaction between the radiation and the sample constituents takes place and the radiation

leaving the sample is collected and analyzed. The absorption and the scattering of the radiation are the main phenomena affecting the reflectance spectrum. Reflectance is often used because NIRS has been used for the non-destructive and direct analysis of solids (Pasquini, 2018).

Donis-González *et al*. (2020) determined table grape and peach quality characteristics by evaluating the performance of two portable spectrometers that are commercially available. Both spectrometers delivered promising PLS prediction models to predict table grape quality characteristics (dry matter (DM) and TSS), but the ones regarding peach did not perform as well as the grapes ones did. Fernandez-Novales *et al*. (2019) went a step further to break down the next wall in precision viticulture by using Visible-Short Wave Near-Infrared (VIS + SW − NIR) spectroscopy as a real substitute to just the regular NIR spectroscopy. These researchers endeavoured to facilitate the decision-making process dealing with grape quality sorting and harvest scheduling. This by making monitoring of grape composition within the vineyard more reliable. The vineyard variability maps these researchers generated for the different dates using this technology with PLS illustrated the ability to monitor the spatiotemporal dynamics and distribution of TSS, anthocyanins and total polyphenols along with grape ripening in a commercial vineyard.

The versatility to monitor fruit grape quality in the packhouse using NIR is shown by the studies done by Magwaza *et al*. (2013). Three different NIR acquisition methods were compared by these researchers, namely a fibre-optic probe for solid samples (SP), an integrating sphere (IS) and an emission head (EH) to measure mass, colour index, TSS, TA, TSS:TA ratio and vitamin C using PLS. Fruit mass, colour index, TSS and vitamin C were predicted with significant accuracy. The spectral acquisition method had a significant influence on the calibration regression statistics and accuracy of prediction. Arendse *et al.* (2018) obtained good prediction performance for nine of the 13 quality parameters evaluated with the contact-less measurement use of an optic fibre coupled emission head (EH) to scan fruit over a distance of 170 mm. Their findings demonstrated that the EH can be implemented as an online tool for the analysis of pomegranate fruit quality.

Monitoring table grape quality during cold storage also becomes probable considering that Giovanelli *et al.* (2014) evaluated the feasibility of NIR spectroscopy to optimise postharvest apple management and to follow changes in fruit quality during storage. Spectral data were elaborated by PLS regression and linear discriminant analysis (LDA) classification techniques with the physicochemical (DM, SSC, colour and firmness) and some nutraceutical characteristics (total phenolics, total flavonoids and antioxidant activity) of Golden Delicious apples. Good correlation models between spectral data and chemical and physical parameters were obtained for SSC, colour and firmness and even higher correlation for indexes related to the antioxidant capacity. A high potential of NIR spectroscopy for the estimation of storage time of apple lots was also found. Pérez-Marín *et al*. (2019) also studied the potential of NIR to see if this technology if used *in situ* whether quality standards and the postharvest shelf-life of

oranges kept in cold storage can be established, as well as to identify substandard produce. Their findings suggest that NIR spectroscopy and the use of spectral distances will enable an innovative quality control system to be developed, based on spectral information that allows the establishment of quality standards in oranges, and the detection of non-standard produce.

### 2.4.2 Hyperspectral imaging (HSI)

The quality of apples, similar to that of grapes, is determined by factors such as weight, SSC colour, and internal browning. Nogales-Bueno *et al*. (2014) used HSI correlated with grape skin total phenolic concentration, sugar concentration, TA and pH wet chemistry data to build models using modified PLS regression and several spectral pre-treatments of intact grapes during ripening. On table grape berries, Baiano *et al*. (2012) used HSI to determine the physicochemical (SSC and TA) and sensory characteristics and Piazzolla *et al*. (2013) to discriminate between different harvest times of table grapes by measuring the TSS, TA, pH, total phenolic content (TPC) and anthocyanin activity (AA). Mo *et al*. (2017) used HSI and PLS regression models to map internal SSC of apples and found that the PLS regression mapping model was enhanced by using the spectrum and SSC measured/averaged from fewer larger areas of the apples rather than from more numerous but smaller localised portions. The SSC mapping models were improved by increasing the measuring area of SSC. In their overall results HSI was demonstrated as a technique that may be valuable for mapping the internal SSC of apples and that it can be utilised for provision of basic data for the design and development of a portable SSC measurement device. This is positive for the table grape industry that can use a similar method to rather measure whole bunches rather than just single berries in the vineyard.

Rajkumar *et al*. (2012) used HSI to study banana fruit quality and maturity stages at three different temperatures, *viz*. 20, 25, and 30°C. Moisture content, firmness and TSS were determined and through correlation with the spectral data analysed using the PLS analysis. These researchers found that the ripening processes accelerated with an increase in temperature and that the change in TSS and firmness of banana fruits stored at the three different temperatures followed a polynomial relationship, while the change in the moisture content of the banana fruits at different maturity stages was found to follow a linear relationship. Zhu *et al*. (2016) endeavoured to develop a non-destructive method for predicting the bruise susceptibility of apples using HSI, since the most common type of mechanical damage to fresh fruit is bruising and prediction of the susceptibility on apples to it can deliver information that is beneficial in the appropriate postharvest handling and storage operations of apples. Fan *et al*. (2017) similarly investigated HSI for blueberry internal bruising detection using LS-SVM. Spectra were extracted from regions of interest (ROIs) at four measurement times (30 min, 2 h, 6 h, and 12 h after mechanical impact). After mechanical impact blueberry internal bruising

could be identified as early as 30 min. Great possibility to distinguish blueberry internal bruising on the packing line was shown through using two wavelengths in the computation of band ratio images. This resonates well with table grapes that are highly sensitive fruit and susceptible to bruising during harvest and packaging, but which is only expressed as browning during and/or after cold storage. HSI, therefore, can be used in the packhouse and during cold storage to monitor quality.

### 2.4.3 Computer vision systems

Computer vision technology has greatly assisted in the development of food grading processes that are completely automated due to their ability to make fast, constant, accurate and unbiased measurements during inspection and grading of fruit and vegetable (Brosnan and Sun, 2002). The tomato fruit, just like table grapes, is very delicate and needs to be handled carefully during grading and packaging. Arakeria and Lakshmana (2016), therefore, developed a computer vision-based fruit grading system to evaluate the quality analysis of tomato in terms of ripeness and defects and obtained good results with the software classifying the tomato images as defective/non-defective and ripe/unripe with an accuracy of 100% and 96.47% respectively. To improve the quality control and sorting of dried figs an automated system based on computer vision was also developed by Benalia *et al*. (2016). First, these researchers addressed the qualitative discrimination of figs by assessing it through comparison to the analysis of colour images acquired using a digital camera with those acquired using conventional instrumental methods such as colourimetry done in laboratories. To achieve real-time sorting of figs they then developed image processing algorithms. All this was to simulate an industrial application, but by using an experimental prototype based on machine vision first. They obtained extremely good results with deteriorated figs being classified 99.5% correctly and light-coloured, good-quality figs 89.0% correctly. Ohali (2011) investigated a date fruit quality assessment and sorting system by looking at the requirements for designing such a computer-mediated system. They developed a technique that could successfully meet those requirements and tested its usefulness on real-life data. They also built a date sorter example and elaborated on its design and performance. In the end, their prototypical computer vision-based date grading system could sort 80% of dates accurately. These two studies and the review of Zhang *et al.* (2014) highlight the importance of knowing exactly what is going to be measured when a computer vision system is envisioned to monitor fruit quality. Knowing exactly where and how the systems need to be set-up and which characteristics will be evaluated, either external or internal alone or both simultaneously and whether measurement in the vineyard is also possible, as can be seen with image processing is, therefore, essential.

### 2.4.4 Image processing

Present-day agriculture has made extensive use of image processing for automation purposes (Behroozi-Khazaei and Maleki, 2017). For application in the vineyard, Pothen and Nuske (2016) demonstrated that it is possible to categorise grape clusters into different grades according to colour development and compute the progression of colour change across a commercial vineyard. Behroozi-Khazaei and Maleki (2017) developed a robust algorithm based on ANN and genetic algorithm (GA) for segmenting grape clusters from leaves and background using colour features near to harvest in the vineyard. Both studies show the complex level and scale of application of this technology in the vineyard, while the study of Cubero *et al*. (2015) showcases its application in the packhouse when they developed a methodology to determine bunch compactness in a non-invasive, objective and quantitative way. Fashi *et al.* (2019) used image processing to show the relationship between the appearance of pomegranate fruit and the colour and size of arils using three different algorithms. A new algorithm to calculate three indices, namely shape, colour and size of strawberries, was also developed by Liming and Yanchao (2010), and Mohammadi *et al*. (2015) developed an algorithm to successfully classify persimmon fruits into three commercial maturity stages. For application during cold storage, Cavallo *et al.* (2019) used a computer vision system together with image processing and machine-learning techniques to evaluate table grape quality contactless and non-destructively.

## 2.5 CONCLUSIONS

Obtaining optimum table grape quality and maintaining it by preventing postharvest defects remains an economically serious challenge for producers in South Africa and all over the world—this particularly since the preparation and logistics involved in achieving and maintaining exceptional quality is so complex. Research has shown that the accumulation of sugar and breakdown of acids in every single berry, bunch, vine, row and block differs from cultivar to cultivar. That is also the case when it comes to postharvest defects like browning where a direct link of a single dominant factor, such as air-forced cooling, repeatedly linking directly to either internal or external browning development could not be found.

The table grape industry still relies on old destructive methods to determine quantitative aspects and for qualitative characteristic classification methods based on visual subjective systems. These also tend to focus on singular parameters measured separately and not together. Also, the focus is sometimes either on certain parts of the value chain, the vineyard, the packhouse or cold storage and/or post-cold storage.

NIR spectroscopy, HSI, computer vision and image processing techniques have been used for many decades in the agricultural industry and instruments have become so modernised,

durable and are capable of excellent accuracy and precision. Unlike the conventional methods, these analysis techniques are fast and multiple parameters can be determined at the same time. Results obtained with these techniques are comparable in accuracy to those of conventional analytical ones.

The purpose of this literature review was, therefore, not to repeat these serious challenges associated with table grape quality, but rather to show the availability of new and improved present-day methods that are available to monitor it throughout the value chain. This entails the possibility of doing measurements from where quality begins, in the vineyard, and then to where it can be further evaluated, the packhouse as well as in cold storage.

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# Chapter 3: Incidence of table grape defects during cold storage


## ABSTRACT

Visual perception of fruit is a very important aspect to consumers. Table grapes thus need to be free of any defects once available on retail shelves. Maintaining grape quality throughout the cold storage period is, therefore, of utmost importance. The current practice is visual inspection of bunches prior to packaging to assess the presence of defects before cold storage and transportation to export markets. The complex nature of table grapes makes it prone to many different types of defects like berry crack, grey mould rot, sulphur dioxide ($SO_2$) damage and browning. There are seventeen different browning phenotypes on white seedless grapes. The aim of this study was thus to investigate the incidence of these different defects as affected by different factors namely cultivar, site, harvest year and cold storage week and a combination of these.. To achieve this the objective three different cultivars (Regal Seedless, Thompson Seedless and Prime) were harvested from three different locations (Wellington, Hex Valley and Kakamas) over two years (2016 and 2017). The results showed that the incidence of the different defects were significantly influenced by a specific factor like cold storage week alone and a combination of factors cultivar, site and harvest year or cultivar, site, harvest and cold storage week while other defects were not. Based on the p-values the incidence of defects such as sunburn and bruising were significant for a combination of cultivarxsitexyear and cold storage week separately but not combined all together (cultivarxsitexyearxweek). The incidence of defects of Prime especially was significantly different from those of the other two cultivars Regal Seedless and Thompson Seedless while that of 2016 was also significantly different from 2017.


## 3.1 INTRODUCTION

The South African table grape industry and economy loses a considerable amount of money every season and this is mainly because of a loss in postharvest quality (Freiboth *et al*., 2013). Table grapes contributes more than R3 billion annually to the South African economy through the export of an average of 63.5 million 4.5 kg-equivalent cartons (Mtshiselwa, 2020). The supply chain logistics of fruit is so complex and multifaceted that the possibility of postharvest quality being significantly transformed (Arendse *et al*., 2014) can occur anywhere along that line. Factors that have a substantial influence on alterations in fruit postharvest quality include temperature, humidity and period of storage (Ekrami-Rad *et al*., 2011). All fruit, especially ones as highly perishable like table grapes, have a limited lifetime and are in a process of deterioration from the moment of harvest. Storage conditions to guarantee decent quality in the

marketplace, therefore, needs to be managed very accurately (Freiboth *et al*., 2013). Cold storage coupled with the use of sulphur dioxide ($SO_2$) has thus far been the greatest technological advances made in maintaining the quality of table grapes and minimising postharvest losses (Dodd *et al*., 2012). The importance of cooling rests in the decreased rate at which the fruit perspires leading to a marginal loss in moisture. It also reduces the growth of fungi and thereby slows down decay (Ngcobo, 2008). Of great importance, however, is that although cold storage helps to preserve the quality and freshness of the product, it does not enhance the quality (Freiboth *et al*., 2013).

Export grapes are usually pre-cooled within a day of harvesting. During this time, grapes cool down from field temperatures that typically range from 21 to 32 °C. Pre-shipment storage and shipment by sea occur at -0.5 °C (Burger *et al*., 2005). Thus, after harvesting and packaging, table grapes are subjected to a period of cold storage that can last up to six weeks (Wolf, 1996; Avenant, 2007). The transport of table grapes can take another three to 22 days from the Southern hemisphere producing countries to the intended Northern hemisphere consumer markets (Burger *et al*., 2005). Monitoring of the cold storage conditions under which grapes are stored is, therefore, just as vital, since these, other than just the initial quality of the grapes before cold storage, will determine for how long the grapes will survive. Table grapes in some instances can be stored at -1 to 0 °C and 90-95% relative humidity for a period of up to four months (Palou *et al*., 2002) or sometimes longer (Zutahy *et al*., 2008).

Proper application of cold storage protocols are always in conjunction with either $SO_2$ fumigation alone (Zutahy *et al*., 2008) or with ethanol as pre-treatment and then $SO_2$ (Guzev *et al.*, 2008); or with ozone exposure (Palou *et al*., 2002; Feliziani *et al*., 2014); or the exogenous application of ethylene (Palou *et al*., 2002) before storage. These other treatments have been investigated because in attempting to control decay using $SO_2$, table grapes often suffer from $SO_2$ damage. Decay, also known as grey mould or rot, is caused by an array of fungi like *Botrytis cinerea*, *Aspergillus niger*, *Rhizopus stolonifera* and *Penicillium* species and is responsible for a large part of the postharvest problems experienced with table grapes when they reach the overseas markets (Abdolahi *et al*., 2010; Castillo *et al*., 2010; Gabler *et al*., 2010). Grey mould and $SO_2$ damage are, however, not the only physiological defects that table grapes suffer from. There is also browning and drying of stems, shrivelling (weight loss) of berries, berry softening (Crisosto *et al*. 2001) as well as berry crack (Zoffoli *et al*., 2008) and table grape browning (Wolf, 1996; Avenant, 2007). Table grape browning, especially, is a severe quality problem in white table grapes worldwide (Moelich, 2010; Rustioni *et al*., 2014; Weksler *et al*., 2015). Different aspects of the browning phenomenon have been studied. Vial *et al.* (2005) investigated the development of postharvest berry browning problems in the cultivar ‘Princess’ to see if it was related to cluster maturity parameters such as soluble solids content (SSC), titratable acidity (TA), the SSC:TA ratio and juice pH. Fortea *et al.* (2009) looked at the characterisation of the enzymes polyphenol oxidase (PPO) and peroxidase (POD) extracted

from Crimson Seedless table grapes since the activity of both these enzymes have been implicated as having a synergistic effect in the enzymatic browning reaction (Subramanian *et al*., 1999; Jiang and Miles, 1993) of grape berries, raisins, grapevine, must, and wine (Kimberley *et al*., 1980; Macheix, 1991; Karadeniz *et al*., 2000). Gónzalez-Barrio *et al*. (2005), however, found that there was no difference in PPO and POD activity between untreated and treated grapes when they irradiated the white seedless table grape cultivar 'Superior' with UV-C to increase the resveratrol concentration in it. From the literature, it is clear that researchers have investigated thoroughly how these defects occur and how different combinations of treatments before and during cold storage can eliminate the development of several of these defects to prolong the postharvest life of table grapes (Walker *et al*., 2001; Deng *et al*., 2005; Moelich, 2010; Ngcobo *et al*., 2013). Fourie (2009) even described several different phenotypes of berry browning that can occur on table grapes. This study, however, offers a new point of view on the problem of postharvest losses by looking at the incidence and intensity of different postharvest defects on different cultivars harvested in different years from different farms in South Africa.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Cultivar and harvest information

The three cultivars (Thompson Seedless, Regal Seedless and Prime), the locations these were harvested from as well as the global positioning system (GPS) coordinates of the three locations are listed in Table 3.1. The harvest week as well as the total soluble solid (TSS) level at which the grapes were harvested are also listed.

**Table 3.1** Cultivars, harvest location, GPS coordinates, harvest week and TSS level of grapes.

| Cultivar | Site | Latitude | Longitude | Altitude | 2016 Harvest Week | 2017 Harvest Week | 2016 TSS[d] | 2017 TSS |
|---|---|---|---|---|---|---|---|---|
| Thompson Seedless | HV[a] | 33°27'53,9"S | 19°39'43,7"S | 907m | W3 | W4 | 16.85 | 15.64 |
| | | | | | W4 | W5 | Stolen | 16.62 |
| Thompson Seedless | W[b] | 33°37'03,5"S | 18°58'05,3"S | 904m | W3 | W3 | 17.49 | 18.72 |
| | | | | | W4 | W5 | 18.62 | Rotten |
| Regal Seedless | HV | 33°27'50,4"S | 19°39'47,6"E | 904m | W3 | W5 | 18.39 | 19.41 |
| | | | | | W5 | W5 | 21.27 | 21.36 |
| Regal Seedless | W | 33°30'14,2"S | 10°50'40,0"E | 904m | W3 | W4 | 15.47 | 14.12 |
| | | | | | W5 | W6 | 16.44 | 16.34 |
| Prime | W | 33°38'22,0"S | 10°50'47,6"E | 900m | W51 | | 10.65 | |
| | | | | | W52 | | 12.02 | |
| Prime | K[c] | 28°37'54,8"S | 20°26'38,6"E | 903m | W48 | | 14.89 | |
| | | | | | W50 | | 16.08 | |

[a]Hex River Valley; [b]Wellington; [c]Kakamas; [d]Total soluble solids in °Brix measured with a handheld refractometer

### 3.2.2 Vineyard treatments

The grapes were prepared according to the standard protocol for export table grapes (Van der Merwe, 2012). This involves shortening, thinning of bunches, followed by application of gibberellic acid ($GA_3$) to bunches. The purpose of $GA_3$ application was for cluster thinning and berry enlargement (Formolo *et al*., 2010; Marzouk and Kassem, 2010; Ozer *et al*., 2012; Abu-Zahra, 2013). Prime Seedless from Wellington had a $1x10^{-6}$ mg/l $GA_3$ spray for thinning, and a 20 $x10^{-6}$ mg/l $GA_3$ plus 1% forchlorfenuron (N-(2-chloro-4-pyridyl)-N-phenylurea or CPPU) sprays for enlargement. Thinning of Thompson Seedless from Hex River Valley occurred by spraying $10x10^{-6}$ mg/l $GA_3$ at 50% bloom, then four days later at 80% and a third spray given at 110% bloom. Thompson Seedless from Wellington received three $7x10^{-6}$ mg/l $GA_3$ thinning sprays at the same stages as the Thompson Seedless from Hex River Valley. For enlargement, Thompson Seedless from Wellington received sprays three times—first spray $20x10^{-6}$ $GA_3$, second spray $30x10^{-6}$ mg/l $GA_3$ + $2x10^{-6}$ mg/l (CPPU) and third spray $20x10^{-6}$ mg/l $GA_3$. Thompson Seedless from Hex River Valley also received three enlargement sprays—first spray $30x10^{-6}$ mg/l $GA_3$, second spray $30x10^{-6}$ mg/l $GA_3$ + $1.5x10^{-6}$ mg/l CPPU, third spray $30x10^{-6}$ mg/l $GA_3$. Regal Seedless from Hex River Valley had no thinning and enlargement sprays while the Regal Seedless from Wellington received no thinning sprays but a $20x10^{-6}$ mg/l $GA_3$ + 2% CPPU enlargement spray. The berries were 4 to 5 mm in diameter when bunches received

enlargement sprays. The wetting agents used during all the spraying was either Sanawet (50ml/100L water) or Allbuff (100 ml/100 l water).

### 3.2.3 Harvesting, packaging and transport

Physical removal of some of the berries and laterals on bunches occurred when the berries were 8 to 10 mm in diameter so that bunches would not be too compact at ripening and harvesting. The grapes were harvested between 9 and 10 am and packed in 4.5 kg closed-top corrugated fibreboard cartons used in the table grape industry. Each bunch was placed in an individual plastic carry bag. The entire carton content was enclosed in a 2-mm perforated low-density polyethene (LDPE) liner bag that contained a corrugated cardboard sheet at the bottom to reduce abrasion damage. An Uvasys® sulphur dioxide ($SO_2$) generator sheet (http://www.uvasys.com/) covered the grapes to control decay. The LDPE liner bag containing the grapes and $SO_2$ sheet was folded, the boxes closed, carried out of the vineyard to the end of each row and placed in the shade until all the other grapes were harvested. Packed boxes were loaded into an air-conditioned vehicle and transported by road to the Chemical Analytical Laboratory of the Institute for Wine Biotechnology, Stellenbosch University, first and then to the table and raisin grape laboratory of the Agricultural Research Council at Nietvoorbij for cold storage and evaluation. A total of 98 boxes containing a total of 1457 bunches were harvested over the two years from the different locations.

### 3.2.4 Grape berry sampling

Each of the harvested boxes was labelled box 1 to 8. A single box was evaluated every week of cold storage (Week 1 to Week 7), except the evaluation of the Week 0 box which was done immediately after harvest. When a bunch was removed from the carry bag (Figure 3.1), the berries that remained in the carry bag were noted down 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 a scissor. Each berry was then evaluated for one type of defect only, berry crack or $SO_2$ damage or chocolate browning or whichever defect that was the most pronounced on it and put into the accordingly marked foam-lite plate (Figure 3.2). In the data collection, the status of each grape berry was not recorded, instead, the weight, in grams, of all the grapes berries with the same specific defect, e.g. berry crack, was recorded. The total weight of each bunch as well as the total weight of the box containing all the grapes were also recorded. For ease of analysis, all bunches were assigned a value of 0 when no defect was present at all and a value of 1 when any defect was present.

**Figure 3.1** A whole Prime table grape bunch taken out of the carry bag in the box for evaluation

**Figure 3.2** Berries from a single bunch separated according to different defects found on them after evaluation

### 3.2.5 Cold storage and inspection

Cold storage of grapes occurred immediately after harvest at 0 °C (90% RH) for seven weeks except for one box (week 0), which was evaluated on the same day as it was harvested. The cold storage regime was followed to simulate storage conditions during the export of grapes by ships, although it is not conventional to remove boxes from cold storage in the industry. This, however, had to be done to determine exactly when the deterioration of the grapes starts and which defects are prevalent in which week during cold storage. After cold storage, boxes were examined for 21 different defects, scored and coded. These defects were loose berries, botrytis, cracks, $SO_2$ damage, as well as the 17 different browning phenotypes (Fourie, 2009): chocolate berry (external stylar end); chocolate berry (external); chocolate berry (internal); water berry; glassy berry (external); glassy berry (internal); fungal infection (mildew); sunburn; bruising; abrasions; peacock spot; styler-end russet spots; stylar-end necrotic spots; and netlike-; contact-; mottled-; friction browning. Each of the defects received a score of 0 if it was not present on a bunch and a score of 1 if it was present. The incidence was calculated by taking

the sum of the 0 and 1 scores that a particular defect received for all the bunches present in a box of a particular cultivar harvested from a particular site in a particular year and cold stored for a particular week and expressed as a percentage.

### 3.2.6 Statistical analyses

Grapes from 10 CultxSitexYear combinations were harvested at two occasions each. Grapes from each harvest were evaluated for defects before (week 0) and after each week of cold storage (week 1-6). In view of this the experimental design was a completely random split plot with CultxSitexYear combination as main plot factor, replicated at random at two harvests, and storage time as subplot factor. All bunches of grapes from a specific harvest of a CultxSitexYear combination evaluated after a particular time of cold storage was considered an experimental unit. For each experimental unit the percentage of a specific defect was calculated as the number of bunches with that defect out of the total number of bunches.

The percentage for each defect was subjected to analysis of variance (Anova) using GLM (General Linear Models) Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality. Fisher's least significant difference was calculated at the 5% level to compare means for significant effects. A probability level of 5% was considered significant for all significance tests.

Principal component analysis (PCA), employing the correlation matrix, was performed using XLStat (Version 2016, Addinsoft; New York, USA) to elucidate the associations amongst CultxSitexYearxStorage combinations and defects.

## 3.3 RESULTS

### 3.3.1 Incidence of defects

The anova p-values obtained using a split plot design and using harvests as random reps are shown in Table 3.1 for the incidence of the different defects (how frequently a specific defect appeared on a specific cultivar, at a specific site, during a specific year during each cold storage week). Figure 3.3 shows the mean plot for the incidence of %rot over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017. In Table 3.1 it can be seen that there was no significant interaction over time (cold storage week) or for a combination of the factors CultivarxSitexYear or CultivarxSitexYearxWeek or week alone on the incidence of chocolate browning external (CBESE) (Figure 3.7), glassy berry external symptoms (GBE) (Figure 3.11), friction browning (FR) (Figure 3.18) and stylar end russet spots (SERS) (Figure 3.20). The p

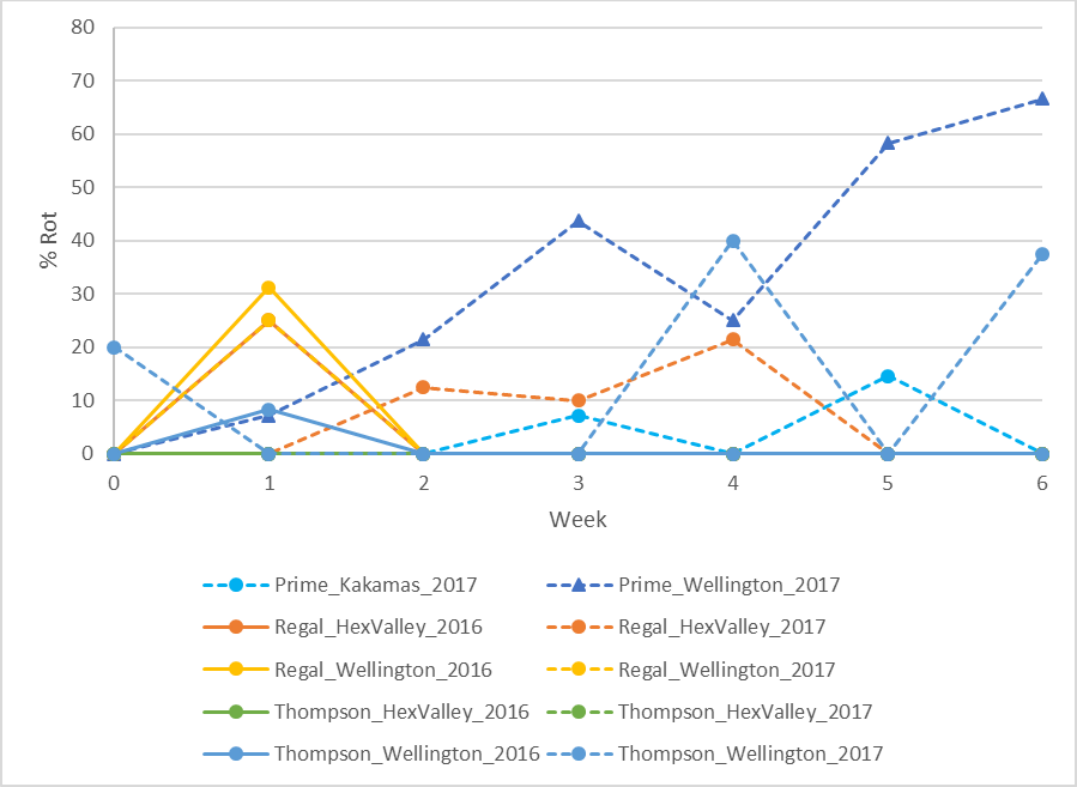
values for all these defects were above 0.005. The Least Significant Difference (LSD) values are shown in the left bottom corner of the plots and was used to select at which level the significance stopped. The factor cold storage week alone had a significant effect on the incidence of chocolate browning external symptoms (CBE) (Figure 3.8), fungal infection mildew (FIM) (Figure 3.13), sunburn (SB) (Figure 3.17) and bruising (BR) (Figure 3.22). The combination of CultivarxSitexYear also had a significant influence on SB and BR ($p < 0.005$) (Table 3.1), as well as on the incidence of rot or Botrytis (B) (Figure 3.3), neck crack (NC) ((Figure 3.4), water berry (WB) (Figure 3.10), contact browning (CT) (Figure 3.15), mottled browning ((MTLD) (Figure 3.16) and stylar end necrotic spots (SENS) (Figure 3.21). The combination of the factors CultivarxSitexYearxWeek had a significant effect on the incidence of berry crack (BC) (Figure 3.5), sulphur dioxide ($SO_2$) damage (Figure 3.6), chocolate berry internal symptoms (CBI) (Figure 3.9), netlike browning (NL) (Figure 3.14), peacock spot browning (PCS) (Figure 3.19) and abrasion damage (ABR) (Figure 3.23).

Figure 3.24 shows the principal component analysis (PCA) plot for the incidence of the different defects over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017. The principal components (PC) F1 and F2 in this instance explains most of the variation. The weight of the defects marked in yellow (CT, GBI, CBI, SENS and CBE) contributes the most to the separation. PC1 purely seperates on the grounds of harvest season (2017 on the left and 2016 on the right) and explains a lot of the variation. The weight of the defects in green (BC, NC, SO2 and ABR) contributes to the separation of PC2. PC2 also seperates Prime from the other two cultivars (Regal Seedless and Thompson Seedless). Thompson Seedless separates from Regal Seedless in 2016 based on PC2. Thompson Seedless at the top and Regal Seedless at the bottom.

**Table 3.2** Defect incidence of all Thompson Seedless, Regal Seedless and Prime bunches evaluated after cold storage during 2016 and 2017

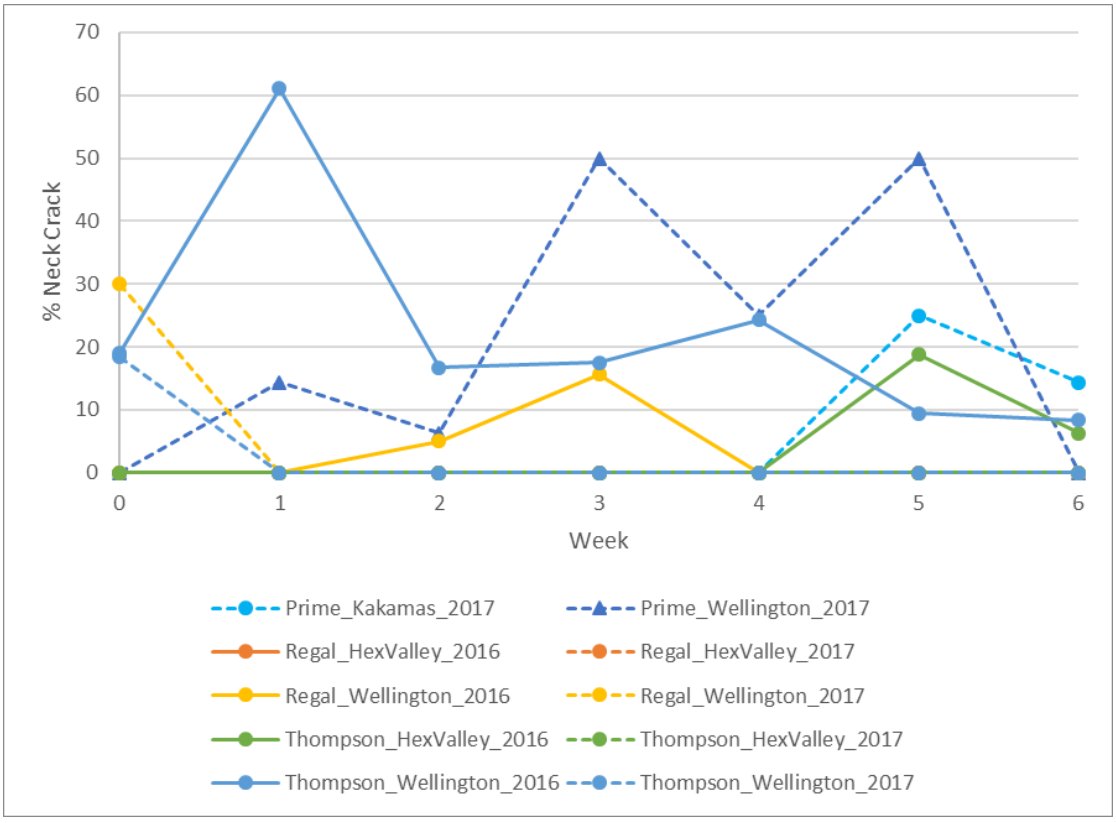
| | DF | CB | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | | B | NC | BC | SO2 | ESE | CBE | CBI | WB | GBE | GBI | FIM | NL | CT | MTLD | SB | FR | PCS | SERS | SENS | BR | ABR |
| CultxSitexYear | 9 | 0.0025 | 0.0374 | 0.0287 | **0.1164** | 0.5786 | 0.6978 | 0.0047 | 0.0071 | 0.5210 | 0.0038 | **0.2149** | 0.0035 | 0.0047 | 0.0021 | **0.0099** | 0.6015 | **0.0753** | 0.7204 | 0.0370 | 0.0002 | **0.0454** |
| Wk | 6 | 0.6156 | 0.7656 | 0.1073 | **<.0001** | 0.4100 | 0.0319 | <.0001 | 0.2900 | 0.3211 | 0.0353 | **0.0003** | <.0001 | 0.2411 | 0.2512 | **0.0487** | 0.1151 | 0.1956 | 0.0695 | 0.2017 | 0.0161 | **0.2285** |
| CultxSitexYearxWk | 53 | 0.1539 | 0.7715 | 0.0011 | 0.0066 | 0.8777 | 0.7942 | <.0001 | 0.0918 | 0.3180 | 0.0079 | 0.1905 | 0.0045 | 0.5955 | 0.0795 | 0.9264 | 0.4170 | 0.0366 | 0.7153 | 0.1052 | 0.5872 | 0.0006 |

| | |
|---|---|
| DF | Degrees of freedom |
| B | Botrytis |
| NC | Neck Crack |
| BC | Berry Crack |
| SO2 | SO2 damage |
| CB ESE | Chocolate berry (external stylar end) |
| CB E | Chocolate berry (external) |
| CB I | Chocolate berry (internal) |
| WB | Water berry |
| GB E | Glassy berry (external) |
| GB I | Glassy berry (internal) |
| FI M | Fungal infection (Mildew) |
| NL | Netlike |
| CT | Contact |
| MTLD | Mottled |
| SB | Sunburn |
| FR | Friction |
| PCS | Peacock spot |
| SERS | Styler-end russet spots |
| SENS | Stylar-end necrotic spots |
| BR | Bruising |
| ABR | Abrasions |



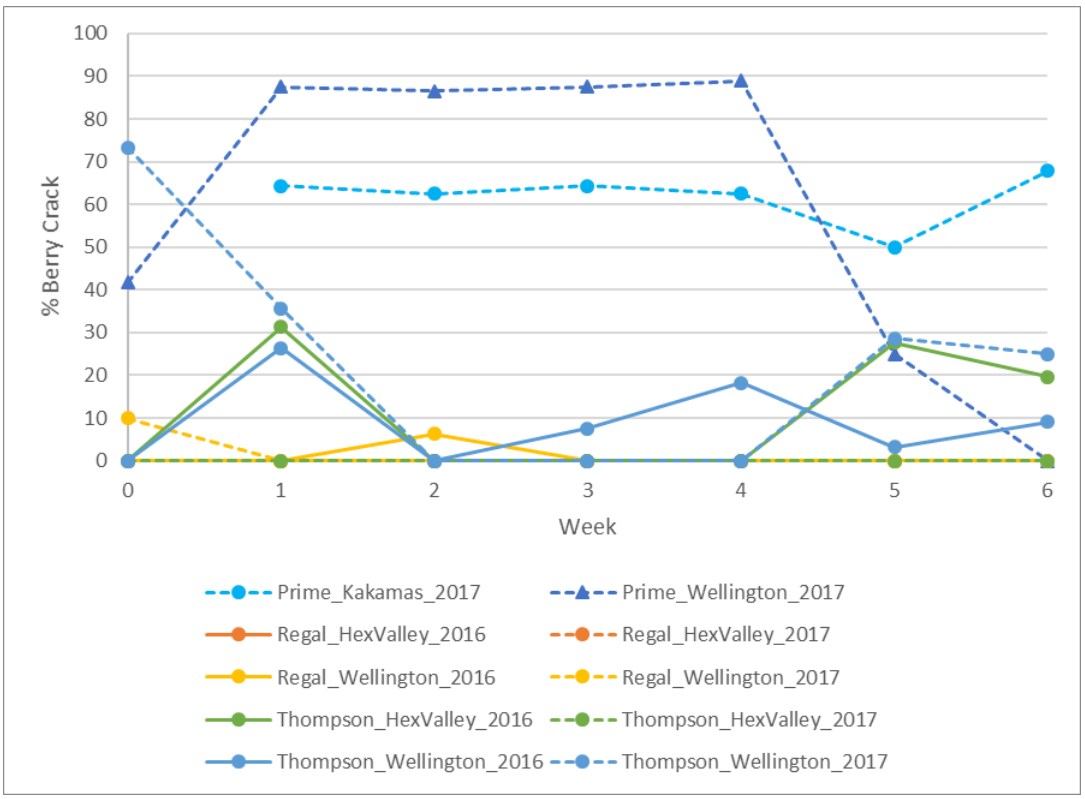

Least Significant Difference = 11.419

**Figure 3.3** Mean plot for the incidence of %rot over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



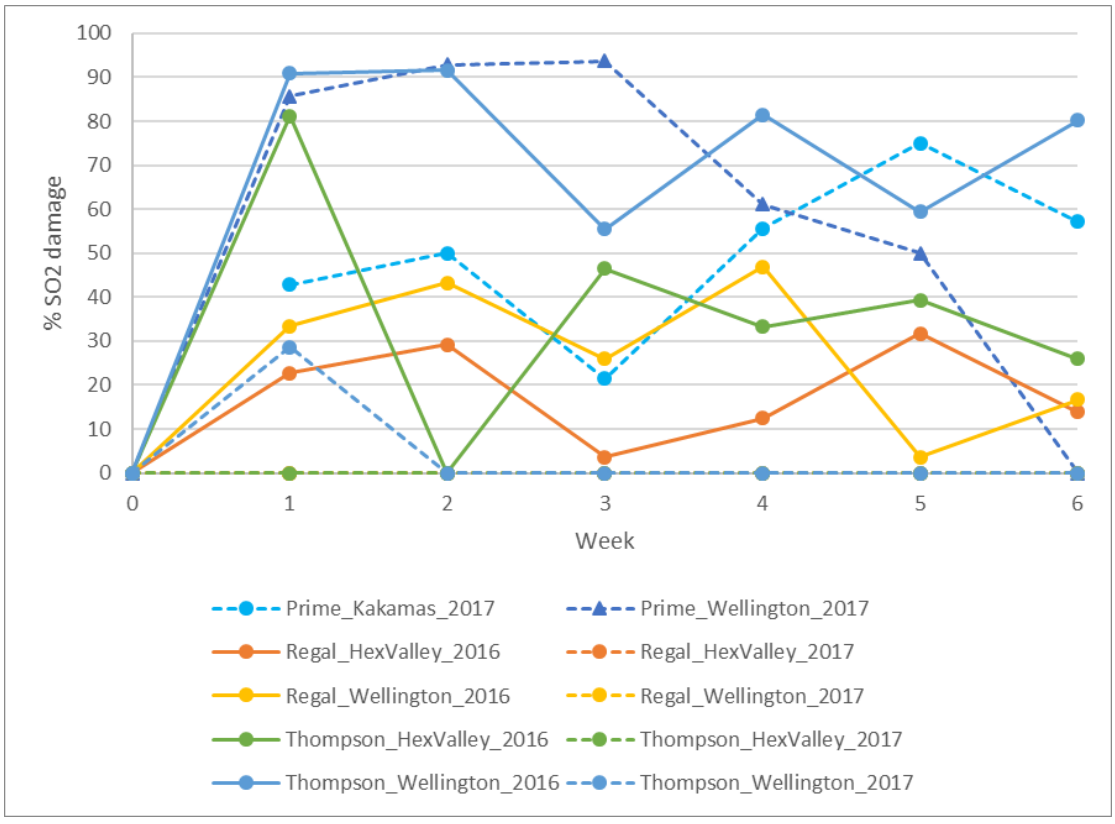

Least Significant Difference = 15.07

**Figure 3.4** Mean plot for the incidence of % neck crack over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



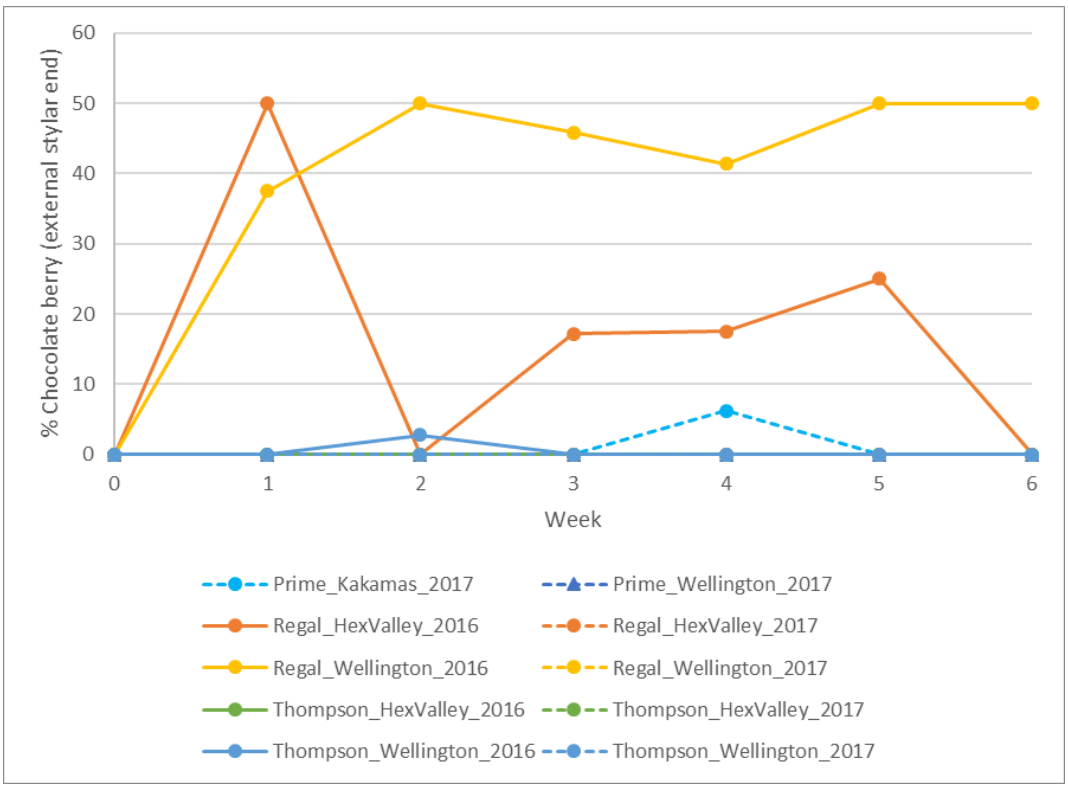

Least Significant Difference = 41.022

**Figure 3.5** Mean plot for the incidence of % berry crack over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



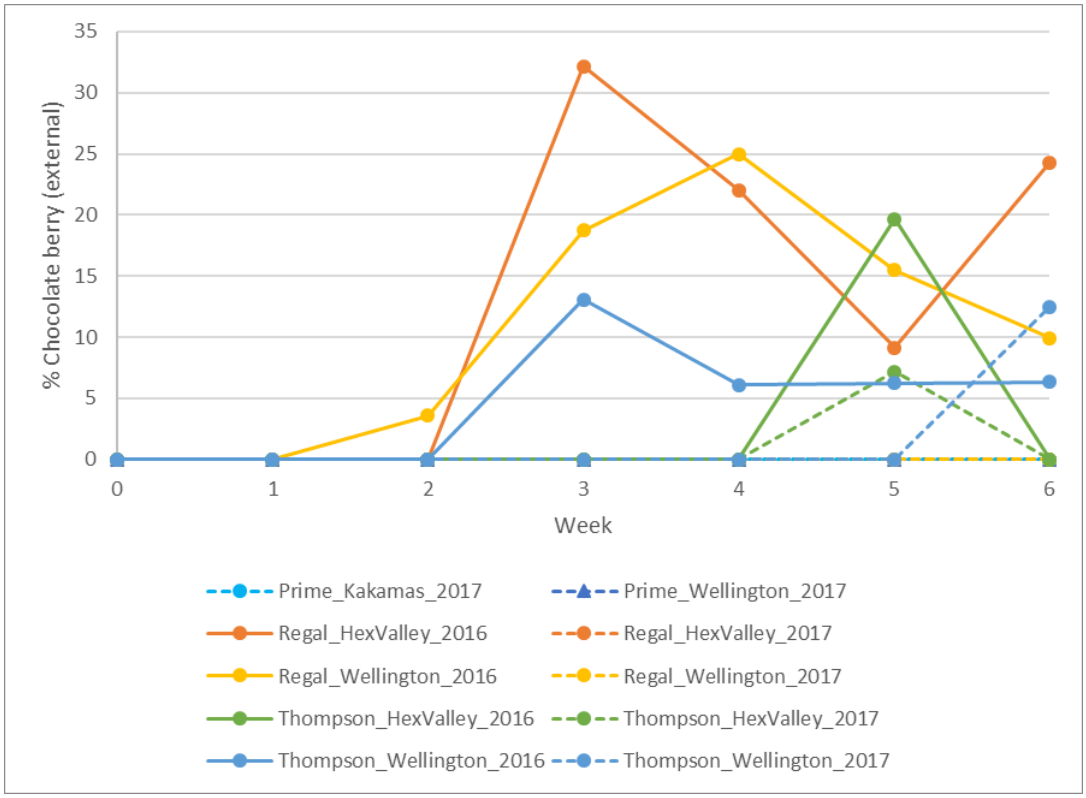

Least Significant Difference = 55.065

**Figure 3.6** Mean plot for the incidence of % $SO_2$ damage over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



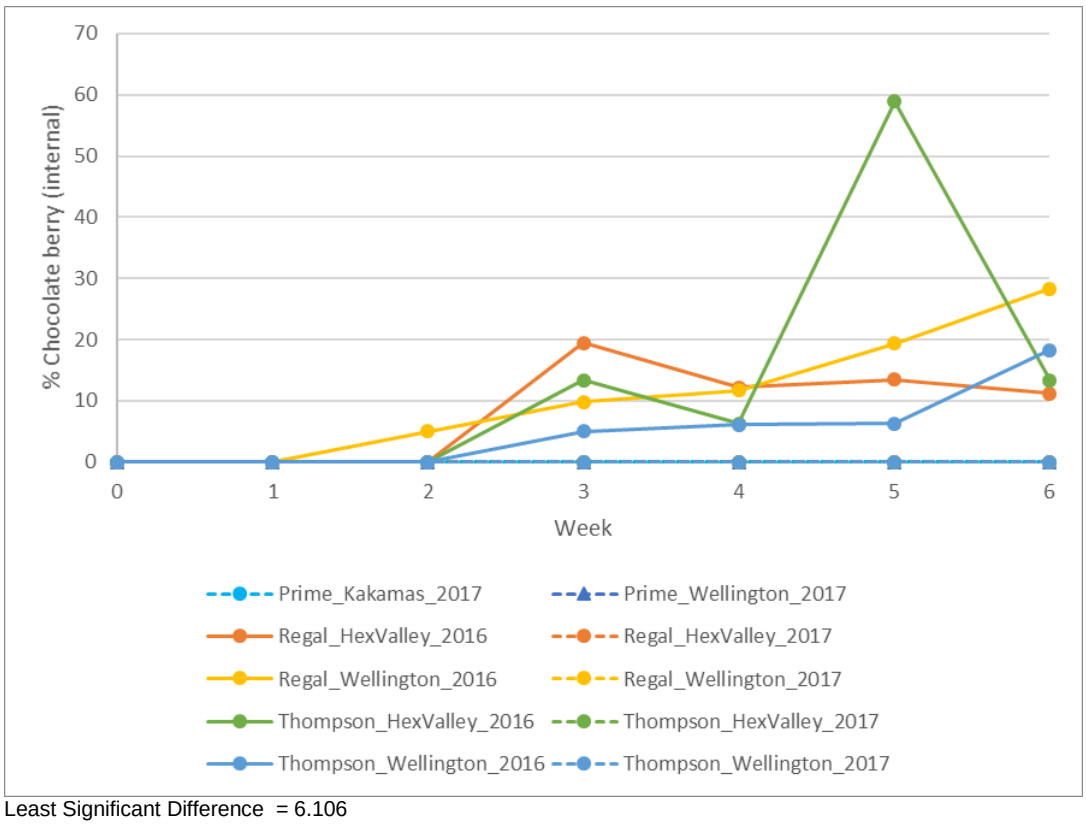

Least Significant Difference = 46.601

**Figure 3.7** Mean plot for the incidence of % chocolate berry (external stylar end) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



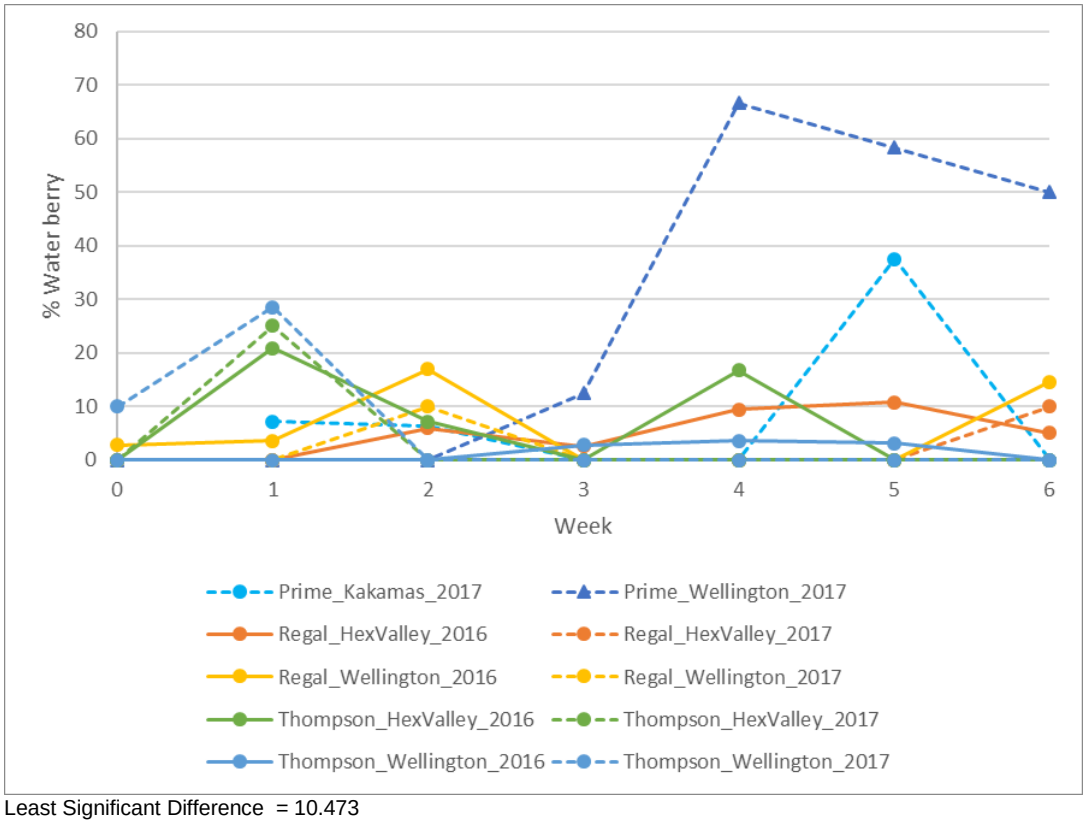

Least Significant Difference = 18.781

**Figure 3.8** Mean plot for the incidence of % chocolate berry (external) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



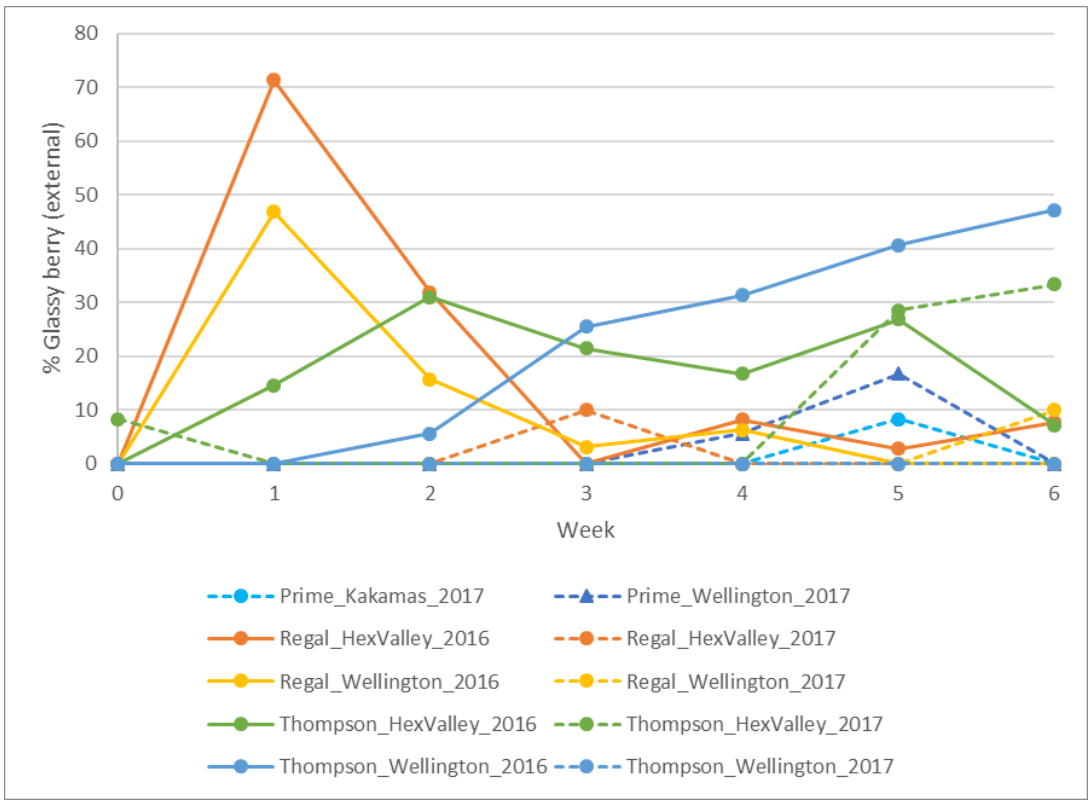

Least Significant Difference = 6.106

**Figure 3.9** Mean plot for the incidence of % chocolate berry (internal) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



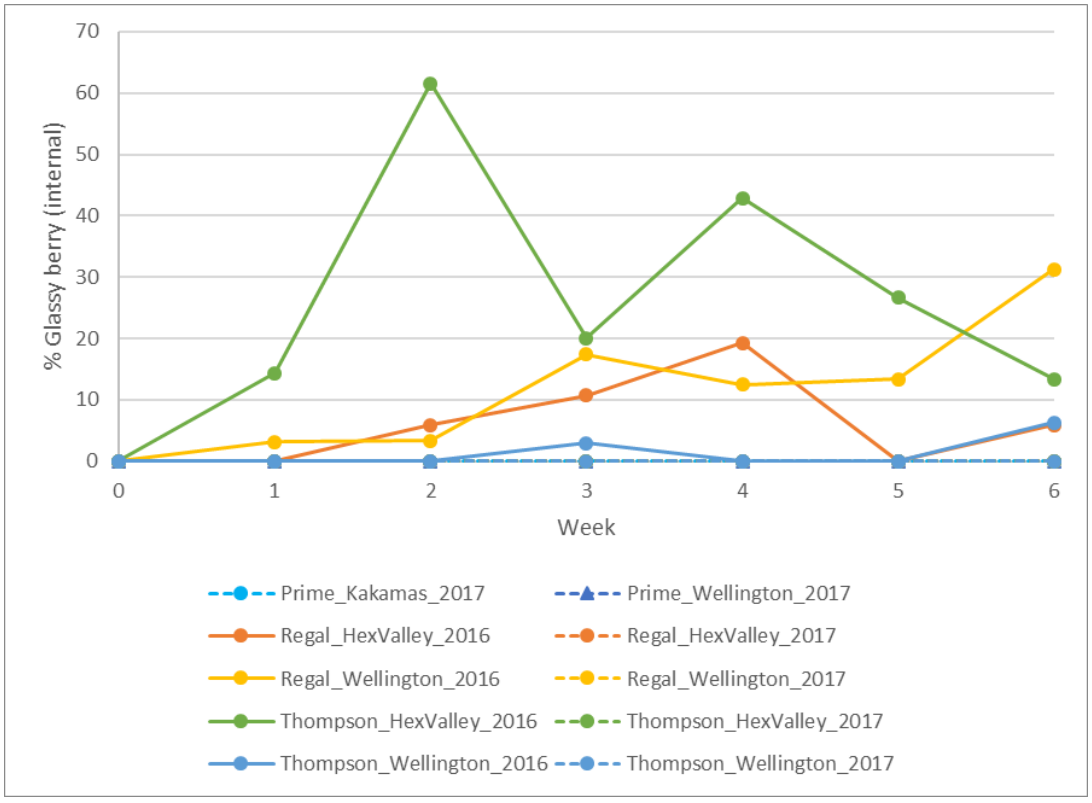

Least Significant Difference = 10.473

**Figure 3.10** Mean plot for the incidence of % water berry (external) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



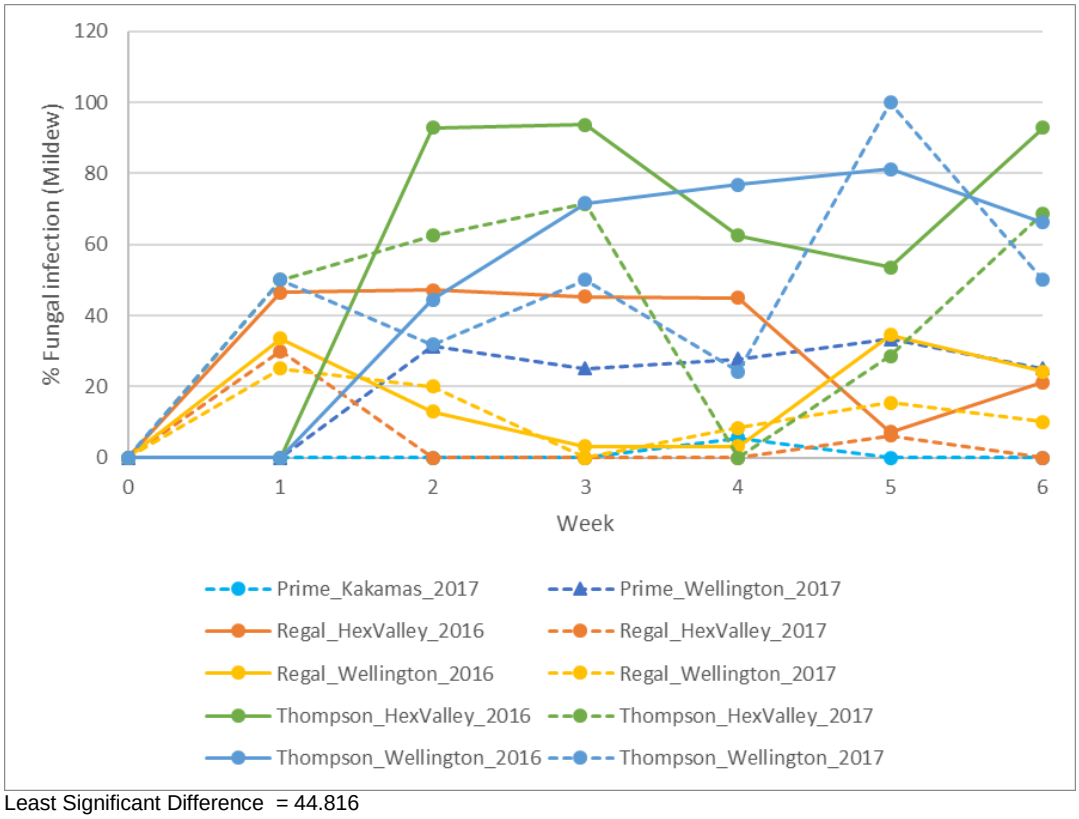

Least Significant Difference = 26.4

**Figure 3.11** Mean plot for the incidence of % glassy berry (external) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



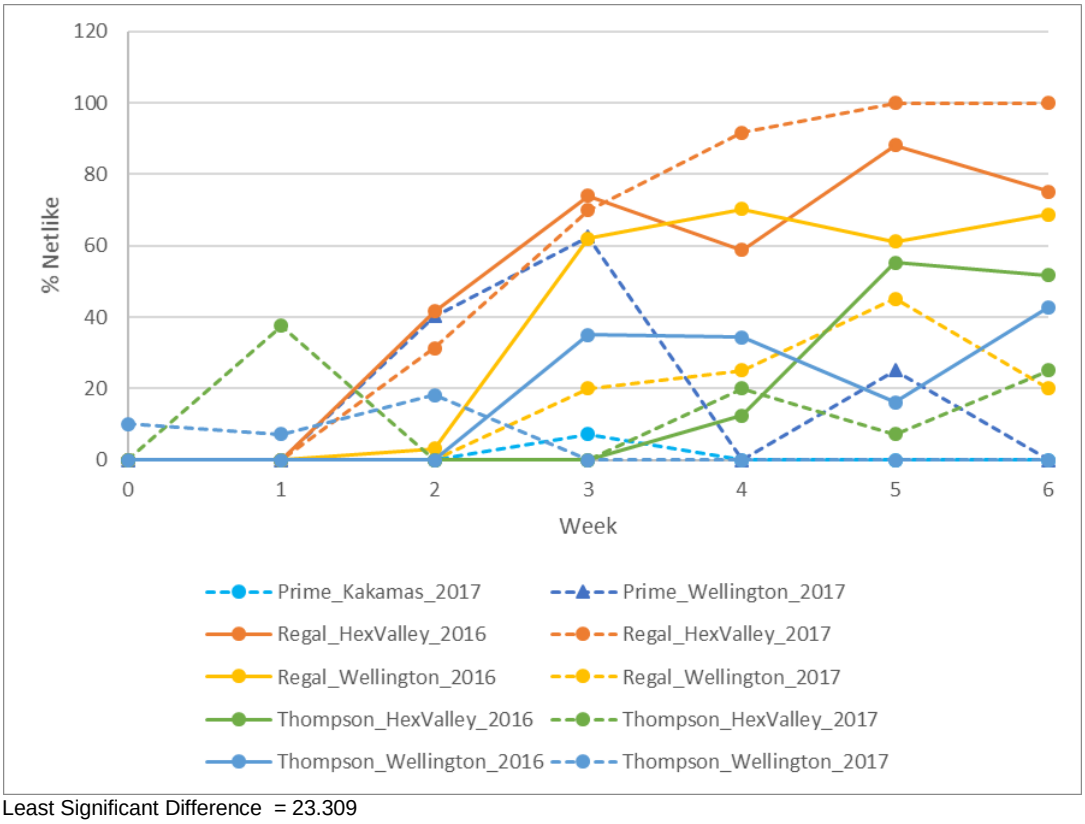

Least Significant Difference = 8.4577

**Figure 3.12** Mean plot for the incidence of % chocolate berry (internal) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



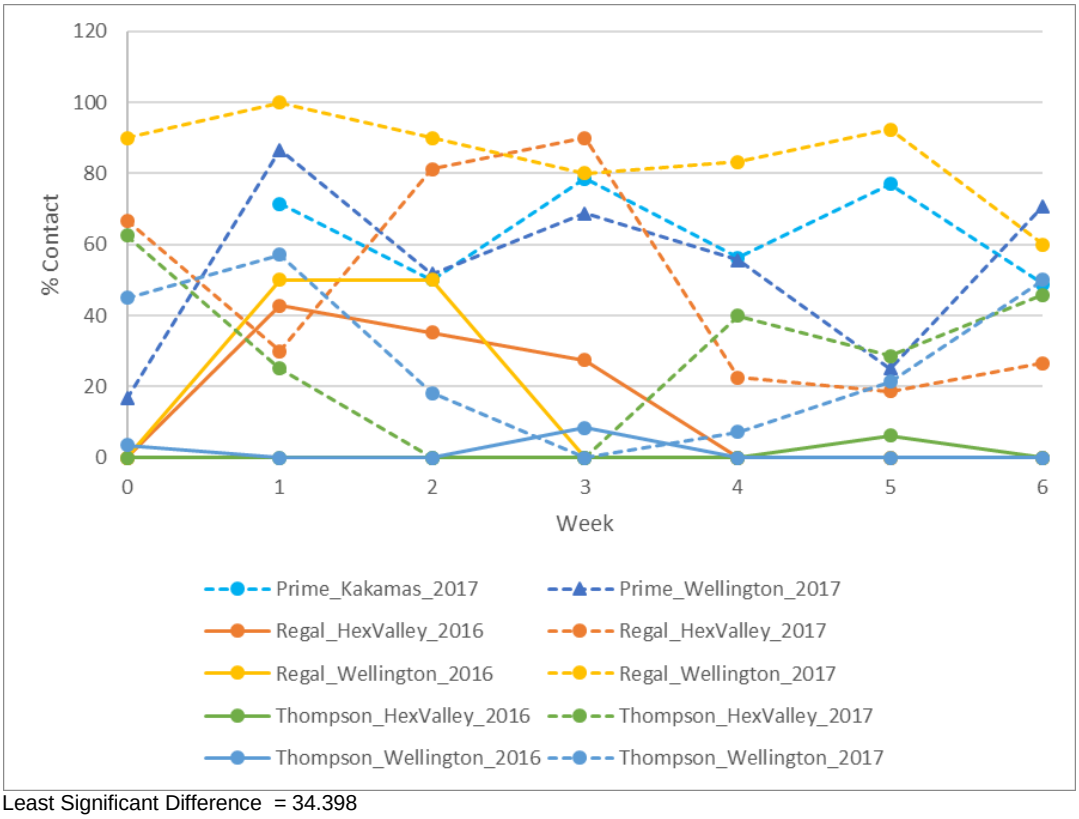

Least Significant Difference = 44.816

**Figure 3.13** Mean plot for the incidence of % fungal infection (Mildew) over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



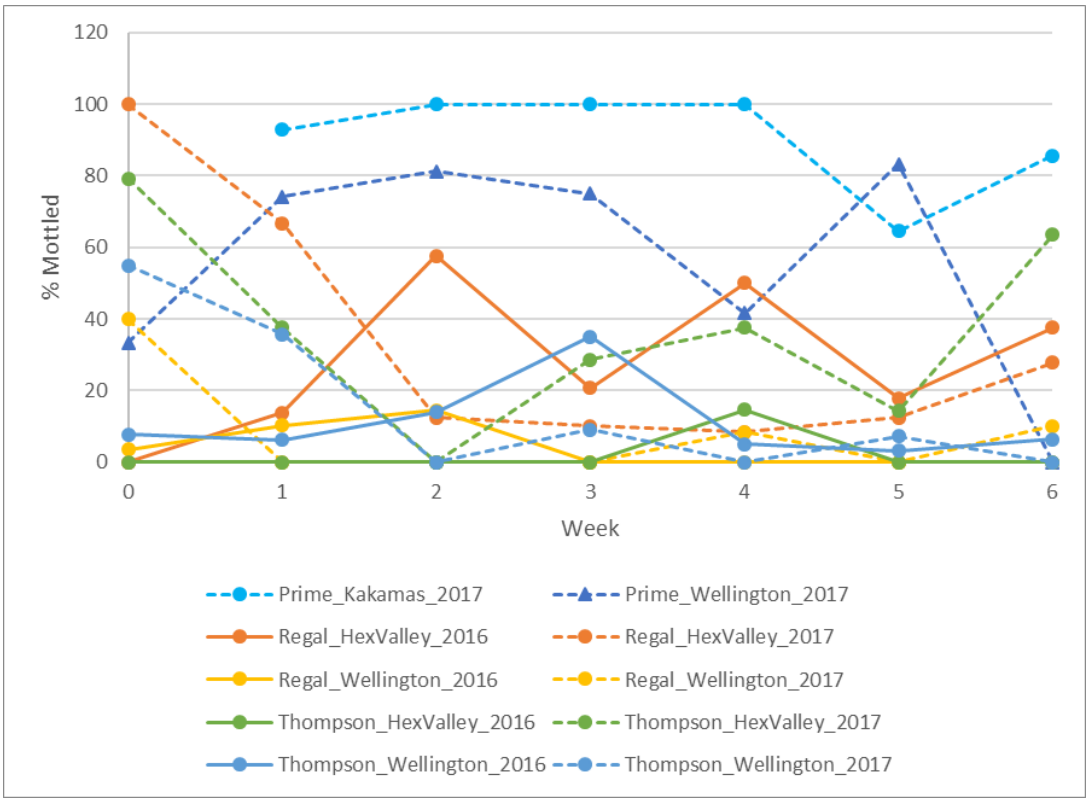

Least Significant Difference = 23.309

**Figure 3.14** Mean plot for the incidence of % netlike browning over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



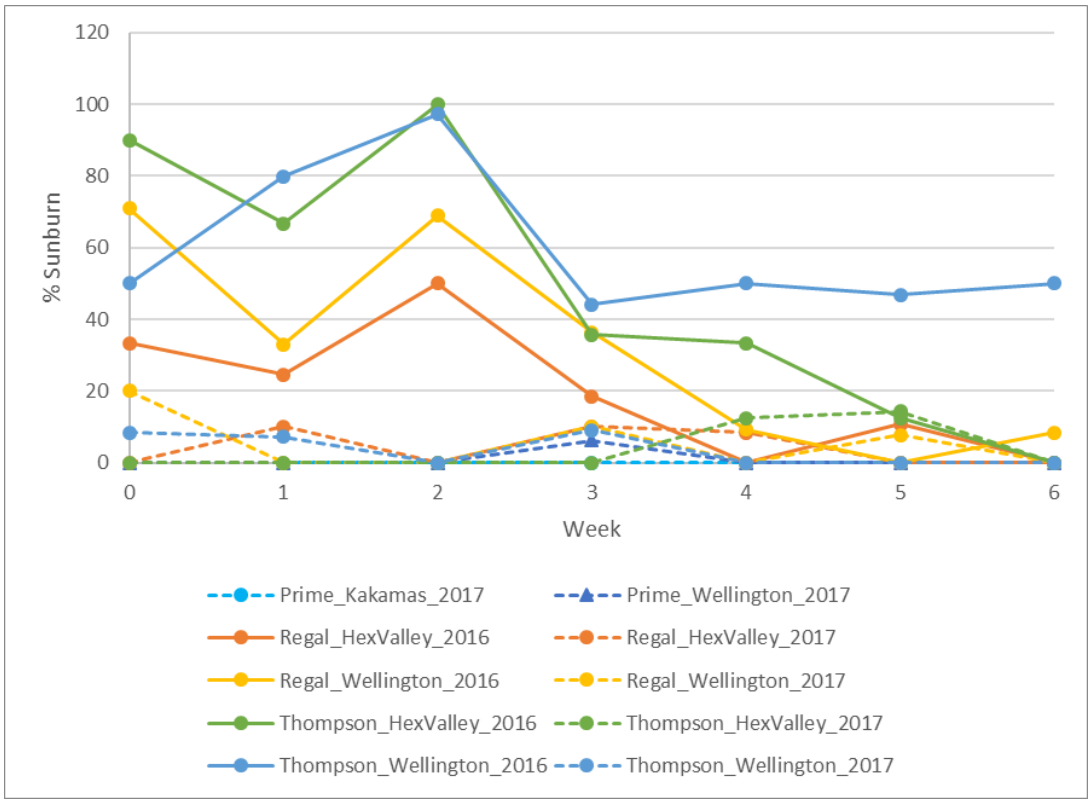

Least Significant Difference = 34.398

**Figure 3.15** Mean plot for the incidence of % contact browning over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



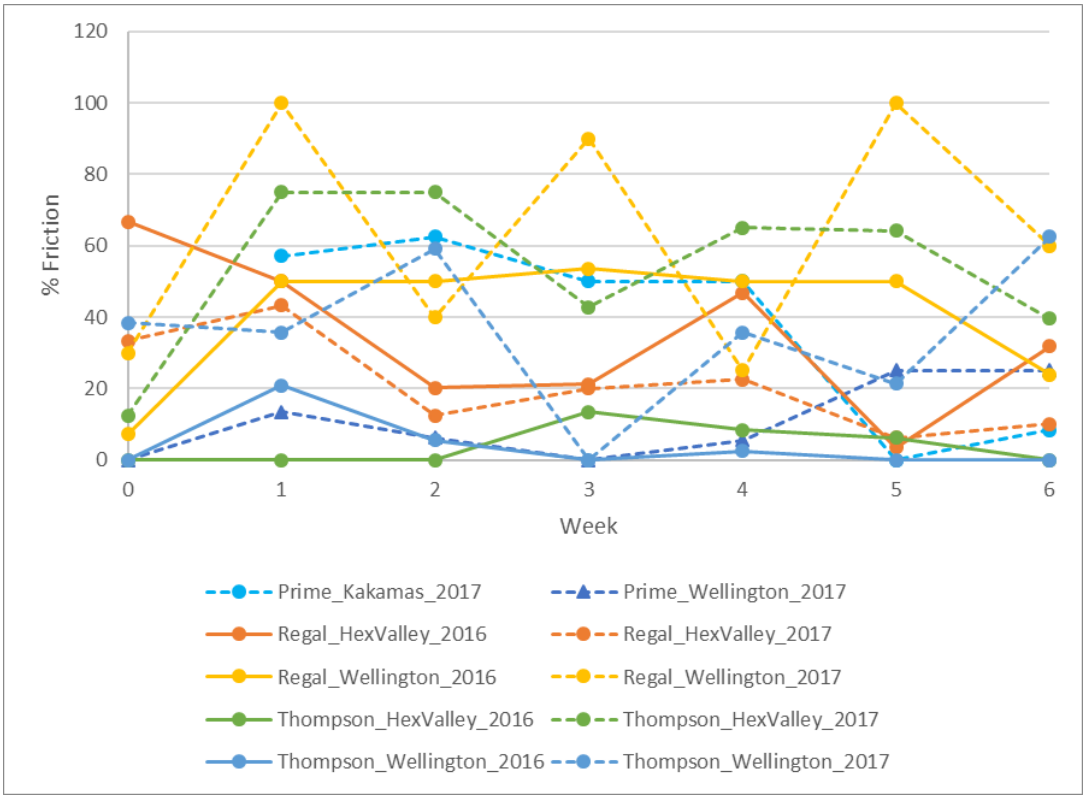

Least Significant Difference = 30.392

**Figure 3.16** Mean plot for the incidence of % mottled browning over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



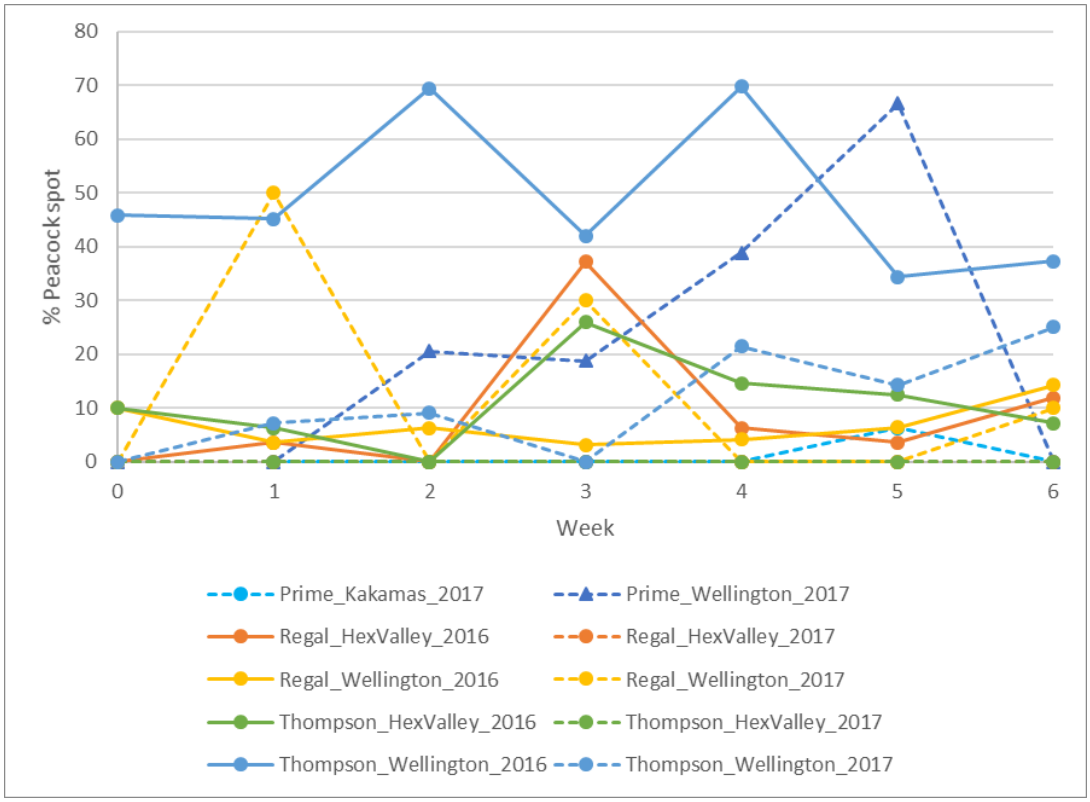

Least Significant Difference = 29.804

**Figure 3.17** Mean plot for the incidence of % sunburn over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



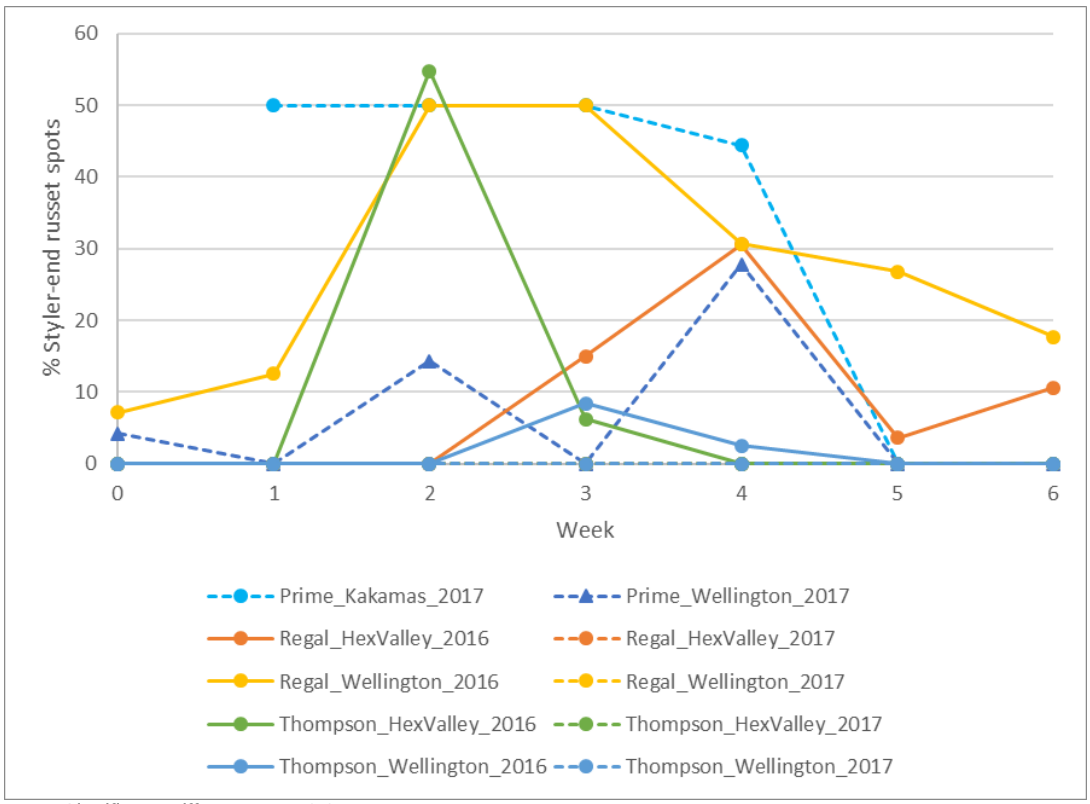

Least Significant Difference = 69.117

**Figure 3.18** Mean plot for the incidence of % friction browning over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



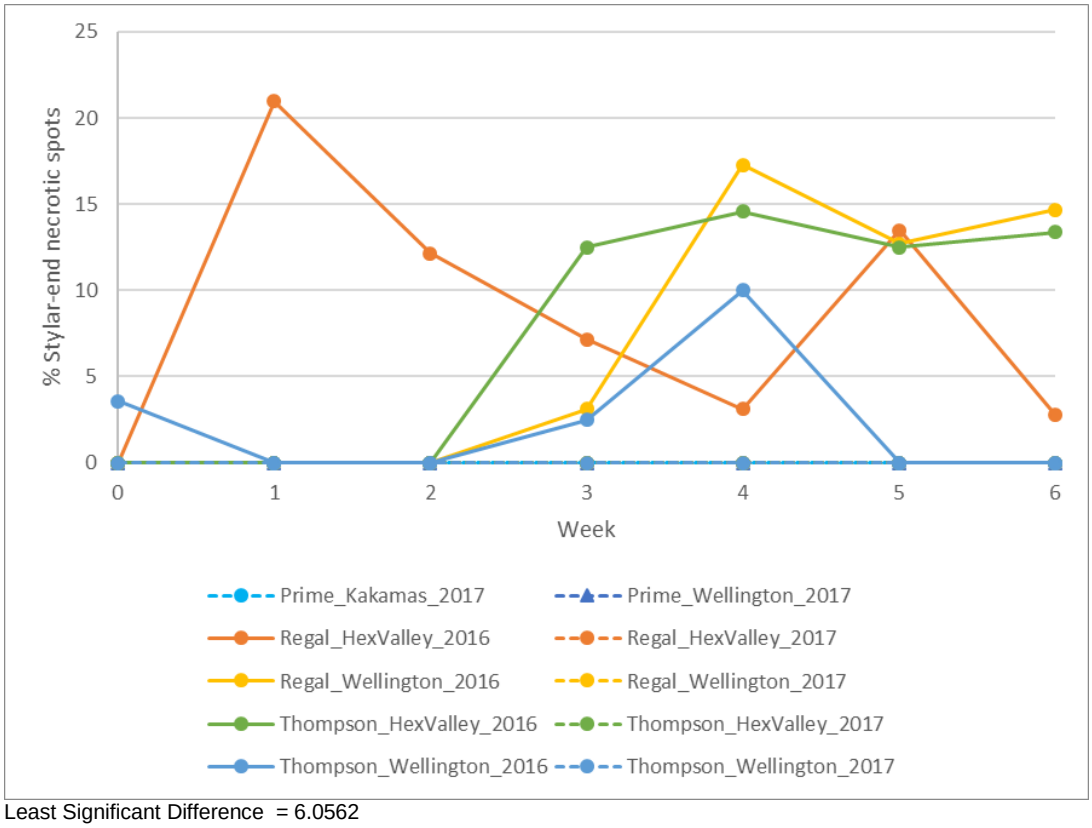

Least Significant Difference = 29.838

**Figure 3.19** Mean plot for the incidence of % peacock spot browning over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



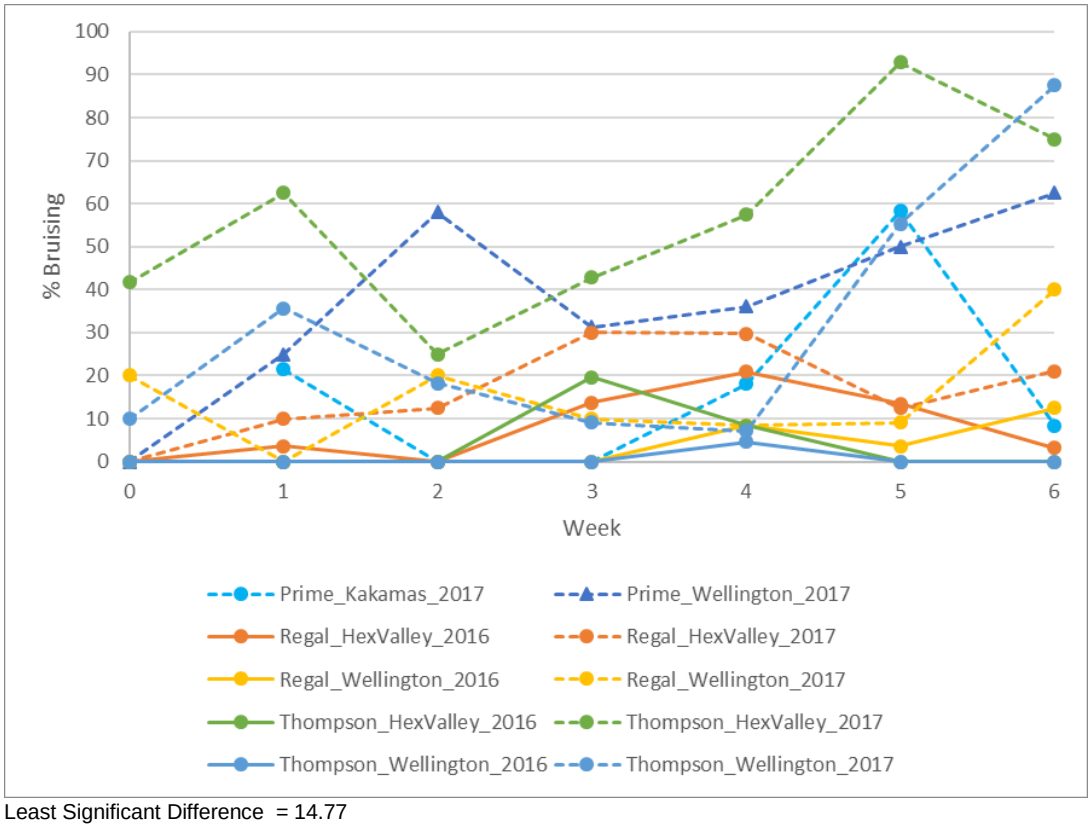

Least Significant Difference = 48.015

**Figure 3.20** Mean plot for the incidence of % styler-end russet spots over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.



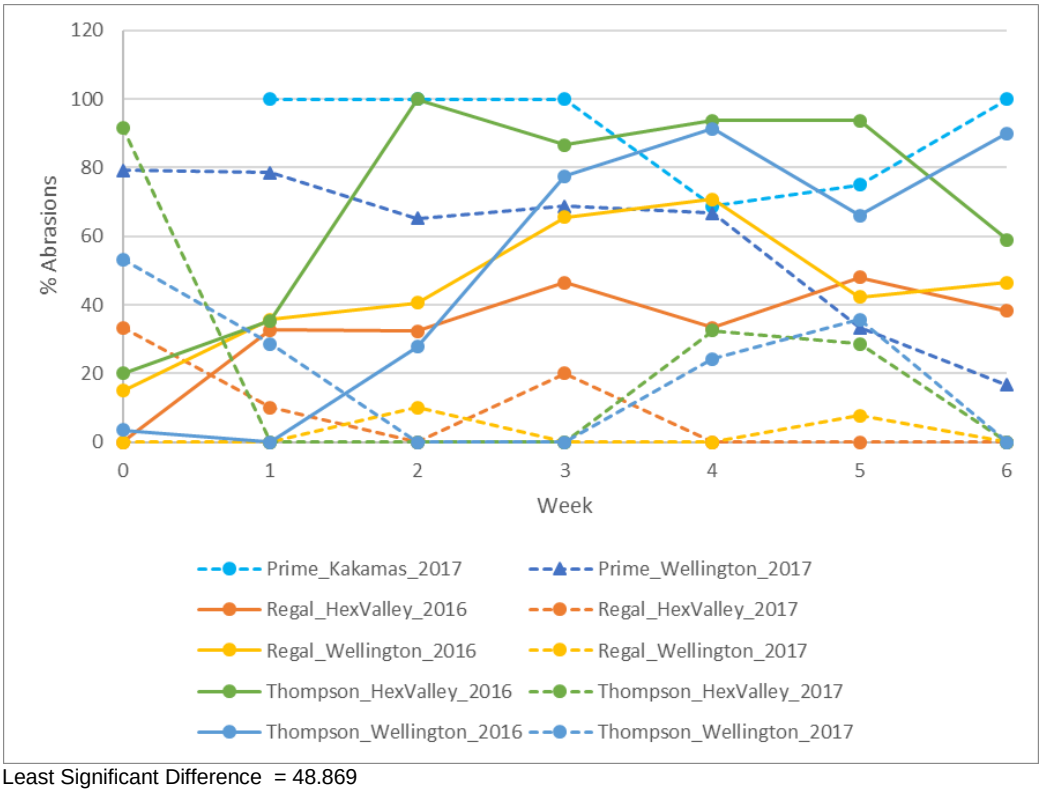

Least Significant Difference = 6.0562

**Figure 3.21** Mean plot for the incidence of stylar end necrotic spots over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.




Least Significant Difference = 14.77

**Figure 3.22** Mean plot for the incidence of % bruising over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.




Least Significant Difference = 48.869

**Figure 3.23** Mean plot for the incidence of % abrasions over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.

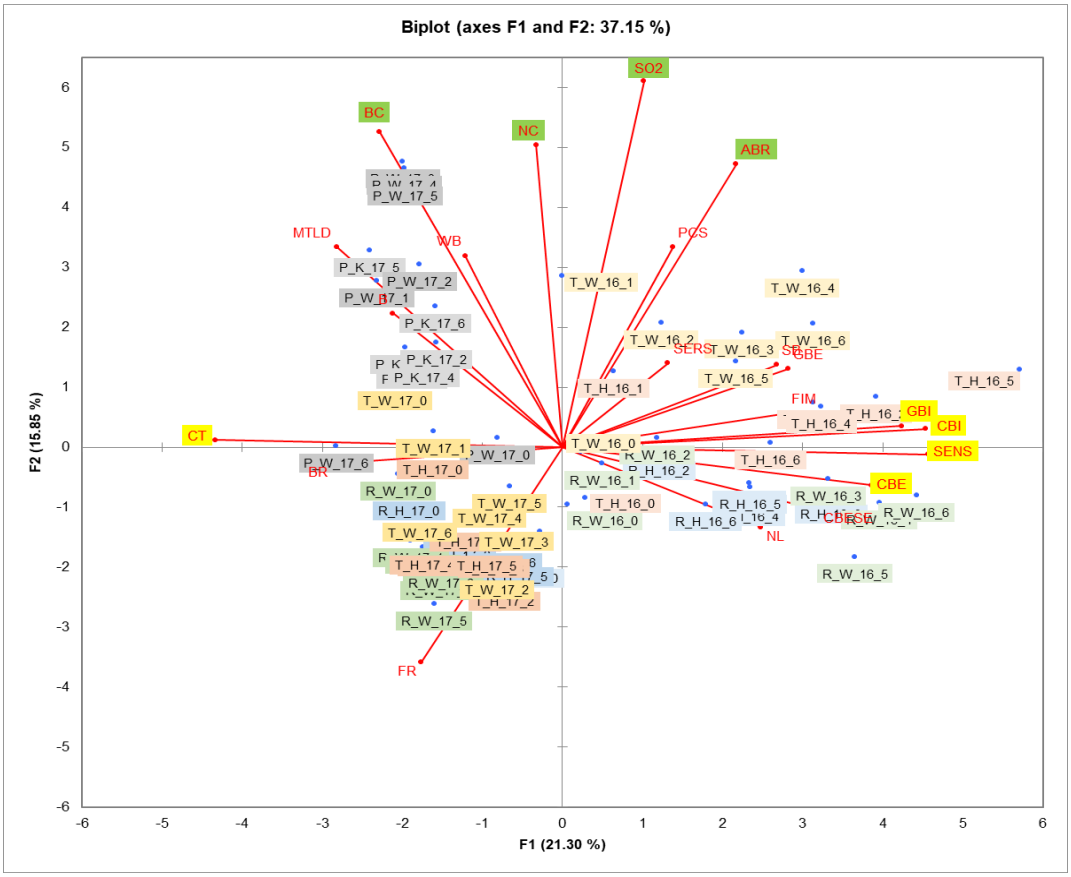



**Figure 3.24** Principal component analysis (PCA) plot for the incidence of the different defects over the different cold storage weeks for Prime, Thompson Seedless and Regal Seedless bunches harvested from Kakamas, Wellington and Hex River Valley in 2016 and 2017.

**Table 3.3** Climate data for the sites from which the grapes were harvested

| Location | Year | Month | Day | Tx | Tn | RHx | RHn | Rain |
|---|---|---|---|---|---|---|---|---|
| Wellington | 2016 | 11 | Average | 28.44 | 14.58 | 80.33 | 26.34 | 0.19 |
| Wellington | 2016 | 11 | Highest | 35.54 | 20.05 | 98.22 | 45.18 | 2.29 |
| Wellington | 2016 | 11 | Lowest | 20.00 | 9.55 | 55.36 | 10.27 | 0.0 |
| Wellington | 2016 | 12 | Average | 31.22 | 15.67 | 88.05 | 26.76 | 0.85 |
| Wellington | 2016 | 12 | Highest | 36.99 | 19.87 | 100.00 | 69.47 | 14.99 |
| Wellington | 2016 | 12 | Lowest | 21.41 | 12.6 | 54.15 | 10.60 | 0.0 |
| Wellington | 2017 | 1 | Average | 31.37 | 16.56 | 83.07 | 26.66 | 0.6 |
| Wellington | 2017 | 1 | Highest | 38.34 | 20.48 | 99.85 | 47.09 | 7.87 |
| Wellington | 2017 | 1 | Lowest | 23.35 | 12.91 | 46.29 | 9.90 | 0.0 |
| Wellington | 2017 | 2 | Average | 32.30 | 17.87 | 79.46 | 25.20 | 0.0 |
| Wellington | 2017 | 2 | Highest | 40.00 | 24.64 | 98.14 | 43.24 | 0.0 |
| Wellington | 2017 | 2 | Lowest | 24.00 | 14.08 | 42.61 | 9.20 | 0.0 |
| Wellington | 2017 | 3 | Average | 30.89 | 14.74 | 85.52 | 25.40 | 0.3 |
| Wellington | 2017 | 3 | Highest | 40.00 | 21.03 | 100.00 | 50.25 | 4.57 |
| Wellington | 2017 | 3 | Lowest | 22.70 | 10.21 | 50.77 | 5.95 | 0.0 |
| Hex River Valley | 2016 | 11 | Average | 28.88 | 9.21 | 89.44 | 22.48 | 0.14 |
| Hex River Valley | 2016 | 11 | Highest | 37.67 | 16.27 | 94.48 | 41.63 | 2.54 |
| Hex River Valley | 2016 | 11 | Lowest | 21.83 | 3.32 | 67.30 | 10.56 | 0.0 |
| Hex River Valley | 2016 | 12 | Average | 33.01 | 11.92 | 88.70 | 16.25 | 0.01 |
| Hex River Valley | 2016 | 12 | Highest | 39.99 | 16.38 | 93.04 | 29.52 | 0.25 |
| Hex River Valley | 2016 | 12 | Lowest | 25.29 | 8.83 | 66.78 | 6.69 | 0.0 |
| Hex River Valley | 2017 | 1 | Average | 32.09 | 12.66 | 88.54 | 21.39 | 0.11 |
| Hex River Valley | 2017 | 1 | Highest | 38.24 | 17.88 | 94.15 | 40.68 | 3.05 |
| Hex River Valley | 2017 | 1 | Lowest | 24.50 | 8.62 | 78.42 | 10.05 | 0.0 |
| Hex River Valley | 2017 | 2 | Average | 33.29 | 12.85 | 89.27 | 19.08 | 0.01 |
| Hex River Valley | 2017 | 2 | Highest | 38.02 | 18.15 | 93.95 | 29.87 | 0.25 |
| Hex River Valley | 2017 | 2 | Lowest | 27.97 | 7.66 | 79.93 | 10.6 | 0.0 |
| Hex River Valley | 2017 | 3 | Average | 31.80 | 10.62 | 88.60 | 17.2 | 0.02 |
| Hex River Valley | 2017 | 3 | Highest | 38.46 | 17.75 | 94.14 | 32.59 | 0.51 |
| Hex River Valley | 2017 | 3 | Lowest | 22.63 | 5.13 | 74.37 | 6.37 | 0.0 |
| Kakamas | 2017 | 11 | Average | 34.47 | 13.96 | 65.53 | 9.94 | 0.16 |
| Kakamas | 2017 | 11 | Highest | 40.88 | 23.48 | 94.00 | 38.49 | 4.83 |
| Kakamas | 2017 | 11 | Lowest | 18.03 | 7.90 | 31.56 | 5.44 | 0.0 |
| Kakamas | 2017 | 12 | Average | 37.37 | 16.17 | 64.73 | 8.11 | 0.2 |
| Kakamas | 2017 | 12 | Highest | 42.24 | 25.31 | 93.70 | 12.76 | 6.1 |
| Kakamas | 2017 | 12 | Lowest | 33.62 | 10.92 | 42.11 | 5.24 | 0.0 |

Tx = Daily Maximum Temperature (°C); Tn = Daily Minimum Temperature (°C); Average Temperature = [[Tx + Tn] / 2] (°C); RHx = Average Daily Maximum Relative Humidity (%); RHn = Average Daily Minimum Relative Humidity (%); Average Relative Humidity [[RHx + RHn] / 2] (%); Total Daily Rainfall (mm)

## 3.4 DISCUSSION

$SO_2$ damage to table grape berries in the form of discolouration, bleaching and hairline cracks on the berries is a well-known defect that occurs when $SO_2$-generating pads are placed on grapes when trying to manage grey mould by (Chervin *et al*., 2005; Abdolahi *et al*., 2010). An in-depth study by Fernández-Trujillo *et al*. (2008) into the effect that single and dual-phase $SO_2$ generator pads had on the quality of table grape cultivars revealed that $SO_2$ does not seem to be responsible for most of the cracks and that berry crack was cultivar dependent. The results observed in this study are, therefore, in agreement with the evidence found in literature in that several factors have an influence on the incidence of both $SO_2$ damage and berry crack. The combination of the factors cultivar, site, year and week at cold storage had a significant effect on both. Prime had the most $SO_2$ damage and berry crack in 2017, whilst that of Thompson Seedless and Regal Seedless remained similar for both cultivars (Figure 3.5 and Figure 3.6). Berry crack was low during 2016 and 2017, but $SO_2$ damage was high in 2016, but very low in 2017. The harvest year seemed to have the most influence on the appearance of $SO_2$ damage. Fungal infection (mildew) was also very high in 2016 for both Thompson Seedless and Regal Seedless and very low for all cultivars in 2017. This can be attributed to the higher relative humidity (RHx) and rainfall that prevailed in 2016 than in 2017 (Table 3.3).

The next defect that was notably higher in 2016 than in 2017 was sunburn. The incidence of sunburn was higher in Regal Seedless than in Thompson Seedless and can be clearly seen in Figure 3.17. A combination of the factors cultivar, site and year had a significant effect on the incidence of this defect ($p<0.05$) and for cold storage week on its own (Table 3.2). Taking into consideration that the appearance and the extent of a disorder such as sunburn are strongly dependent on factors such as grape variety and bunches directly exposed to sunlight (Krasnow *et al*., 2010), it is understandable why sunburn was prevalent on Thompson Seedless and Regal Seedless from Hex River Valley in 2016, but not on these same cultivars from Wellington and also not in 2017. This is revealed by the environmental conditions (climate) that differed between 2016 and 2017 between these areas. The difference in temperature between these two seasons (Table 3.4), therefore, probably had an influence. During the ripening and harvest period, the average maximum daily temperatures were higher and the average minimum temperature were mostly lower. This influence of climate on the incidence of browning phenotypes is further supported by what Weksler *et al.* (2015) found when they investigated the connotation of browning in Thompson Seedless table grapes with cracking were heightened by the application of organophosphate insecticides. These researchers specifically looked at ‘streak browning’, referred to as netlike browning in this study. They, similarly, also found that the incidence of this defect varied widely over the different years and, therefore, turned to the

potential effect of climate to evaluate it by looking at maximum and minimum temperatures as well as relative humidity in certain months of the year.

The incidence of defects based on different stages of the grape development was not looked at in this study, but an investigation on Princess table grapes by Vial *et al*. (2005) showed that harvest maturity influenced skin browning development, but that vineyard location and management practices had a bigger impact on its development, further supporting the results observed in this study and those in the study of Weksler *et al.* (2015). Defect intensity for Regal Seedless grapes was higher for grapes from Wellington than those from Hex River Valley in both years (Table 3.3).

The table grape industry has always been interested in identifying the several factors that may associate with the incidence of browning in white table grape cultivars and to determine factors that may predispose these cultivars to berry browning (Moelich, 2010). The browning reaction results from mechanical injury during post-harvest storage or the processing of fruits (Fortea *et al*., 2009). Table grape processing/packaging after harvest is a very complex process and involves much handling of the fruit during which damage such as abrasion damage can result. The incidence of abrasion damage was very high for all three cultivars (Table 3.2), although very, very high in 2016 for Thompson Seedless and Regal Seedless but not so in 2017.

Moelich (2010) found that there was a noticeable variation in browning incidence between different bunches sourced from the same box when the possible role of delivery air temperature (DAT) and influence of forced-air cooling (FAC) practices on the development of berry browning in table grapes was investigated. No consistent, repeatable effect of DAT and FAC duration on berry incidence, however, was demonstrated. The misconceptions that might exist concerning which temperature or storage regimes may be conducive to or preventative against the development of browning on certain cultivars was highlighted. That seemed to resonate well with what was found in this study, where the incidence of peacock spot was high for Thompson Seedless and Regal Seedless in 2016, but for none of the three cultivars in 2017. Contact and mottled browning were very high for Prime in 2017, while contact browning for Thompson Seedless was low in 2016 but very high for Regal Seedless in 2016. In 2017, contact browning for Thompson Seedless was higher than in 2016 but lower for Regal Seedless than in 2016. The incidence of mottled browning was higher in 2017 for Thompson Seedless than in 2016 and lower for Regal Seedless in 2017 than in 2016.

These varying incidences of defects on different cultivars and intensities from the different harvest locations prove that good management practices in the vineyard before and during harvest as well as cold storage practices after harvest and during cold storage should always be followed to ensure that good-quality products are delivered. This especially since there are still so many unidentified factors indicated as the primary cause of berry browning. Even the in-detail investigation of the role of the phenolic-related oxidative enzymes, polyphenol oxidase

(PPO) and peroxidase (POD) by Gonzalez-Barrio *et al.* (2005) showed that UV-C-induced browning in white table grapes does not seem to be a 'general' unwanted side effect, but it seems to be subject to the features of the cultivar exposed to it (skin thickness, chlorophyll content, etc.). This is why Rustioni *et al*. (2014) rather proposed using a chlorophyll index threshold as a marker for grapes' predisposition to sunburn browning. They showed that with the developing fast, non-invasive, and cheap methods such as reflectance spectroscopy support could be granted to grapevine variety characterisation concerning sunburn vulnerability, as well as the study of the physiological processes involved in the symptoms' presence.

## 3.5 CONCLUSION

The results clearly show that the incidence of specific defects ($SO_2$ damage, FI, SB, PCS and ABR damage) can be very high for Thompson Seedless Regal Seedless in one year (2016) and be very, very low for the same cultivars in the following year (2017). This highlights the important influence that environmental factors such as temperature can have on the development of certain defects; similarly with the intensity of defects that were also very high for both Thompson Seedless and Regal Seedless in 2016, but much lower in 2017. The intensity also highlighted cultivar differences with Prime from Kakamas and Wellington both having a higher intensity in 2017 than Thompson Seedless and Regal Seedless from Hex River Valley and Wellington. For the table grape industry the implications of these results are such that quicker decisions can be taken regarding the quality of the grapes, assisting to decide which classes grapes should be placed in and to which export markets grapes should be shipped. If it is, for example, repeatedly found that berries with severe defects are from the same cultivar from the same vineyard, these grapes could then be sold on the local market instead of being exported. The potential contribution of the soil and water to this phenomenon is presently unknown, especially when looking at the intensity of the defects from the different vineyards (Regal Seedless from Wellington having almost double the intensity than Regal Seedless from Hex River Valley in 2016). Further studies at the vineyard level should reveal patterns that may give rise to defects like the external browning phenotypes (contact, mottled, sunburn, friction, styler-end russet spots and styler-end necrotic spots) that might offer a better interpretation of the factors involved. Examination of other factors of the berries such as firmness, sugar level and the synergistic effect of these together with cold storage regimes, etc. might also offer better holistic insights into the incidence and intensity observed.

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# Chapter 4: Measuring internal maturity parameters contactless on intact table grape bunches using NIR spectroscopy


## ABSTRACT

The determination of internal maturity parameters of table grape is usually done destructively using manual methods that are time-consuming. The possibility was investigated to determine whether key fruit attributes, namely, total soluble solids (TSS); titratable acidity (TA), TSS:TA ratio, pH, and BrimA could be determined on intact table grape bunches using Fourier transform near-infrared (FT-NIR) spectroscopy and a contactless measurement mode. Partial Least Squares (PLS) regression models were developed for the maturity and sensory quality parameters using grapes obtained from two consecutive harvest seasons. Different spectral pre-processing techniques were applied to the original spectral and baseline corrected spectra. The combination of Savitzky-Golay first derivative coupled with multiplicative scatter correction on the original spectra delivered the best models. Decision of the best model was based on the lowest number of latent variables (LV) used to build the model as well as which one had the highest prediction correlation coefficient ($R^2_p$) and root mean square error of prediction ($RMSE_P$). For the respective parameters TSS, TA, TSS:TA ratio, pH, and BrimA, the number of LV used when the models were build according to a random split of the calibration and validation set were 6, 4, 5, 5 and 10, the $R^2_p$ = 0.81, 0.43, 0.66, 0.27, and 0.71, and the RMSEP = 1.30 °Brix, 1.09 g/L, 7.08, 0.14, and 1.80. When 2016 was used as the calibration set and 2017 as the validation set in model building the number of LV used were 9, 5, 5, 4 and 4, and the $R^2_p$ = 0.44, 0.06, 0.17, 0.05 and 0.05, and the RMSEP = 3.22 °Brix, 2.41 g/L, 14.53, 0.21, and 8.03 for the respective parameters. This study provides the first steps towards a completely nondestructive and contactless determination of internal maturity parameters of intact table grape bunches.


## 4.1 INTRODUCTION

The logistics of table grape harvest and shipment to intended consumer markets are complex and challenging. Table grapes (*Vitis vinifera* L.) is a non-climacteric fruit, which does not ripen further, nor does the quality improve after harvest (Sonego *et al*., 2002). Therefore, grapes must be at the desired maturity level when harvested and the eating quality of packed produce must be retained during several weeks of cold storage and ultimate shipment to markets. Traditionally, fruit maturity is expressed in terms of total soluble solids (TSS), also referred to as soluble solids content (SSC), which primarily reflects the sugar content, and titratable acidity (TA), which reflects the tartaric acid content (Nelson *et al*., 1963). Although pH is usually

included as part of the routine chemical analysis to assess the maturity and sensory characteristics of grapes, no clear link has yet been established between pH and grape maturity (Walker *et al*., 2001; Reynolds *et al*., 2006). TSS is typically measured in the vineyard with a handheld refractometer and expressed as °Brix, while TA is determined in the laboratory by wet chemistry methods. Worldwide, TSS and sugar:acid ratios (TSS:TA) serve as primary indices for the quality of export fruit. Minimum requirements are specified for TSS concentrations and TSS:TA ratios for each cultivar, for example by the Agricultural Product Standards Act, 1990 (Act No. 119 of 1990) of South Africa, section 4(3)(a)(ii). Harvested table grape bunches are packed and exported either as individual bunches in punnets, or individually wrapped and packed in a box together with other bunches. When table grape consignments reach the harbour of the exporting country, random spot checks are done on packed fruit. If any sample is found to be at the incorrect TSS and/or TSS:TA ratio, whole export consignments can be rejected, or even returned once they have reached the intended market. Given that the popularity of table grapes makes it one of the most consumed fruits in the world (Piazolla *et al*., 2013), anything that affects quality negatively and leads to losses should be avoided. All the aforementioned laboratory measurements are done–destructively and are time-consuming. Furthermore, the measurement of TA requires both specialised equipment and chemicals and creates chemical waste. Opportunities for the table grape industry to move away from destructive techniques to determine key maturity parameters (TSS, TA, TSS;TA ratio and pH) already exist. Fourier transform near infrared (FT-NIR) spectroscopy has long been used with success to determine a wide variety of parameters in fruit. Non-destructive postharvest determination of TSS, TA and pH have been reported on apricots (Camps and Christen, 2009), pears (Liu *et al*., 2008), mandarins (Liu *et al*., 2010), plums (Pérez-Marín *et al*., 2010), blueberries (Sugiyama *et al*., 2010), avocados (Wedding *et al*., 2010), wine grapes (González-Caballero *et al*., 2010; Kemps *et al*., 2010; Barnaba *et al*., 2013), and individual table grape berries (Cao *et al*., 2010; Omar, 2013).

Challenges related to quality evaluation of intact bunches include the complexity of their morphology which includes the number of berries on the bunch and, the shape and compactness of the bunch (Mattheou *et al*., 1995; May, 2000), which in turn have been shown to be dependent on the grape cultivar (Balic *et al*., 2014). Other factors which add to the challenge of scanning intact bunches include the within-bunch and between-bunch heterogeneity in sugar and maturity levels (Mattheou *et al*., 1995; Šuklje *et al*. (2012). These aspects are known to be influenced by the seasonal effects as well as the geographical location of the vineyards (Sonega *et al*., 2002). The double sigmoidal growth curve associated with grape development and ripening stages has been thoroughly discussed by several authors (Dokoozlian and Kliewer, 1996; Wheeler *et al*., 2009) and recently on table grapes by Sonnekus (2015). It is, however, important to emphasize the complex role temperature plays in the ripening (Kuhn *et al*., 2014) and hence quality of grapes (Coombe, 1987). Fluctuations in the

maximum and minimum temperatures during consecutive seasons can lead to grapes either ripening earlier or later than might be anticipated. This has serious consequences on the marketability of table grapes for the producers.

In this study, the potential of NIR spectroscopy to quantify TSS, TA, TSS:TA ratio and pH non-destructively on intact bunches is explored. Individual bunches were scanned contactless using diffuse reflectance FT-NIR spectroscopy. To enrich the information gathered on the mentioned quality parameters, another sensory-based parameter, namely BrimA (calculated as TSS - *k* x TA), and originally proposed by (Jordan, 2001), was also included in the analysis. BrimA is an alternative parameter for determining the palatability of table grapes. Jordan et al. (2001) argued that the TSS/TA ratio does not fully reflect the major influence that acid has on the tastiness prediction of table grapes. The human tongue does not have the same sensitivity for sugar than it has for acidity. However, Jayasena and Cameron (2007), argued that TSS:TA ratio is a better indicator of consumers' taste acceptance of Crimson Seedless table grapes than TSS, TA and BrimA alone. Fawole and Opara (2013) also reported that both the TSS:TA ratio and BrimA are useful to create a dependable index for evaluating optimal fruit maturity of pomegranates. The inclusion of both TSS:TA ratio and BrimA as sensory parameters in this study was, therefore, of utmost importance to pave the way for non-destructive evaluation of the taste acceptability of grapes. To our knowledge, this is the first report on analysis of completely intact bunches using FT-NIR spectroscopy.

## 4.2 MATERIALS AND STRATEGIES

### 4.2.1 Grape sampling

The data collection and analysis plan in Figure 4.1 shows the harvest years, cultivars, location of the vineyard cultivars were harvested from, number of bunches harvested per location and per year, as well as the two strategies followed to build PLS models for the parameters under investigation. Our experimental design aimed to include variability resulting from seasonal effects, vineyard geographic location, ripeness levels and grape cultivar. Grapes were harvested from three locations over two seasons (2016 and 2017), and at two ripening stages. Three white seedless table grape cultivars were used, Prime Seedless, Thompson Seedless and Regal Seedless, which are amongst the top 20 cultivars exported from South Africa (SATI, 2018).

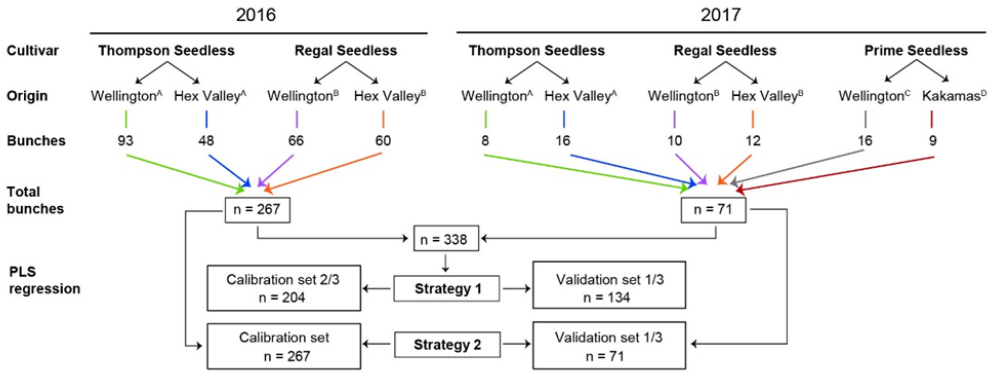



**Figure 4.1 The** data collection and analysis plan for the 338 intact bunch table grapes subjected to FT-NIR spectroscopy. [A]Grapes harvested from the same vineyard block in both years; [B]Grapes harvested from the same vineyard block in both years; [C,D] Grapes harvested from these two new vineyard block in 2017.

Table 4.1 shows the GPS co-ordinates, harvest week and the TSS level for the three cultivars at the individual locations. Grapes were harvested from the fifth row of each block starting from the third section of the row. The vines were marked so that grapes could be harvested from the same vines in the two consecutive years. The rationale for this step was to reduce the number of factors that would play a role in each year. Soils, as well as the micro-climate which influences the development of bunches (accumulation of sugar and breakdown of acids) may vary within a block (Šuklje *et al*., 2012). Bunches were randomly selected from the vines on both sides of the canopy and each cultivar was harvested twice on two separate dates from each location. The respective distances from the vineyards to the laboratory in Stellenbosch are Kakamas 840 km, Wellington 42 km and Hex Valley 139 km. Grapes were harvested and packed in the morning before 10h00 and kept at 20 $^{o}$C during transport to the laboratory. A total of 338 grape bunches was scanned on the infrared spectrometer within twelve hours after harvest.

**Table 4.1** GPS co-ordinates, harvest week and TSS level of grapes.

| Cultivar | Site | Latitude | Longitude | Altitude | 2016 Harvest Week | 2017 Harvest Week | 2016 TSS[d] | 2017 TSS |
|---|---|---|---|---|---|---|---|---|
| Thompson Seedless | HV[a] | 33°27'53,9"S | 19°39'43,7"S | 907m | W3 | W4 | 16.85 | 15.64 |
| | | | | | W4 | W5 | Stolen | 16.62 |
| Thompson Seedless | W[b] | 33°37'03,5"S | 18°58'05,3"S | 904m | W3 | W3 | 17.49 | 18.72 |
| | | | | | W4 | W5 | 18.62 | Rotten |
| Regal Seedless | HV | 33°27'50,4"S | 19°39'47,6"E | 904m | W3 | W5 | 18.39 | 19.41 |
| | | | | | W5 | W5 | 21.27 | 21.36 |
| Regal Seedless | W | 33°30'14,2"S | 10°50'40,0"E | 904m | W3 | W4 | 15.47 | 14.12 |
| | | | | | W5 | W6 | 16.44 | 16.34 |
| Prime Seedless | W | 33°38'22,0"S | 10°50'47,6"E | 900m | W51 | | 10.65 | |
| | | | | | W52 | | 12.02 | |
| Prime Seedless | K[c] | 28°37'54,8"S | 20°26'38,6"E | 903m | W48 | | 14.89 | |
| | | | | | W50 | | 16.08 | |

[a]Hex Valley; [b]Wellington; [c]Kakamas;[d]Total soluble solids in °Brix measured with a handheld refractometer.

Table 4.2 shows the lowest, highest and average daily temperatures for the different locations taken from weather stations in the nearest vicinity of the blocks from which grapes were harvested from during the two seasons. These weather stations were Hex Valley PP with latitude = -33,46609, longitude = 19,66304 and altitude = 459 for Hex Valley; Eureka with latitude = -33,69301, longitude = 18,95259 and altitude = 161 for Wellington and Kromhout Boerdery with Latitude = -28,7869, Longitude 18,95259 and Altitude = 161 for Kakamas. The values in bold indicate where the daily average maximum and minimum temperatures were higher in the second season and the underlined values indicate where the daily average maximum and minimum temperatures were lower in the second season. The influence this had on the maturity and sensory parameters will be discussed further down in the manuscript.

**Table 4.2** Temperature data for the sites from which the grapes were harvested.

| Site | Month | Day | Tx[a] | Tn[b] | Tx | Tn | Tx | Tn |
|---|---|---|---|---|---|---|---|---|
| | | | 2015 | | 2016 | | 2017 | |
| HV[d] | 11 | Lowest | 19.75[c] | 6.38 | 21.83 | 3.32 | | |
| HV | 11 | Highest | 37.64 | 22.65 | 37.67 | 16.27 | | |
| HV | 11 | Average | 27.18 | 17.8 | **28.88** | 9.21 | | |
| HV | 12 | Lowest | 26.63 | 19.9 | 25.29 | 8.83 | | |
| HV | 12 | Highest | 35.37 | 28.42 | 39.99 | 16.38 | | |
| HV | 12 | Average | 30.23 | 23.65 | **33.01** | 11.92 | | |
| HV | 1 | Lowest | | | 33.36 | 26.1 | 24.5 | 8.62 |
| HV | 1 | Highest | | | 33.55 | 28.31 | 38.24 | 17.88 |
| HV | 1 | Average | | | 33.46 | 27.45 | 32.09 | 12,66 |
| HV | 2 | Lowest | | | 26.02 | 8.13 | 27.97 | 7.66 |
| HV | 2 | Highest | | | 39.91 | 19.6 | 38.02 | 18.15 |
| HV | 2 | Average | | | 31.46 | 12.29 | **33.29** | **12.85** |
| W[e] | 11 | Lowest | 17.55 | 8.02 | 20 | 9.55 | | |
| W | 11 | Highest | 38.66 | 20.05 | 35.54 | 20.05 | | |
| W | 11 | Average | 27.34 | 13.73 | **28.44** | **14.58** | | |
| W | 12 | Lowest | 23 | 12.44 | 21.41 | 12.6 | | |
| W | 12 | Highest | 41.09 | 22.34 | 36.99 | 19.87 | | |
| W | 12 | Average | 30.94 | 16.46 | **31.22** | 15.67 | | |
| W | 1 | Lowest | | | 24.26 | 15.82 | 23.35 | 12.91 |
| W | 1 | Highest | | | 39.97 | 25.3 | 38.34 | 20.48 |
| W | 1 | Average | | | 33.99 | 20.92 | 31.37 | 16.56 |
| W | 2 | Lowest | | | 25.14 | 12.5 | 24 | 14.08 |
| W | 2 | Highest | | | 38.41 | 24.52 | 40 | 24.64 |
| W | 2 | Average | | | 31.49 | 17.53 | **32.3** | **17.87** |
| K[f] | 11 | Lowest | | | 30.25 | 8.9 | | |
| K | 11 | Highest | | | 41.14 | 21.33 | | |
| K | 11 | Average | | | 36.15 | 14.37 | | |
| K | 12 | Lowest | | | 33.18 | 11.72 | | |
| K | 12 | Highest | | | 44.05 | 22.76 | | |
| K | 12 | Average | | | 38.71 | 16.97 | | |

[a]Daily Maximum Temperature; [b]Daily Minimum Temperature, [c]Unit=°C; [d]Hex Valley; [e]Wellington; [f]Kakamas

### 4.2.2 Fourier transform near-infrared spectroscopy

The laboratory measurement setup was designed so that diffuse reflectance FT-NIR spectra of intact table grape bunches were obtained in a contactless mode by using the MATRIX-F FT-NIR spectrometer connected via a fibre optic cable (1 m) to a NIR emission head (Bruker Optics,

Ettlingen, Germany), as shown in Figure 4.2. Each bunch was placed on the sample platform directly below four air-cooled tungsten NIR light sources (12V, 5W each) housed in the emission head (230 mm diameter, 185 mm height), and scanned individually. Upon illumination of the grapes, the diffuse reflected light was collected and guided back to the spectrometer by the optic cable. The focal point of the lights was 170 mm and the area illuminated on bunches was 80 mm in diameter. The detecting emission head also housed a sensitive, thermoelectric cooled and temperature-controlled InGaAs diode detector. The scanning procedure per sample took 40 s in which time 32 repeat scans (resolution, 2 $cm^{-1}$; scanner velocity, 10 kHz) were collected in the wavenumber range 800 to 2500 nm (12000 to 4000 cm-1), and averaged into a single absorbance spectrum using OPUS software (OPUS version 7.2 Bruker Optics, Ettlingen, Germany). OPUS works by default in wavenumbers thus 12000 to 4000 $cm^{-1}$. Each spectrum consisted of 1,801 data points. A background spectrum was collected using a spectralon in the same way prior to scanning the grape bunches and at hourly intervals during the operation of the spectrometer. The spectralon is situated on the sample platform and is covered with a black lid when the sample is being scanned. The Log (1/R) transformed absorbance spectra were processed using OPUS and saved after the spectral acquisition. Each bunch was scanned on two opposite sides, denoted top or bottom respectively, by turning the bunch manually.

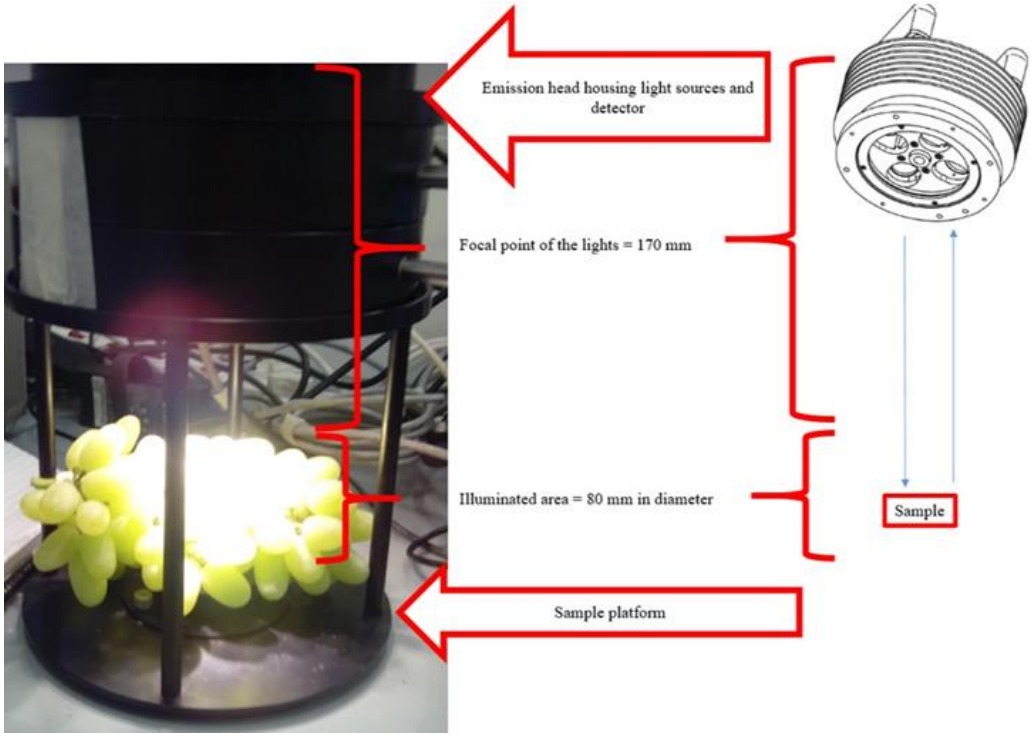



**Figure 4.2.** An intact Thompson Seedless table grape bunch scanned contactless with the MATRIX-F NIR spectrometer. Important parts of the instrument are also illustrated.

### 4.2.3 Reference measurements

A sampling of twenty grape berries (ten each bunch side - the top and bottom sides) from within the focus area of the NIR light sources (Figure 4.2) was done after the spectra of bunches were generated. Free flowing juice was collected by crushing the berries by hand, for 1 minute in a plastic bag, followed by filtration using cheesecloth. TSS of the juice was determined using a handheld digital refractometer (ATAGA Palete Digital Refractometer PR-32 Alpha, Tokyo, Japan). TA and pH were determined with a TIM 865 Titration Manager (Radiometer Analytical, Villeurbanne Cedex, France) automatic titrator. The TSS:TA ratio was calculated by dividing the TSS value of each juice sample by that of the percentage TA (°Brix ÷ %Acid) (Jayasena and Cameron, 2008). BrimA was calculated as TSS – k x TA. The constant k shows that the tongue is more sensitive to acid than it is to sugar. Due to different fruit containing different ratios of acids and sugars the k value range from 2 to 10. A k value of 5 is suggested for table grapes and was accordingly used in this study (Jordan et al., 2001).

The standard error of laboratory (SEL) for respectively, TSS (±0.03), TA (±0.05) and pH (±0.20) were based on those reported by the Wine Analytical Laboratory of the Agricultural Research Council, Infruitec-Nietvoorbij in Stellenbosch, South Africa where the samples were analyzed. Certified standards for each parameter were tested daily in triplicate. SEL was calculated as the average of the difference between the true value of the certified standard and the measured result (triplicate measurements). Grape samples were analysed once.

### 4.2.4 Data analysis

To investigate the relationship between the spectral information of the intact bunches and the content of TSS, TA, TSS:TA ratio, pH and BrimA, PLS regression was implemented in the R statistical environment (R Core Team, 2016) using the ‘pls’ package (Mevik *et al*., 2016). PLS is a bilinear modelling strategy (Naes *et al*. 2004) which was used to find the correlation between the spectra taken of the intact table grape bunches and the reference values that was obtained for the maturity parameters TSS, TA, TSS:TA ratio, pH and BrimA. The data matrix, therefore, consisted of a set of independent X variables (NIR spectral data) and five dependent Y variables TSS, TA, TSS:TA ratio, pH and BrimA.

Two strategies were used to design calibration and validation sample sets. In Strategy 1 as can be seen in Figure 4.1, a model was created with data from one year (2016) and tested on data from another year (2017). In Strategy 2 the calibration set and the validation sample sets consisted of randomly selected data from both years combined (2016 and 2017). In Strategy 1 the 2016 data (n=267) was used as the training set and the 2017 data (n=71) was used as the test set. In Strategy 2 the data sets for 2016 and 2017 were combined (n=338) and randomly divided into two sub-datasets, the training set containing 60% of the data (n=204) and

the testing set containing 40% of the total data set (n=134) for each parameter. A full cross-validation process was applied to build the PLS regression models using the training data set for each parameter

The regression models were evaluated using the coefficient of determination ($R^2$) and the Root Mean-Square Error of Calibration (RMSEC) or Validation (RMSECV when cross-validation is used and RMSEP when test set validation is used). The $R^2$ value, which represents the proportion of explained variance of the response variable in the calibration set ($R^2_c$) or validation set ($R^2_{cv}$ or $r^2$ when cross-validation is used and $R^2_p$ when test set validation is used). This value needs to be as high as possible for a good model. It differs from the correlation coefficient (r) which only shows how strong the relationship between two variables is (Taylor, 1990) and $R^2$ is a multiple of it (Nagelkerke, 1991). RMSECV is the term indicating the prediction error of the model and the RMSEP value gives the average expected uncertainty for predictions of future samples and both need to be as close as possible to zero (Saeys *et al*., 2005; Brown *et al*., 2005; Esbensen, 2006). The residual prediction deviation (RPD) value is defined as the ratio of the standard deviation of the reference data of the validation set to the standard error of prediction and gives some indication of the efficiency of a calibration (Williams and Norris, 2001). The RPD value has to be between 1.5 and 2 for the model to discriminate low from high values of the response variable; a value between 2 and 2.5 to indicate that course quantitative predictions are possible, and a value between 2.5 and 3 or above to show good and excellent prediction accuracy (Saeys *et al*., 2005). The standard error of calibration (SEC); standard error of performance (SEP); limit control for SEP (LC_SEP) and limit control for bias (LC_bias) were also calculated. The SEC and SEP, as well as the control limits, also have to be as close as possible to zero to give good working models.

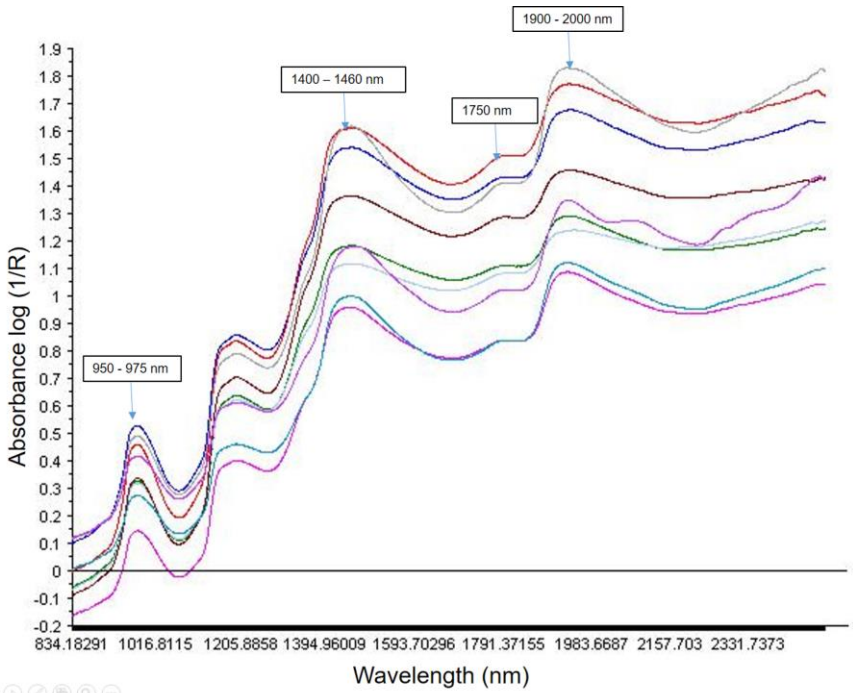
Further, the original data (no spectral pre-processing), as well as five spectral pre-processing techniques, were evaluated for each parameter when the models were built. These were baseline correction, multiplicative scattering correction (MSC), standard normal variate (SNV) moving window smoothing (MWS) and Savitzky-Golay first derivative (SG1d) (Rinnan *et al*., 2009). A combination of each of the last three spectral pre-processing techniques were used in combination with MSC as follows MSW+MSC, SNV+MSC and SG1d+MSC.

## 4.3 RESULTS AND DISCUSSION

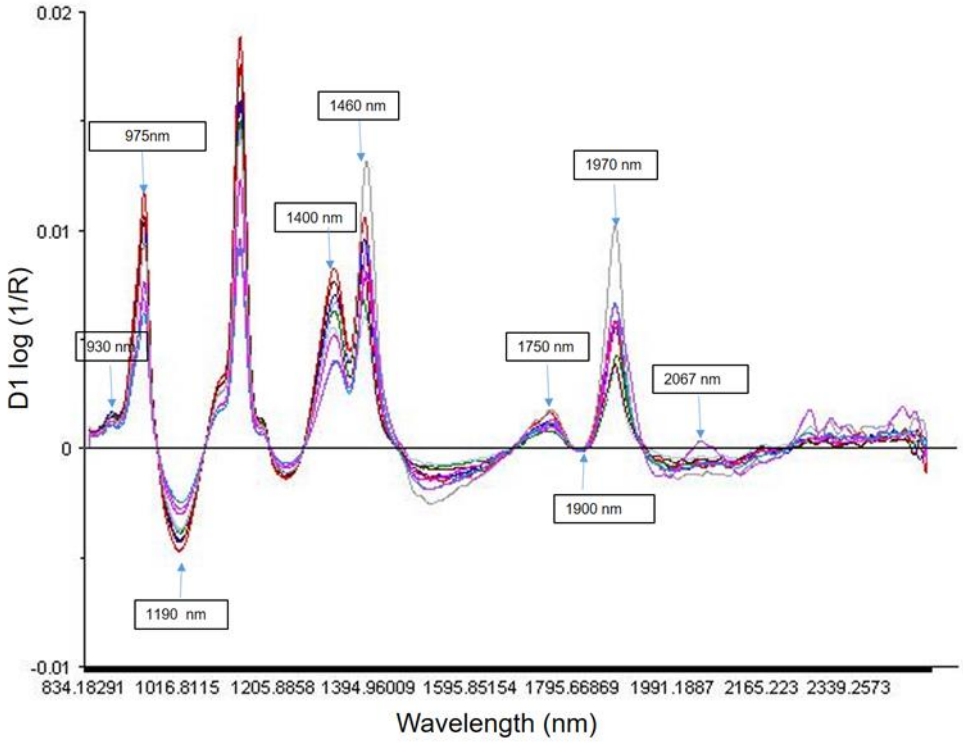
### 4.3.1 Intact bunch spectral features

In Figure 4.3 the characteristic log (1/R) spectra of intact bunches (A) and the spectral pre-processed spectra (B) are displayed. Similar as in González-Caballero *et al*. (2010) the first derivative of the spectra was taken and the effect can clearly be seen through the overlapping absorption bands being separated and absorbance peaks being displayed more clearly (B). The

near infrared region contains absorption bands corresponding to overtones and combinations of fundamental CH, OH and NH vibrations (Nogales-Bueno et al., 2017). Water-related absorption bands are also found at around 950 and 1460 nm, which are related to the third overtone of OH, as is usually the case for fruits and vegetables, particularly grapes which has 70–80% water (González-Caballero *et al*., 2010). Many other authors also mention several peaks due to absorption by water and carbohydrate related to the combination bands of OH in water such as the obvious peak centered at 975 nm (Cao et al., 2010), the peaks at 1050 nm and 1400 nm (Chen et al., 2015). González-Caballero et al. (2012) also found sugar-related overtones at around 1,750 and 2,067 nm and with water peaks at around 1,900 and 1,970 nm when they used NIR spectroscopy for on-vine monitoring of grape ripening. Kemps et al. (2010) found the first peak around 1190 nm (a negative peak) that is observed corresponding to an increase in absorption with increasing sugar concentration (OH) in case of Syrah when they assessed the quality parameters in grapes using VIS/NIR spectroscopy and that sugar related phenomena contributed to the prediction of OH. Thus in case of grapes this peak can be attributed to sugars. Omar, (2013) also mentions the 910 nm (CH band) and 950 (OH band) as the most important wavelengths for prediction of SSC in grapes and star fruit and the wavelengths 922-923 nm and 990-995 nm for pH prediction. In a study by Omar et al. (2012) looking at the NIR spectroscopic properties of aqueous acids solutions they managed to quantify the pH of aqueous acids solutions, citric, tartaric, malic and oxalic, (organic acids that are naturally present in grapes) through the application of NIR spectroscopy between 700 nm and 1,000 nm. The most important water absorbance wavelength in their study was also 975 nm, for the pH measurement with the most important wavelengths located at 918–925 nm and 990–996 nm and the peak response wavelength for citric acid at 850 nm, tartaric at 900 nm and 960 nm, malic at 965 nm and oxalic at 850 nm. As can be seen in Figure 4.3 A it is very difficult to discern all of these peaks in the log (1/R) spectra, but most of them peaks can be distinguished very clearly in the Savitzky-Golay First Derivative (SG1d) spectra in Figure 4.3 B.




(A)




(B)

**Figure 4.3** The log (1/R) spectra of intact bunches (A) and spectra of intact bunches after Savitzky-Golay First Derivative (SG1d) spectral pre-processing was applied (B).

### 4.3.2 Reference data statistics

A large portion of the soluble solids in grapes is sugars that account for more than 90% of TSS at harvest (Muñoz-Robredo *et al*., 2011). Kliewer (1967) found that the range of TSS in mature grapes varied widely from 13.7 to 31.5°Brix. Table 4.3 shows the statistical analysis of training sample sets of 2016 and 2017 respectively for all the parameters (Strategy 1) and Table 4.4

shows them for the training set and the testing set when the two years are combined (Strategy 2). In Strategy 2 the training set contains two-thirds of the data (n=204) and the testing set contains one-third of the data (n=137). The minimum value was 10.18 °Brix in 2016 and 6.58 °Brix in 2017. This was exceptionally low particularly in 2017 given that the intended TSS that the grapes were to be harvested at was 14.0 °Brix for Prime Seedless and 16.0 °Brix for Regal Seedless and Thompson Seedless according to the standards and requirements regarding control of the export of table grapes (ACT No. 119 OF 1990 of South Africa). However, when the mean (17.59 °Brix in 2016 and 15.62 °Brix), as well as the range values (14.22 °Brix in 2016 and 15.60 °Brix in 2017), are considered, they seem to be on par with the standards. Both the standard deviations (SD) and coefficients of variation (CV) values were higher in 2016 (2.37 and 0.13 respectively) compared to 2017 (3.75 and 0.24) as shown in Table 4.3. The trend repeats in Table 4.4 when the combined two years and the training and testing sets selection is random. Harvesting of the grapes was at two stages in both years. The second harvest is at a higher TSS level shown by the maximum values contained in Table 4.3 (24.40 °Brix in 2016 and 22.18 °Brix in 2017). This might explain the high coefficient of variation (CV) values as was the case when González-Caballero *et al*. (2010) also harvested the grapes in their study over different ripening periods.

Grapes also contain significant amounts of organic acids. These are very important components of grape juice since they are responsible for the tart taste and have a marked influence on juice stability, colour and pH (Fahmi *et al*., 2012). During berry development, TA usually decreases as TSS increases. The juice pH is a measure of the hydrogen ion concentration in the berry generally related to juice acidity. Although there is no direct relationship between TA and pH, higher acid levels in fruit are often associated with lower pH values and vice versa as can be seen in Table 4.3 especially in terms of the maximum values in 2016 (TA = 7.62 g/L and pH = 4.07) and 2017 (TA = 10.99 g/L and pH = 4.29). The juice pH of Thompson Seedless grapes usually ranges between 3.5 and 3.9 at harvest. Vial *et al*. (2005) obtained a mean of 3.46 in the experiments they conducted and Fahmi *et al*. (2012) one of 4.05 in theirs. This falls within the range obtained in 2016 (4.73 g/L) but not in 2017 (8.02 g/L) and in Strategy 2 (8.10 g/L and 7.39 g/L respectively for the training and testing sets). This is due to the higher minimum (2.97 g/L) and maximum (10.99 g/L) values that were obtained in 2017. This highlights a very significant effect that seasons can have on the development of grapes as could also be clearly seen in the minimum and maximum values of TSS which were lower in 2017 (6.58°Brix and 22.18° Brix) than in 2016 (10.18°Brix and 24.40°Brix). The temperature difference between these two seasons (Table 4.2) probably played a role with the average maximum daily temperatures being mostly higher and the average minimum temperature being mostly lower during the ripening and harvest period. All metabolic processes in plants such as photosynthesis responsible for carbohydrate manufacturing (TSS) are temperature-dependent (Berry and Björkman, 1980).

According to the South African standards and requirements regarding control of the export of table grapes (ACT No. 119 OF 1990), the acceptable TSS:TA ratio values for table grapes are 22 for Prime Seedless, 24 for Regal Seedless and 21 for Thompson Seedless. When the minimum values are considered, they are below these and the maximum values are above these, with the mean value being much higher in 2016 (39.24) than in 2017 (29.01) and the range the other way around for the two years 49.20 in 2016 and 59.21 in 2017. Jayasena *et al*. (2008) obtained values of up to 40 for Crimson Seedless in their study similar to the mean of this study in 2016.

BrimA is not a widely used parameter for table grapes and has only thus far been proposed by Jordan *et al*. (2001) and evaluated by Jayasena *et al*. (2008) who found that it could not give better predictive results for the sensory qualities of Crimson Seedless table grapes than what TSS:TA ratio could. However, BrimA has been reported as a valuable maturity index and quality parameter for a wide range of fruits including mango (Wongkhot *et al*., 2012), pomegranate (Fawole and Opara, 2013, Arendse *et al*., 2014), citrus (Ncama *et al*., 2017) and grapefruit (Olarewaju *et al*., 2018). The acceptable minimum and maximum values as well as median and ranges is, therefore, still to be established and may differ from the ones achieved in Table 4.3 and 4 when other table grape cultivars are added.

**Table 4.3** Statistical analysis of sample sets for the table grape quality parameters TSS, TA, TSS:TA ratio, pH and BrimA under study collected in the 2016 and 2017 harvesting seasons to incorporate seasonal changes

| Training | 2016 | | | | | 2017 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Statistic | | | | | | | | | | |
| Parameter | TSS[a] | TA[b] | TSS/TA Ratio | pH | BrimA | TSS | TA | TSS/TA Ratio | pH | BrimA |
| N | 267 | 267 | 267 | 267 | 267 | 71 | 71 | 71 | 71 | 71 |
| Mean | 17.59 | 4.67 | 39.24 | 3.78 | 5.82 | 15.62 | 6.15 | 29.01 | 3.78 | 12.55 |
| Median | 17.54 | 4.40 | 38.66 | 3.77 | 5.88 | 15.70 | 5.55 | 27.67 | 3.74 | 12.45 |
| Min[c] | 10.18 | 2.89 | 15.08 | 3.31 | 2.63 | 6.58 | 2.97 | 6.93 | 3.36 | 1.83 |
| Max[d] | 24.40 | 7.62 | 64.28 | 4.07 | 10.43 | 22.18 | 10.99 | 66.14 | 4.29 | 19.58 |
| Range | 14.22 | 4.73 | 49.20 | 0.76 | 7.80 | 15.60 | 8.02 | 59.21 | 0.93 | 17.75 |
| Standard Deviation | 2.37 | 0.90 | 9.66 | 0.14 | 1.35 | 3.75 | 2.05 | 13.45 | 0.22 | 4.20 |
| Coefficient of Variation | 0.13 | 0.19 | 0.25 | 0.04 | 0.23 | 0.24 | 0.33 | 0.46 | 0.06 | 0.33 |

[a]Total soluble solids, [b]Titratable acidity, [c]Minimum, [d]Maximum

**Table 4.4** Statistical analysis of randomly selected training (two-thirds of data) and test (one-third of data) sets for the combined 2016 and 2017 data sets of the table grape quality parameters TSS, TA, TSS:TA ratio, pH and BrimA under study

| Training Statistic | Training set | | | | | Testing set | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | TSS[a] | TA[b] | TSS/TA Ratio | pH | BrimA | TSS | TA | TSS/TA Ratio | pH | BrimA |
| N | 204.00 | 204.00 | 204.00 | 204.00 | 204.00 | 134.00 | 134.00 | 134.00 | 134.00 | 134.00 |
| Mean | 17.07 | 4.99 | 36.89 | 3.78 | 7.11 | 17.17 | 4.89 | 36.57 | 3.78 | 7.18 |
| Median | 17.45 | 4.62 | 37.40 | 3.77 | 6.23 | 17.40 | 4.64 | 37.18 | 3.77 | 6.23 |
| Min[c] | 6.58 | 2.89 | 6.93 | 3.31 | 2.63 | 6.58 | 2.89 | 6.93 | 3.34 | 2.63 |
| Max[d] | 24.40 | 10.99 | 66.14 | 4.29 | 19.32 | 22.96 | 10.28 | 61.92 | 4.29 | 18.63 |
| Range | 17.82 | 8.10 | 59.21 | 0.98 | 16.69 | 16.38 | 7.39 | 54.99 | 0.95 | 16.00 |
| Standard Deviation | 2.94 | 1.41 | 11.47 | 0.16 | 3.35 | 2.94 | 1.13 | 11.21 | 0.16 | 3.49 |
| Coefficient of Variation | 0.17 | 0.28 | 0.31 | 0.04 | 0.47 | 0.17 | 0.23 | 0.31 | 0.04 | 0.49 |

[a]Total soluble solids, [b]Titratable acidity, [c]Minimum, [d]Maximum

### 4.3.3 Performance of calibration models

Daniels *et al*. (2018) showed that the best calibration models were obtained when the average spectra of table grape bunches were used to construct the respective models. Table 4.5 shows the results of the calibration models for TSS, TA, TSSTA ratio, pH and BrimA (Strategy 1). Table 4.6 shows the results for the same parameters but built using Strategy 2. Construction of models was with data of the original spectra as well as the baseline-corrected spectra, but only results of the models with the original spectra are shown since they always performed better. The best model was selected in terms of which spectral pre-processing technique or combination of techniques gave the most appropriate values for the statistics used to measure the strength of the model. The model in which the lowest number of LV was used was chosen as the best.

**Table 4.5** Performance of PLS models for table grape quality parameters using 2016 data as the training set (n=267) and 2017 as the testing set (n=71). Also shown are the pre-processing techniques that gave the best model.

| Pre-processing technique | Average | MSC[a] | MSW[b] | MSW-MSC | SG[c] | SG-MSC | SNV[d] | SNV-MSC |
|---|---|---|---|---|---|---|---|---|
| | | | | TSS[e] | | | | |
| LV[f] | 21 | 18 | 22 | 21 | 9 | **6** | 23 | 22 |
| $R^2_c$[g] | 0.93 | 0.92 | 0.92 | 0.95 | 0.93 | **0.85** | 0.94 | 0.95 |
| $R^2_{cv}$[h] | 0.84 | 0.80 | 0.80 | 0.88 | 0.80 | **0.73** | 0.81 | 0.82 |
| $R^2_p$[i] | 0.82 | 0.81 | 0.80 | 0.71 | 0.77 | **0.81** | 0.82 | 0.81 |
| Sec[j] | 0.74 | 0.78 | 0.82 | 0.61 | 0.77 | **1.08** | 0.64 | 0.62 |
| Sep[k] | 1.28 | 1.34 | 1.21 | 1.50 | 1.32 | **1.29** | 1.29 | 1.25 |
| LC_Sep[l] | 0.96 | 1.01 | 1.06 | 0.79 | 0.99 | **1.40** | 0.83 | 0.81 |
| LC_bias[m] | 0.44 | 0.47 | 0.49 | 0.36 | 0.46 | **0.65** | 0.38 | 0.37 |
| $RMSE_c$ (°Brix)[n] | 0.73 | 0.78 | 0.82 | 0.61 | 0.76 | **1.08** | 0.64 | 0.62 |
| $RMSE_p$ (°Brix)[o] | 1.28 | 1.34 | 1.21 | 1.52 | 1.31 | **1.30** | 1.29 | 1.25 |
| $RPD_c$[p] | 0.94 | 3.52 | 3.62 | 4.72 | 3.80 | **2.59** | 4.26 | 4.50 |
| $RPD_p$[q] | 0.95 | 2.04 | 2.45 | 1.89 | 2.21 | **2.14** | 2.10 | 2.25 |
| | | | | TA[r] | | | | |
| LV | 23 | 9 | 30 | 14 | 8 | **4** | 15 | 15 |
| $R^2_c$ | 0.80 | 0.40 | 0.93 | 0.62 | 0.79 | **0.63** | 0.62 | 0.68 |
| $R^2_{cv}$ | 0.47 | 0.19 | 0.43 | 0.37 | 0.37 | **0.44** | 0.26 | 0.35 |
| $R^2_p$ | 0.33 | 0.34 | 0.31 | 0.30 | 0.46 | **0.43** | 0.43 | 0.42 |
| Sec | 0.67 | 0.98 | 0.36 | 0.83 | 0.61 | **0.80** | 0.81 | 0.75 |
| Sep | 1.08 | 1.22 | 1.33 | 1.22 | 1.18 | **1.09** | 1.10 | 1.11 |
| LC_Sep | 0.87 | 1.27 | 0.47 | 1.08 | 0.79 | **1.03** | 1.05 | 0.98 |
| LC_bias | 0.40 | 0.59 | 0.22 | 0.50 | 0.36 | **0.48** | 0.49 | 0.45 |
| $RMSE_c$ | 0.67 | 0.98 | 0.36 | 0.83 | 0.60 | **0.79** | 0.81 | 0.75 |
| $RMSE_p$ | 1.09 | 1.23 | 1.33 | 1.21 | 1.19 | **1.09** | 1.11 | 1.12 |
| $RPD_c$ | 2.26 | 1.30 | 3.72 | 1.62 | 2.22 | **1.66** | 1.62 | 1.77 |
| $RPD_p$ | 1.38 | 1.03 | 1.01 | 1.10 | 1.12 | **1.21** | 1.19 | 1.19 |
| | | | | TSS:TA ratio[s] | | | | |
| LV | 19 | 10 | 11 | 11 | 5 | **5** | 11 | 12 |
| $R^2_c$ | 0.78 | 0.67 | 0.65 | 0.65 | 0.76 | **0.70** | 0.69 | 0.67 |
| $R^2_{cv}$ | 0.53 | 0.56 | 0.56 | 0.55 | 0.61 | **0.53** | 0.61 | 0.55 |
| $R^2_p$ | 0.63 | 0.49 | 0.53 | 0.52 | 0.57 | **0.66** | 0.49 | 0.59 |
| Sec | 5.37 | 6.72 | 6.84 | 6.88 | 5.31 | **5.84** | 6.50 | 6.57 |
| Sep | 6.96 | 7.81 | 7.63 | 7.67 | 7.86 | **7.10** | 8.09 | 7.42 |
| LC_Sep | 6.98 | 8.74 | 8.89 | 8.95 | 6.91 | **7.59** | 8.45 | 8.54 |
| LC_bias | 3.22 | 4.03 | 4.10 | 4.13 | 3.19 | **3.50** | 3.90 | 3.94 |
| $RMSE_c$ | 5.35 | 6.70 | 6.82 | 6.87 | 5.30 | **5.83** | 6.48 | 6.55 |
| $RMSE_p$ | 6.96 | 7.97 | 7.66 | 7.64 | 7.83 | **7.08** | 8.12 | 7.50 |
| $RPD_c$ | 2.14 | 1.74 | 1.70 | 1.70 | 2.05 | **1.84** | 1.82 | 1.74 |
| $RPD_p$ | 1.64 | 1.46 | 1.51 | 1.53 | 1.39 | **1.52** | 1.45 | 1.52 |
| | | | | pH | | | | |
| LV | 11 | 9 | 9 | 11 | 7 | **5** | 10 | 9 |
| $R^2_c$ | 0.34 | 0.29 | 0.29 | 0.34 | 0.66 | **0.51** | 0.33 | 0.27 |
| $R^2_{cv}$ | 0.08 | 0.11 | 0.09 | 0.08 | 0.21 | **0.21** | 0.12 | 0.07 |
| $R^2_p$ | 0.12 | 0.20 | 0.14 | 0.13 | 0.28 | **0.27** | 0.17 | 0.20 |
| Sec | 0.13 | 0.14 | 0.13 | 0.14 | 0.09 | **0.11** | 0.13 | 0.13 |
| Sep | 0.16 | 0.14 | 0.16 | 0.14 | 0.14 | **0.14** | 0.15 | 0.15 |

| Pre-processing technique | Average | MSC | MSW | MSW-MSC | SG | **SG-MSC** | SNV | SNV-MSC |
|---|---|---|---|---|---|---|---|---|
| LC_Sep | 0.17 | 0.18 | 0.17 | 0.18 | 0.12 | **0.15** | 0.17 | 0.17 |
| LC_bias | 0.08 | 0.08 | 0.08 | 0.08 | 0.06 | **0.07** | 0.08 | 0.08 |
| $RMSE_c$ | 0.13 | 0.14 | 0.13 | 0.14 | 0.09 | **0.11** | 0.13 | 0.13 |
| $RMSE_p$ | 0.16 | 0.14 | 0.16 | 0.14 | 0.14 | **0.14** | 0.15 | 0.15 |
| $RPD_c$ | 1.24 | 1.19 | 1.19 | 1.24 | 1.72 | **1.44** | 1.23 | 1.17 |
| $RPD_p$ | 1.04 | 1.14 | 1.00 | 1.23 | 1.13 | **1.16** | 1.12 | 0.99 |
| | | | | BrimA | | | | |
| LV | 25 | 23 | 28 | 24 | 10 | **10** | 22 | 22 |
| $R^2_c$ | 0.93 | 0.95 | 0.95 | 0.95 | 0.94 | **0.92** | 0.93 | 0.95 |
| $R^2_{cv}$ | 0.74 | 0.82 | 0.78 | 0.78 | 0.77 | **0.72** | 0.79 | 0.76 |
| $R^2_p$ | 0.83 | 0.81 | 0.75 | 0.77 | 0.71 | **0.77** | 0.80 | 0.85 |
| Sec | 0.97 | 0.83 | 0.80 | 0.75 | 0.93 | **0.95** | 0.98 | 0.78 |
| Sep | 1.44 | 1.52 | 1.81 | 1.81 | 1.80 | **1.75** | 1.46 | 1.49 |
| LC_Sep | 1.26 | 1.08 | 1.04 | 0.98 | 1.21 | **1.24** | 1.27 | 1.01 |
| LC_bias | 0.58 | 0.50 | 0.48 | 0.45 | 0.56 | **0.57** | 0.59 | 0.47 |
| $RMSE_c$ | 0.97 | 0.83 | 0.80 | 0.75 | 0.93 | **0.95** | 0.97 | 0.77 |
| $RMSE_p$ | 1.44 | 1.51 | 1.81 | 1.80 | 1.80 | **1.76** | 1.50 | 1.49 |
| $RPD_c$ | 3.73 | 4.39 | 4.43 | 4.57 | 3.97 | **3.65** | 3.88 | 4.33 |
| $RPD_p$ | 2.51 | 2.40 | 1.96 | 1.90 | 2.05 | **1.98** | 2.52 | 2.24 |

[a]Multiplicative scatter correction, [b]Moving smoothing windows, [c]Savitzky-Golay first derivative, [d]Standard Normal Variate, [e]Total soluble solids, [f]Latent variables, [g]Coefficient of determination for the calibration set, [h]Coefficient of determination for cross validation, [i]Coefficient of determination for prediction, [j]Standard error of calibration, [k]Standard error of performance, [l]Limit control for Sep (LC_Sep), [m]Limit control for bias, [n]Root mean square error of calibration, [o]Root mean square error for prediction, [p]Residual prediction deviation for calibration, [q]Residual prediction deviation for prediction, [r]Titratable acidity, [s]TSS:TA ratio.

**Table 4.6** Performance of PLS models for table grapes quality parameters of randomly selected training (n=204) and test set (n=134) samples of the combined 2016 and 2017 data. Also shown are the pre-processing techniques that gave the best model.

| Pre-processing technique | Average | MSC[a] | MSW[b] | MSW-MSC | SG[c] | SG-MSC | SNV[d] | SNV-MSC |
|---|---|---|---|---|---|---|---|---|
| | | | | TSS[e] | | | | |
| LV[f] | 21 | 19 | 20 | 20 | 10 | **9** | 20 | 19 |
| $R^2_c$[g] | 0.90 | 0.92 | 0.90 | 0.91 | 0.91 | **0.90** | 0.92 | 0.92 |
| $R^2_{cv}$[h] | 0.85 | 0.83 | 0.84 | 0.83 | 0.78 | **0.77** | 0.83 | 0.83 |
| $R^2_p$[i] | 0.73 | 0.70 | 0.74 | 0.72 | 0.39 | **0.44** | 0.71 | 0.70 |
| Sec[j] | 0.73 | 0.68 | 0.76 | 0.70 | 0.69 | **0.75** | 0.68 | 0.68 |
| Sep[k] | 2.05 | 2.11 | 2.02 | 2.05 | 2.95 | **2.86** | 2.09 | 2.10 |
| LC_Sep[l] | 0.95 | 0.89 | 0.99 | 0.90 | 0.90 | **0.98** | 0.88 | 0.88 |
| LC_bias[m] | 0.44 | 0.41 | 0.46 | 0.42 | 0.42 | **0.45** | 0.41 | 0.41 |
| $RMSE_c$[n] | 0.73 | 0.68 | 0.76 | 0.69 | 0.69 | **0.75** | 0.68 | 0.68 |
| $RMSE_p$[o] | 2.21 | 10.29 | 2.18 | 8.26 | 3.26 | **3.22** | 2.18 | 2.20 |
| $RPD_c$[p] | 3.26 | 3.49 | 3.13 | 3.41 | 3.42 | **3.15** | 3.51 | 3.49 |
| $RPD_p$[q] | 1.07 | 0.23 | 1.09 | 0.29 | 0.73 | **0.74** | 1.09 | 1.08 |
| | | | | TA[r] | | | | |
| LV | 21 | 18 | 19 | 18 | 5 | **5** | 18 | 18 |
| $R^2_c$ | 0.62 | 0.65 | 0.57 | 0.62 | 0.52 | **0.53** | 0.66 | 0.65 |
| $R^2_{cv}$ | 0.26 | 0.36 | 0.32 | 0.35 | 0.38 | **0.36** | 0.32 | 0.27 |
| $R^2_p$ | 0.11 | 0.18 | 0.29 | 0.19 | 0.04 | **0.06** | 0.16 | 0.18 |
| Sec | 0.55 | 0.53 | 0.59 | 0.55 | 0.62 | **0.61** | 0.52 | 0.53 |
| Sep | 1.95 | 1.87 | 1.88 | 1.87 | 2.00 | **1.98** | 1.89 | 1.87 |
| LC_Sep | 0.72 | 0.69 | 0.76 | 0.72 | 0.81 | **0.80** | 0.67 | 0.69 |
| LC_bias | 0.33 | 0.32 | 0.35 | 0.33 | 0.37 | **0.37** | 0.31 | 0.32 |
| $RMSE_c$ | 0.55 | 0.53 | 0.58 | 0.55 | 0.62 | **0.61** | 0.52 | 0.53 |
| $RMSE_p$ | 2.45 | 2.67 | 2.32 | 3.35 | 2.44 | **2.41** | 2.51 | 2.42 |
| $RPD_c$ | 1.62 | 1.70 | 1.53 | 1.62 | 1.44 | **1.47** | 1.73 | 1.70 |
| $RPD_p$ | 0.37 | 0.33 | 0.39 | 0.27 | 0.37 | **0.37** | 0.36 | 0.37 |
| | | | | TSS:TA ratio[s] | | | | |
| LV | 21 | 16 | 21 | 18 | 6 | **5** | 20 | 17 |
| $R^2_c$ | 0.63 | 0.59 | 0.61 | 0.61 | 0.55 | **0.53** | 0.67 | 0.62 |
| $R^2_{cv}$ | 0.32 | 0.37 | 0.30 | 0.36 | 0.37 | **0.37** | 0.32 | 0.30 |
| $R^2_p$ | 0.33 | 0.11 | 0.41 | 0.26 | 0.15 | **0.17** | 0.14 | 0.29 |
| Sec | 5.83 | 6.21 | 6.05 | 6.01 | 6.47 | **6.59** | 5.50 | 5.97 |
| Sep | 11.26 | 12.61 | 10.58 | 11.65 | 12.36 | **12.19** | 12.55 | 11.43 |
| LC_Sep | 7.58 | 8.07 | 7.86 | 7.81 | 8.41 | **8.57** | 7.15 | 7.77 |
| LC_bias | 3.50 | 3.72 | 3.63 | 3.61 | 3.88 | **3.96** | 3.30 | 3.58 |
| $RMSE_c$ | 5.82 | 6.19 | 6.03 | 6.00 | 6.46 | **6.58** | 5.49 | 5.96 |
| $RMSE_p$ | 15.07 | 15.12 | 13.19 | 17.54 | 15.17 | **14.53** | 19.86 | 15.40 |
| $RPD_c$ | 1.66 | 1.56 | 1.60 | 1.61 | 1.50 | **1.47** | 1.76 | 1.62 |
| $RPD_p$ | 0.64 | 0.64 | 0.73 | 0.55 | 0.64 | **0.66** | 0.49 | 0.63 |
| | | | | pH | | | | |
| LV | 13 | 16 | 16 | 11 | 4 | **4** | 11 | 11 |
| $R^2_c$ | 0.29 | 0.62 | 0.32 | 0.25 | 0.31 | **0.31** | 0.30 | 0.27 |
| $R^2_{cv}$ | 0.04 | 0.30 | 0.05 | 0.08 | 0.16 | **0.17** | 0.07 | 0.06 |
| $R^2_p$ | 0.09 | 0.29 | 0.09 | 0.09 | 0.07 | **0.05** | 0.11 | 0.09 |
| Sec | 0.12 | 5.97 | 0.12 | 0.12 | 0.12 | **0.12** | 0.12 | 0.12 |
| | Average | MSC | MSW | MSW-MSC | SG | **SG-MSC** | SNV | SNV-MSC |

| Pre-processing technique | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sep | 0.21 | 11.43 | 0.21 | 0.21 | 0.21 | **0.21** | 0.21 | 0.21 |
| LC_Sep | 0.16 | 7.77 | 0.15 | 0.16 | 0.15 | **0.15** | 0.16 | 0.16 |
| LC_bias | 0.07 | 3.58 | 0.07 | 0.07 | 0.07 | **0.07** | 0.07 | 0.07 |
| $RMSE_c$ | 0.12 | 5.96 | 0.12 | 0.12 | 0.12 | **0.12** | 0.12 | 0.12 |
| $RMSE_p$ | 0.22 | 15.40 | 0.21 | 1.01 | 0.21 | **0.21** | 0.21 | 0.21 |
| $RPD_c$ | 1.19 | 1.62 | 1.22 | 1.16 | 1.21 | **1.21** | 1.20 | 1.17 |
| $RPD_p$ | 0.64 | 0.63 | 0.67 | 0.14 | 0.68 | **0.67** | 0.68 | 0.67 |
| | | | | BrimA | | | | |
| LV | 11 | 11 | 11 | 11 | 6 | **4** | 14 | 12 |
| $R^2_c$ | 0.30 | 0.27 | 0.28 | 0.31 | 0.43 | **0.34** | 0.38 | 0.34 |
| $R^2_{cv}$ | 0.16 | 0.06 | 0.15 | 0.14 | 0.18 | **0.19** | 0.13 | 0.13 |
| $R^2_p$ | 0.01 | 0.09 | 0.01 | 0.03 | 0.11 | **0.05** | 0.12 | 0.04 |
| Sec | 1.13 | 0.12 | 1.14 | 1.12 | 1.02 | **1.10** | 1.06 | 1.10 |
| Sep | 4.16 | 0.21 | 4.15 | 4.48 | 4.02 | **4.10** | 4.69 | 4.57 |
| LC_Sep | 1.47 | 0.16 | 1.48 | 1.46 | 1.33 | **1.43** | 1.38 | 1.43 |
| LC_bias | 0.68 | 0.07 | 0.68 | 0.67 | 0.61 | **0.66** | 0.64 | 0.66 |
| $RMSE_c$ | 1.13 | 0.12 | 1.14 | 1.12 | 1.02 | **1.09** | 1.06 | 1.10 |
| $RMSE_p$ | 8.26 | 0.21 | 8.31 | 4.53 | 7.99 | **8.03** | 8.35 | 8.17 |
| $RPD_c$ | 1.20 | 1.17 | 1.19 | 1.20 | 1.33 | **1.23** | 1.28 | 1.23 |
| $RPD_p$ | 0.16 | 0.67 | 0.16 | 0.30 | 0.17 | **0.17** | 0.16 | 0.17 |

[a]Multiplicative scatter correction, [b]Moving smoothing windows, [c]Savitzky-Golay first derivative, [d]Standard Normal Variate, [e]Total soluble solids, [f]Latent variables, [g]Coefficient of determination for the calibration set, [h]Coefficient of determination for cross validation, [i]Coefficient of determination for prediction, [j]Standard error of calibration, [k]Standard error of performance, [l]Limit control for Sep (LC_Sep), [m]Limit control for bias, [n]Root mean square error of calibration, [o]Root mean square error for prediction, [p]Residual prediction deviation for calibration, [q]Residual prediction deviation for prediction, [r]Titratable acidity, [s]TSS:TA ratio.

### 4.3.4 TSS, TA. TSS:TA ratio, pH and BrimA

The best predictive results for all the parameters was obtained with the combination SG1d+MSC as spectral pre-processing technique with both strageties. When Gonzalez-Caballero *et al*. (2010) scanned whole wine grape bunches to assess the SSC the authors obtained value of 0.57 and higher SEP, LC_SEP and LC_bias values of 1.63, 0.62, and 1.35 respectively. Cao *et al*. (2010) found r and RMSEP of 0.91 and 0.96 for SSC and similarly did Baiano *et al*. (2012) and Omar (2013) who found $R^2$ and RMSE of 0.94 and 0.06 and 0.95 and 0.18 respectively. The $R^2$ value is higher and the RMSE values lower because they scanned single table grape berries and not intact table grape bunches as in this study. This is also clearly illustrated in the study of Parpinello *et al*. (2013) that found values for $r^2$ = 0.85, RMSECV = 1.08, SECV = 1.08 and RPD = 2.6 when using cross-validation instead of test set validation. The data in all the other experiments were also collected from a single year and not over two years as in this study.

Baiano *et al*. (2012) found the $R^2$ and RMSE to be 0.95 and 0.06 for TA using 5 LV for the construction of their models. In this study 4 LV were used in Strategy 1 and 5 LV in Strategy 2.

González-Caballero *et al*. (2010) also scanned intact bunches for amongst others SSC, TA and pH, but it was of wine grapes and the physiology of wine grape bunches are different from those of table grapes. Wine grape bunches and berries are much smaller than those of table grapes and the berries are also situated much closer together (more compact) than table grape bunches. Table grape bunches tend to be looser due to not only having longer pedicels, but also due to the bunch preparation that were done on them such as thinning and removal of small and uneven berries before harvesting.

Cao *et al*. (2010) found r and RMSEP were 0.98 and 0.13 for pH, and 0.91 and 0.96 for SSC respectively in the prediction set. Baiano *et al*. (2012) found the pH validation values for $R^2$ and RMSE to be 0.80 and 0.06 and Omar (2013) found $R^2$ = 0.763 and RMSE = 0.11. Gonzalez-Caballero *et al*. (2010) made use of test set validation and found the best predictive values for pH ($r^2$ = 0.51, SEP = 0.19, LC_BIAS = 0.06, LC_SEP = 0.13). These values were similar to those in this study except the $r^2$ that was lower (0.28).

The RPD values obtained for the TSS and BrimA models in Strategy 1 were the highest overall and indicated that these models will be able to discriminate low from high values of the response variable (Saeys *et al*., 2005). For TA and pH, this value indicated that the models are not ready yet to be used for discrimination purposes since it is below 1.5 (Saeys *et al*., 2005) in Strategy 1. The TSS:TA ratio model for Strategy 1, however, had a RPD value of 1.52, indicating its ability for use in discriminating low from gigh values for this parameter. All the RPD values for Strategy 2 were below 1. RPD was rarely reported in the published literature as a statistic to evaluate the strength of calibration models for the parameters of interest. Parpinello *et al*. (2013) reported a RPD value of 2.6 for SSC for single table grape berries. In the present work on intact bunches, higher RPD values for the calibration stage ($RPD_c^p$) were obtained for TSS (2.59 in Table 4.5 for Strategy 1 and 3.15 in Table 4.6 for Strategy 2). In one study on intact wine grape bunches, González-Caballero *et al*. (2010) reported RPD values for SSC ranging from 2.92 to 3.18 depending on the spectral range used to establish the calibration models. A comparison of RPD values obtained for TA and pH showed that the results obtained in the present study were comparable to those reported by González-Caballero *et al*. (2010). The $R^2$ values obtained in the present study for BrimA were considerably better than those found for the TSS:TA ratio in Strategy 1, but not in Strategy 2. This was also the case in the research work of Jordan *et al*. (2001). The $R^2$ values for BrimA were mostly above 70% where those for the TSS:TA ratio were always just above 60%.

The major difference in the results of the two different calibration sample selection strategies was the much higher RMSEP values that were obtained for all the parameters, except BrimA with Strategy 2. Low RPD values were also obtained with Strategy 2 (Table 4.5 and 4.6). A major contributor towards this difference may have been the higher maximum values for all the parameters, except TSS that was present in the 2017 dataset that was used for validation. Samples with similar or higher values should have been present in the calibration

dataset (2016) as well. For TSS the minimum value of 2017 again was not present in the calibration set and similar samples would thus not have been able to be predicted.

The SEL values were in all instances much lower than the RMSEC and RMSEP values obtained with the models, highlighting the fact that the accuracy of models constructed using data captured through NIR spectroscopy can never be as good as the standard reference method used. These results underscore the importance of updating calibration models with samples from future harvests (Guthrie *et al*., 2005) as well as the use of different calibration ranges as was done in González-Caballero *et al*. (2010).

### 4.3.5 Effects of spectral pre-processing techniques

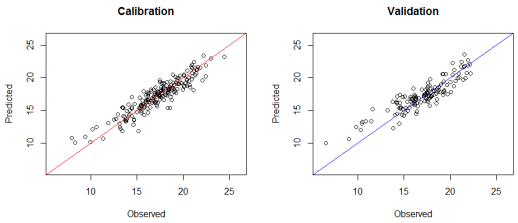
The set of mathematical procedures that are applied on spectra before developing a calibration model are known as pre-treatments or spectral pre-processing methods. Noise or background information (smoothing techniques) are reduced and the signal from the chemical information (differentiation) increased by applying these mathematical pre-treatment of spectra. Any pre-treatment must lead a robust model with good predictive ability. Baseline correction normalization, signal enhancement, and statistical filtering of signal noise can all basically, be classified as pre-processing methods (Agelet and Hurburgh Jr. 2010). Multiplicative scattering correction (MSC) is perhaps the most commonly used spectral pre-processing technique followed by standard normal variate (SNV) (Rinnan *et al*., 2009). These first three are used to correct for any shift that might have occurred in the baseline of the samples and in that way minimise the inconsistency between the samples because of light scatter (Rinnan *et al*., 2009). To enhance the signal-to-noise ratio the moving window smoothing (MWS) method is used. This is the standard and easiest one and makes use of a function that smoothes the original data by computing a moving average on a fixed-size spectral window. Before the average can be computed, points outside the spectral window are determined by second-order polynomial extrapolation on both ends of the spectrum (Chau *et al*., 2004). Savitzky-Golay first derivative (SG1d) also uses smoothing of the spectra before computing the derivative. This is to minimise the negative influence that conventional fixed-difference derivatives would have on the signal-to-noise ratio (Rinnan *et al*., 2009). All the spectral pre-processing techniques and combination with MSC had various effects on the results obtained for each parameter (Table 4.5 and 4.6). Parpinello *et al*. (2013) also evaluated five spectral pre-processing techniques but does not show the effect each specific spectral pre-processing technique had on each model, but states that a combination of mean normalization (MN)+MSC delivered the best model for SSC when discriminant analysis (DA) was performed. Baiano *et al*. (2012) also evaluated second derivatives. They, however, found that not any of the spectral pre-processing technique could create a better model than the original spectra. Cao *et al*. (2010) just made use of averaging and not any specific spectral pre-processing technique. It is however clear from the results

shown here that a specific spectral pre-processing technique will not always deliver all the desired statistical values that constitute for a good model. Thus one spectral pre-processing technique or combination with another, for example, SNV or MSW alone or each combined with MSC will not always deliver the highest $R^2$ and RPD values and lowest SEP, RMSEP and control limits for a parameter as desired. This can most probably be contributed to the different regions or areas of the spectrum that is highly associated with the chemistry of each parameter, which was not evaluated in this study. In Poblete-Echeverría *et al*. (2018), however, a decrease in predictive accuracy was obtained with variable selection in both the artificial neural network (ANN) and PLS models, but a good result was obtained with spectral pre-processing applied in the final PLS model.

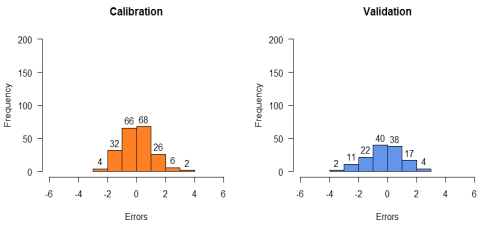
### 4.3.6 Latent variables

The number of LV used to construct the best model for the parameters varied from as little as four for TA and as high as 30 (Table 4.5). The optimum number of principal components (latent variables) in case of PLS seems to be three at the lowest level of residual validation variance (Jha *et al*., 2006). A relatively low number of LV are generally desirable to avoid modelling noise signals (Fernández-Novales *et al*., 2009). This especially not to compromise the robustness of the models for future predictions. The lowest number of LV should thus be that which always gave the lowest error as to not make the models too complex by using more factors that are necessary (Rinnan *et al*., 2009). This is, however, not always possible as can be seen in this study. Parpinello *et al*. (2013) obtained the best model with 17 LV for SSC when monitored in each berry of intact bunches in order to evaluate intra-bunch distribution and variability. The model in which the lowest number of LV was used was chosen as the best in this study. Baiano *et al*. (2012) used nine, seven and nine for SCC, pH and TA respectively. Numbers that are comparable to the ones chosen as the best in this study. Only when SG1d was used in combination with MSC were such low number of LV used, but they did not give the lowest errors.

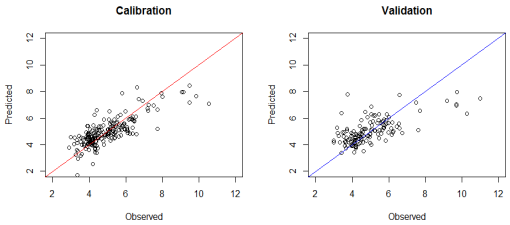
Figure 4.4 shows the calibration and validation plots of the models obtained for the five parameters and the spectral pre-processing strategy applied to the raw spectra during the construction process as well as the distribution of the errors obtained with each model. It can be seen in the calibration plots that the samples are not always spread evenly along the regression line in the validation plots as they are in the calibration plots. The same way that the frequency and the spread of the errors are, not the same in the calibration and validation bar plots. This shows clearly that the models should thus not only be evaluated on the numerical values of the statistics but also on the visual distribution of the samples and/or errors.



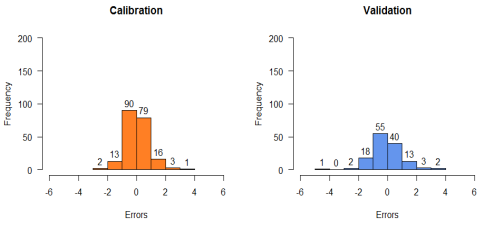

(A) TSS (SG1d+MSC)



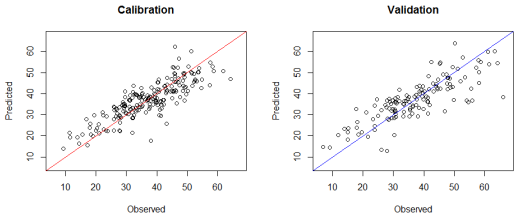

(F) TSS (SG1d+MSC))



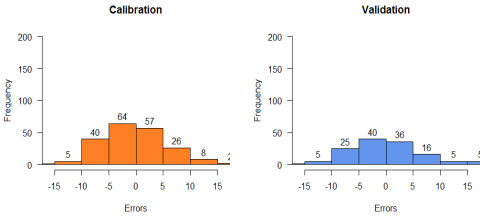

(B) TA (SG1d+MSC)



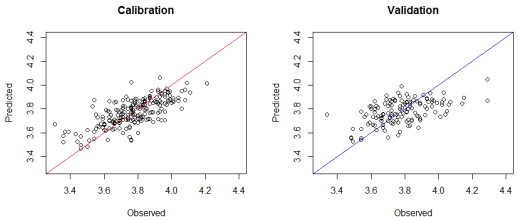

(G) TA (SG1d+MSC)



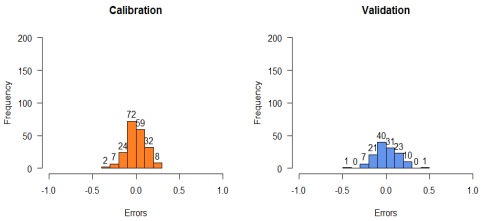

(C) TSS:TA ratio (SG1d+MSC)



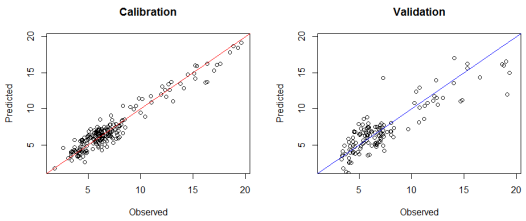

(H) TSS:TA ratio (SG1d+MSC)



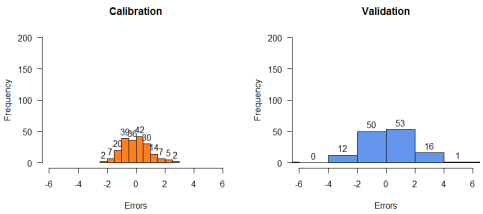

(D) pH (SG1d+MSC)




(I) pH (SG1d+MSC)




(E) BrimA (SG1d+MSC)




(J) BrimA (SG1d+MSC)

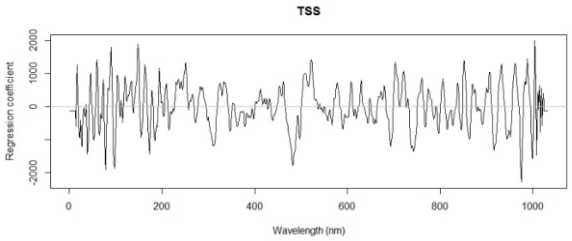
**Figure 4.4** Calibration and validation plots of the models obtained for the five parameters and the spectral pre-processing methods applied to the raw spectra during the construction process. (A) TSS, (B) TA, (C) TSS/TA ratio, (D) pH and (E) BrimA as well as the distribution of the errors obtained for each model (F) TSS, (G) TA, (H) TSS:TA ratio, (I) pH and (J) BrimA.

### 4.3.7 Calibration ranges

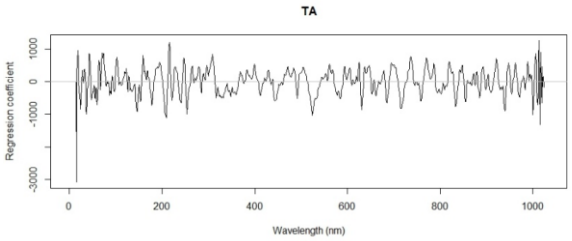
The better prediction statistics obtained for TSS are due to not only the higher concentration level of TSS present in the grapes, but also due to the wide range over which it spreads (6.58-24.40). The values of TA and pH spreads over a very narrow range, 2.89-10.99 g/L for TA and 3.31-4.29 for pH making the construction of a proper calibration model extremely difficult as can be seen in Figure 4.4. Also given that NIR spectra contain overtones and combinations derived from fundamentals which appear in the infrared region (Skoog, 1997) and measures the vibrational transitions of molecular bonds, such as the O-H bonds in water, and bonds such as C-N, N-H and C=O, characteristic to organic matter (Rinnan and Rinnan, 2007). TSS is predominantly consisting of water and sugar, making the creation of a good calibration easier unlike pH that cannot actually be measured directly seeing that the activity of single ion (H+) is involved (Covington *et al*., 1985). Its accuracy, therefore, depends on the operation used to measure it, usually in a liquid state, as done during the reference measurements in this experiment and not non-destructively and intact as set out in this experiment.

Due to the fact that table grapes mainly consist of water like many other fruit and vegetables, NIR spectra are complex and are dominated by the water peaks (Nicolaï *et al*., 2007) in the wavelength ranges from 1400-1440 nm and 1900 to 1950 nm (Bünning-Pfaue, 2003), as can be seen in Figure 4.4A. Since grape sugars are dissolved in water, the wavelengths that are strongly associated with the O-H and C-H first and second overtones associated with sugar are usually masked in those areas (Cozzolino *et al*., 2006; Dambergh*s et al*., 2006). First derivative of the spectra using the Savitzky-Golay algorithm as was done in this study to enhance these peaks (Figure 4.4B).

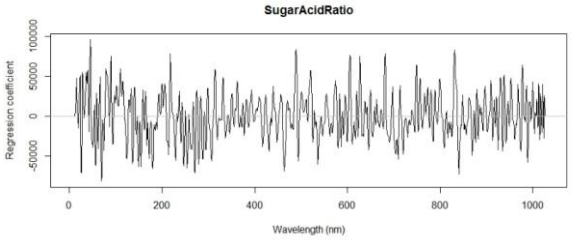
The PLS beta coefficient is also a very good indication of which wavelengths play a dominant role in the calibration model (Maghirang *et al*., 2003: Nagle *et al*., 2010). In Figure 4.5 the regression coefficients for all the best models for the wavelength region up to 1000 nm are shown and the peaks at 950 nm and 980 nm are strongly associated with TSS and that at 980 nm for pH as in González-Cabalero (2010). Giovenzana *et al* (2014) identified 670 nm, 730 nm and 780 nm as being highly associated with TSS in wine grapes. It is not uncommon to use the entire NIR region (Jarén *et al*., 2001) during calibration as was done in this study, although the use of specific regions has also been reported (Herrera *et al*., 2003; Bellincontro *et al*., 2011). The regression plots for TA highlighted the difficulty of assigning a specific wavelength to this parameter, since it is made up of several different acids, and likewise the TSS/TA ratio and BrimA parameters which are calculated from the TSS and TA values.



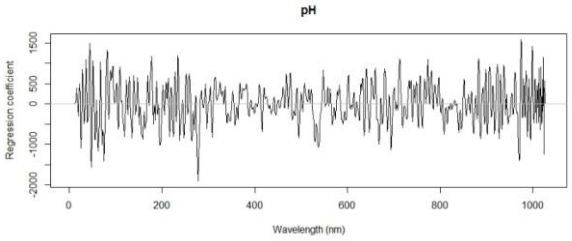

(A) TSS (MSW+MSC)



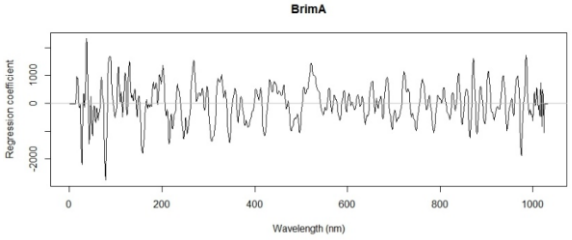

(B) TA (No spectral pre-processing)




(C) TSS/TA ratio ($SG_{1d}$)




(D) pH ($SG_{1d}$)




(E) BrimA (MSW+MSC)

**Figure 4.5** PLS beta-coefficient plots obtained during the calibration construction process of (A) TSS, (B) TA, (C) TSS/TA ratio, (D) pH and (E) BrimA.

It is important to note when comparing the results obtained here to those on berry experiments of the work of other authors (Parpinello *et al*., 2013; Baiano *et al*., 2012 and Omar, 2013) that the focus area of the light source on their samples was short, and not 17 cm as in this experiment. It is thus remarkable that the spectra could capture enough of the information in the grape bunches. This not only because of the heterogeneous nature of grape bunches which consists of a rachis berries, and pedicels, but also due the usually low penetration depth of NIR light into a sample.

## 4.4 CONCLUSIONS

The development of models with RPD values which can discriminate between high and low values of TSS, TA and TSS:TA ratio together with low RMSEP values, can greatly help minimize the losses suffered by producers due to the incorrect determination and classification of grapes for the export market based on these parameters. Although the use of the RPD as a measure of model performance has been criticized, mainly because it does not have statistical bases to make meaningful conclusions, it still, however, in use as can be seen in the studies conducted over the past five years on different fruit. Olarewaju et al. (2016) used it to predict dry matter and moisture content (MC) of avocado fruit with values 2.00 and 2.13 respective. Li et al. (2017) used it to predict 'Friar' plum's quality parameters; SSC, TA, pH, firmness, SSC:TA ratio and flesh color (L*, a*, b*) with RPD values of 4.43, 2.18, 2.37, 2.38, 2.10, 2.68, 1.62) and 3.19 respectively. Arendse et al. (2018) to predict firmness, colour components, TSS, pH, TA, TSS:TA ratio, BrimA, total phenolics, total anthocyanin and vitamin C of intact pomegranate fruit with RPD values = 2.43, 3.34, 2.43, 2.17, 2.12, 2.08, 2.91 and 2.06 respectively. Theanjumpol et al. (2019) obtained values of 2.00, 1.62 and 1.71 when they analyzed MC, SSC and TA of tangerine fruit and Agussabti et al. (2020) values of 3.05 and 2.62 respectively for MC and fat content when they determined these parameters of intact cocoa bean samples. All these positive resuls with this statistic prompted and motivated its use to evaluate model performance in this study.

Another implication of these results for the table grape industry is much quicker decisions taken over the quality of the grapes either using one of the parameters or all of them collectively to determine which class and which export markets table grapes should be send to. This especially with the inclusion of BrimA which can now help producers with the sensory quality of table grapes, so they can market them accordingly based on consumers' palates, e.g. low sweetness-high acidity, neutral, high sweetness-low acidity tasting grapes etc.

Future work will be to build better models for especially pH and TA. This will be explored through the selection of specific wavelengths strongly associated with these two parameters. When different strategies are used to build NIRS models, sampling should be done in such a way that in the end both the calibration and validation sets contain samples that are represented in each.

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# Chapter 5: Evaluation of handheld NIR spectrometers for quantitative purposes on whole berries


## ABSTRACT

Determining the correct harvest maturity parameters of table grapes is one of the most essential steps before harvesting. Measuring a parameter such as total soluble solids (TSS) is, however, very time consuming and destructive. Developing faster, non-destructive and more accurate ways of determining TSS in the vineyard or during any of the postharvest stages can lead to a decrease in losses suffered by industry at the postharvest stage. This study focussed on the development of a non-destructive way of determining harvest maturity and quality of table grapes in the vineyard before and after harvesting over two years using handheld and benchtop spectrometers on intact table grape berries. Spectra taken in the laboratory (lab) with the MicroNIR were more homogenous than the ones taken in the vineyard with the same spectrometer. The results obtained with the MPA were not as good as those obtained with the MicroNIR in the lab. The model constructed with the combined data of 2016 and 2017 taken in the lab with the MicroNIR had the best statistics in terms of the $R^2_p$ (0.74) and the $RPD_p$ (1.97). The model constructed with the 2017 data obtained in the lab with the MicroNIR had the lowest prediction error (RMSEp = 1.13). Both instruments delivered spectra that has the potential to predict TSS in the vineyard and in the laboratory and the advantages associated with using handheld instruments is evident. Although the MicroNIR showed higher accuracy in terms of prediction the effects of pre-processing and selection of wavelengths needs exploring.


## 5.1 INTRODUCTION

Table grape berries are individual fruit within which metabolic processes such as the accumulation of sugar and degradation of acids occur completely separately and at a different rate from those next to it on a bunch (Dai *et al*., 2013). The accumulation of sugar in the form of total soluble solids (TSS) or soluble solid content (SCC) (Shiraishi, 2000) and colour are the main factors that determine the berry readiness for harvest to meet commercial demand (Bahar *et al*., 2012). These TSS levels at which a specific cultivar may be harvested are defined by thresholds in the specific exporting country (Sonego *et al*., 2002). In South Africa, these thresholds are guided by section 4(3)(a)(ii) of the Agricultural Product Standards Act, 1990 (Act No. 119 of 1990). This means that grape harvest may be selective by requiring either early harvest or storage on the vines up until reaching the desired threshold (Bahar *et al*., 2012). The first problem with this is the negative effects of post-harvest treatments or pests and diseases by harvesting early or late (Crisosto *et al*. 2002). The second problem is the increase in time

and labour due to the individual measurement of grape berries on bunches for TSS through the traditional use of a refractometer. Thirdly, creating a lot of waste and destroying the integral structure and shape of the bunch seeing that many berries would have to be removed and squashed from a lot of bunches and vines. This especially since not all berries on a bunch, all bunches on a vine and all vines in a block are at the same maturity level for harvest (Šuklje *et al.*, 2012). The need for technology or an instrument that can help to overcome this problem so that TSS can be measured fast, in real-time and on the bunch and the vine is, therefore, needed.

Near-infrared (NIR) spectroscopy has proven to be such a technique or technology. It combines many sought-after characteristics such as speed, convenience, flexibility, preciseness, safe, cheap and non-destructive measurement/analysis of different fruit quality attributes with no harm to the environment (Nicolaï *et al*., 2007; Sánchez *et al*., 2013). Its incorporation into any industry always greatly helps to enable various decision-making steps through continuous monitoring in the pre-harvest, harvest and post-harvest of essential attributes such as TSS (Nicolaï *et al*., 2007). This is mostly because a wide range of portable or handheld NIR instruments is now available which offers other great advantages such as size, weight, robustness, spectral range and optical design options when choosing which instrument to use for the analysis (dos Santos *et al*., 2013; Entrenas *et al*. 2019).

Portable or handheld visible (VIS)-NIR spectrometers, just like their benchtop counterparts, have been used to non-destructively estimate a wide variety of attributes on a wide variety of products. On animal products, for example, Garrido-Varo *et al*. (2018) used one for the analysis of individual pork carcasses on-site. Bellincontro et al. (2012) used one for on-field prediction of phenolic compounds of ripening olives and during olive oil production. Pérez-Marín *et al.* (2010) assessed the post-harvest quality and refrigerated storage performance in plums on-site and non-destructively. Blakey and Rooyen (2011) used one for moisture content of Fuerte and Hass, and possibly other cultivars, across the full range of avocado maturity. Most studies used it on fruit to determine maturity status such as TSS on Mandarin (Antonucci *et al*., 2010). Other parameters, however, like titratable acidity (TA), vitamin C and colour of Nanfeng mandarin (Xudong *et al*., 2009); TA and firmness of apricots (Camps and Christen, 2009) and weight, diameter and flesh firmness in nectarines (Sánchez *et al*., 2011) have also simultaneously been investigated with TSS. Sánchez *et al.* (2013) even expanded these to include equatorial and axial diameters, colour, maximum penetration force, pericarp thickness, juice weight, juice content and maturity index during an on-tree ripening and at harvest investigation on intact oranges. Regarding grapes, studies included monitoring the ripening evolution of the Italian red wine grape variety Sangiovese by Barnaba *et al.* (2013) and determination of dry matter content (DM) and TSS in fresh table grapes and peach fruits by Donis-González *et al*. (2020). There is, however, a gap when it comes to table grapes as seen in the list of reported applications of portable NIR instruments for fruit and vegetable analysis in the review by (dos Santos *et al*.

(2013). This study plans to fill it by not only looking at the comparison of different instruments but by also incorporating two different scenarios (vineyard vs laboratory) as well as different harvest years.

The objective was thus to evaluate the quality of TSS prediction models obtained using two NIR instruments: a handheld MicroNIR device ideal for measuring fruits intact on the vine and a benchtop spectrometer well suited for measuring any sample that does not need any sample preparation in two harvest different years.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Grape sampling

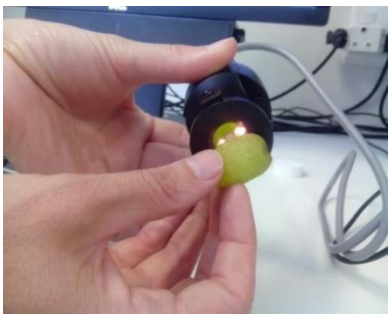
Regal Seedless and Thompson Seedless bunches were randomly selected from the vines on both sides of the canopy from two different vineyards during 2016 and 2017, located respectively in the Wellington region (33°37'03,5" S, 18°58'05,3" E) and the Hex River Valley region (33°27'53,9"S, 19°39'43,7"E) of the Western Cape, South Africa. Every berry on the selected bunches was numbered with a permanent marker (Figure 5.1a). Scanning of the attached berries to the bunch happened in the vineyard. Harvest of these same berries occurred for scanning in the laboratory. Packing of bunches was in 4.5 kg closed-top corrugated fibreboard cartons used in the table grape industry, with each bunch placed in an individual plastic carry bag. The enclosure of the entire carton content was in a 2-mm perforated low-density polyethene (LDPE) liner bag that contained a corrugated cardboard sheet at the bottom. An Uvasys® sulphur dioxide ($SO_2$) generator sheet (http://www.uvasys.com/) covered the grapes to control decay. Packed boxes were loaded into an air-conditioned vehicle and transported by road to the Department of Food Science analytical laboratory and afterwards to the analytical laboratory of the Institute for Wine Biotechnology, Stellenbosch University.

### 5.2.2 Fourier transform near-infrared spectroscopy

Spectral data collection of the intact table grape berries was collected in reflectance mode (log 1/R) using a handheld NIR instrument and a benchtop NIR instrument. The handheld instrument was the MicroNIR Pro 1700 ES Lite spectrometer (Viavi, San Jose, California, USA), from here on just referred to as the MicroNIR. The benchtop instrument was the solid probe of Bruker's Multi-purpose analyser (MPA) (Bruker Optics, Ettlingen, Germany), from here on just referred to as the MPA. Scanning of individual whole berries in the vineyard and the laboratory was with the MicroNIR. This instrument weighs only 64 grams, measures <50 mm in diameter and <45 mm in height within which a light source, collection optics, electronics and linear variable filters (LVF), as the dispersing element, are contained. The illumination source has two integrated

vacuum tungsten lamps with a bulb life span of > 40 000 hours. The sample working distance is 0 mm to 15 mm from the window (3 mm optimal). The detector has a 128-pixel InGaAs photodiode array one with pixel-size/ pitch of 30 µm x 250 µm/50 µm. Measurements occurred in the first and second overtone spectral region of 950 to 1650 nm (10,526 to 6060 $cm^{-1}$) with a pixel-to-pixel interval of 6.2 nm for 950 to 1650 nm. Its spectral bandwidth (FWHM) is <1.25% of centre wavelength (1% typical) (for example, @1000 nm, resolution is <12.5 nm). Its analogue-to-digital converter was 16 bit and its dynamic range equalled 1000:1. The measurement time per sample was 0.25 to 0.5 s with a signal-to-noise ratio of 23,000 (average of 100 scans). The integration time was 10 ms (minimum 10 µsec). The mode used was in diffuse reflection by connecting the USB cable to a laptop. Reference measurements occurred every 20 samples with a spectralon®. All berry samples adjusted to room temperature (20° in the laboratories) before scanning. Two spectra were collected per berry sample at the centre location of each berry (Figure 5.1a and b), and the average for each sample was calculated.

The MPA spectrometer was equipped with a fibre-optic probe for scanning solid samples. Scanning of whole table grape berries in the laboratory happened using this solid probe. It was a pistol-like accessory with an external trigger attached to the instrument with a fibre optic cable that was 60 cm long. The length of the probe's head was 80 mm and its diameter was 5 mm. The NIR source of the MPA was 20 W tungsten-halogen lamps with high energy, air-cooled by using an extremely steady RockSolidTM always-aligned interferometer. The wave number precision of the interferometer was better than 0.1 $cm^{-1}$ and the wave number reproducibility was better than 0.04 $cm^{-1}$. To ensure a highly reflective exterior and one that was inactive, the mirrors in the interferometer were gold coated. The make of the beam splitter required a quartz substrate with proprietary coating and to ensure correct calculation of position and velocity of the movable mirror, a He-Ne laser Class 1 was used. Guiding of light to the sample was by source fibres and the reflected light received with the detector fibres. Due to the bifurcated optical configuration of the fibre optic probe, a thermoelectrically cooled InGaAs-detector that had a high sensitivity was used. The placing of fruit was in contact directly in front of the fibre-optic probe. The resolution used to scan was 8 $cm^{-1}$ in the 780 nm to 2500 nm wavelength (12500 to 4000 $cm^{-1}$) region. It took about 16 seconds to scan each fruit 32 times. Taking a reference spectrum after every 20 samples occurred with a spectralon®. Measurement of samples was in duplicate.

(a)  (b)  (c) 

### 5.2.3 Reference measurements

Berry removal from the bunch occurred after scanning and crushing by hand so that the juice trickled directly onto the sample plate of a handheld digital refractometer (ATAGO Palette Digital Refractometer PR-32 Alpha, Tokyo, Japan) to determine the TSS. The recording of each value (in °Brix) for each berry was according to the number assigned to it on the bunch.

### 5.2.4 Data analysis

To investigate the relationship between the spectral information of the intact berries and TSS content, PLS regression was implemented in the R statistical environment (R Core Team, 2016) using the PLS package (Mevik *et al*., 2016). To find the correlation between the spectra taken of the intact table grape berries and the reference values obtained for TSS, the use of PLS which is a bilinear modelling strategy (Naes *et al*. 2004) occurred. The data matrix, therefore, consisted of a set of independent X variables (NIR spectral data) and a dependent Y variable TSS. The creation of different models happened. First, this was done with data collected from samples in 2016 with the MicroNIR in the laboratory; second, of the same 2016 samples collected with the MPA in the laboratory; third, with samples collected in 2017 in the vineyard with the MicroNIR; fourth, with the same samples collected with the MicroNIR in the laboratory; fifth, by combining the samples collected in 2016 and 2017 with the MicroNIR; and lastly, using the 2016 data collected with the MicroNIR in the laboratory as the training set and the 2017 data collected with the MicroNIR as the test set. With the last model where the datasets for 2016 and 2017 were combined (n=3559), the entire dataset was randomly divided into two sub-datasets, the training set containing 2/3 of the data (n=2373) and testing set containing 1/3 of the total dataset (n=1186) for TSS. A full cross-validation process was applied to build the PLS regression models using the training dataset.

The regression models were evaluated using the coefficient of determination ($R^2$) and the Root Mean-Square Error of Calibration (RMSEC) or Validation (RMSECV when cross-validation is used and RMSEP when test set validation is used). The $R^2$ value, which represents the proportion of explained variance of the response variable in the calibration set ($R^2_c$) or validation set ($R^2_{cv}$ or $r^2$ when cross-validation is used and $R^2_p$ when test set validation is used). This value

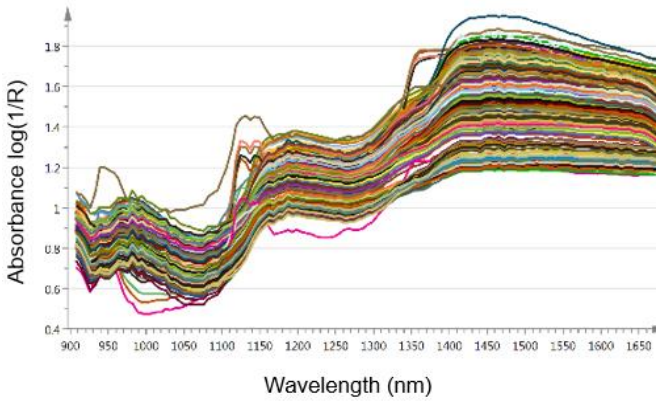
needs to be as high as possible for a good model. It differs from the correlation coefficient (r) which only shows how strong the relationship between two variables is (Taylor, 1990) and $R^2$ is a multiple of it (Nagelkerke, 1991). RMSECV is the term indicating the prediction error of the model and the RMSEP value gives the average expected uncertainty for predictions of future samples and both need to be as close as possible to zero (Saeys *et al*., 2005; Brown *et al*., 2005; Esbensen, 2006). The residual prediction deviation (RPD) value is defined as the ratio of the standard deviation of the reference data of the validation set to the standard error of prediction and gives some indication of the efficiency of a calibration (Williams and Norris, 2001). The RPD value has to be between 1.5 and 2 for the model to discriminate low from high values of the response variable; a value between 2 and 2.5 to indicate that course quantitative predictions are possible, and a value between 2.5 and 3 or above to show good and excellent prediction accuracy (Saeys *et al.,* 2005). The standard error of calibration (SEC); standard error of performance (SEP); limit control for SEP (LC_SEP) and limit control for bias (LC bias) were also calculated. The SEC and SEP, as well as the control limits, also have to be as close as possible to zero to give good working models.

## 5.3 RESULTS AND DISCUSSION

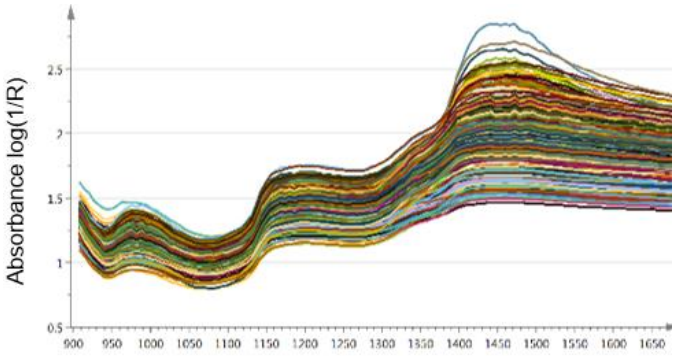
### 5.3.1 Intact berry spectral features

In Figure 5.2 a-c, the characteristic log (1/R) spectra of whole berries are shown. Figure 5.2a shows the spectra of whole berries scanned with the MicroNIR in the vineyard and Figure 5.2b those obtained in the laboratory with the same spectrometer. Figure 5.2c shows spectra of whole berries obtained in the laboratory with the MPA's solid probe.

(a)




(b)




(c)

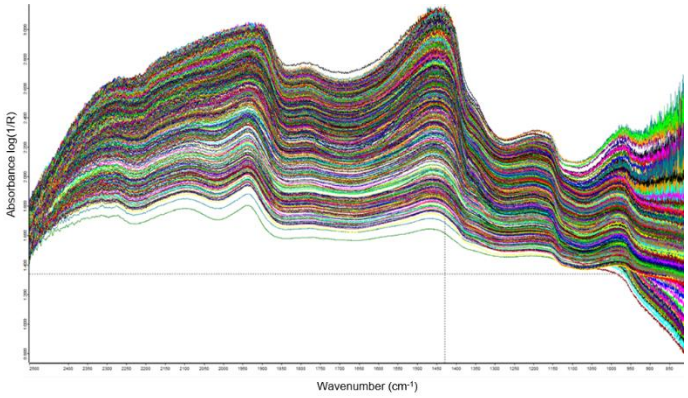



**Figure 5.2** Absorbance log (1/R) spectra of whole table grape berries taken with the MicroNIR spectrometer in the vineyard (a) and in the laboratory (b) and (c) with the solid probe of the MPA in the laboratory. Spectra of the MicroNIR was measured in wavelength (nm) and those of the MPA in wavenumber ($cm^{-1}$).

All the spectra show clearly defined peaks with very little noise. Spectra taken in the laboratory with the MicroNIR (Figure 5.2b) appear more homogenous than when taken in the vineyard with the same spectrometer (Figure 5.2a). A possible reason for this may be that in the laboratory the measurement was uniform due to each berry being in front of the instrument, other than in the vineyard where the placement of the instrument was against the berries still attached to the bunch. Manipulation of the instrument has to be in such a way to get proper contact with the berries, even those situated in the back or in awkward positions. The instrument, therefore, did not make complete contact with them, allowing stray light in.

### 5.3.2 Reference data statistics

Table 5.1 shows the statistical analysis of training datasets and Table 5.2 that of the testing datasets of TSS in 2016 taken with the MicroNIR and the MPA in the laboratory respectively and in 2017 taken with the MicroNIR in the vineyard and the laboratory as well as these two datasets combined. The minimum value for the 2016 MicroNIR laboratory dataset and the 2016 MPA dataset was 10.10 °Brix as well as that for the combined 2016 and 2017 MicroNIR laboratory datasets. The maximum value for the 2016 MicroNIR laboratory dataset was 25.20°Brix and for the 2016 MPA and the 2016 and 2017 MicroNIR laboratory datasets it was 26.70°Brix. In 2017, the minimum was 16.60 °Brix and the maximum value 25.50 °Brix for the 2017 MicroNIR laboratory and vineyard datasets. The minimum values for the 2016 MicroNIR laboratory and 2016 MPA laboratory datasets was exceptionally low particularly in 2016 given that Regal Seedless and Thompson Seedless harvest occurs at 16 °Brix (ACT No. 119 OF 1990 of South Africa). The mean, however (17.56 °Brix and 17.28 °Brix, respectively) as well as the range for the MPA (16.70 °Brix respectively) were still in equivalence with the standards. For 2017, the minimum value was higher (16.60 °Brix) and the maximum value lower (25.50 °Brix). Both, however, still in line with the standards but bringing the range down to 8.90°Brix for the 2017 MicroNIR vineyard dataset. The standard deviations (SD) and coefficients of variation (CV) values were higher in 2016 (2.38 and 0.14 respectively) compared to 2017 vineyard values (1.45 and 0.07), as shown in Table 5.1. Temperature plays a very important role in producing carbohydrates (TSS) through photosynthesis (Berry and Björkman, 1980). Daniels *et al.* (2019) already showed how the difference in temperature between two seasons probably played a role in the differing values found in the datasets for grapes over two separate harvest years. The average maximum daily temperatures tended to be generally higher and the average minimum temperature generally lower during the ripening and harvesting periods.

**Table 5.1** Statistical analysis of the training dataset for TSS[a] collected in 2016 in the laboratory with the MicroNIR and the MPA and in 2017 in the vineyard and the laboratory with the MicroNIR only, as well as these combined

| Training Statistic | 2016 MicroNIR Laboratory | 2016 MPA Laboratory | 2017 MicroNIR Vineyard | 2017 MicroNIR Laboratory | 2016 MicroNIR combined with 2017 MicroNIR Laboratory |
|---|---|---|---|---|---|
| N | 3120 | 2110 | 381 | 381 | 3559 |
| Mean | 17.56 | 17.28 | 20.72 | 20.74 | 17.93 |
| Median | 17.60 | 17.30 | 20.80 | 20.80 | 18.00 |
| Min[b] | 10.10 | 10.10 | 16.60 | 16.60 | 10.10 |
| Max[c] | 25.20 | 26.70 | 25.50 | 25.50 | 26.70 |
| Range | 15.10 | 16.60 | 8.90 | 8.79 | 16.60 |
| Standard Deviation | 2.38 | 2.56 | 1.45 | 1.47 | 2.57 |
| Coefficient of Variation | 0.14 | 0.15 | 0.07 | 0.07 | 0.14 |

[a]Total soluble solids, [b]Minimum, [c]Maximum

**Table 5.2** Statistical analysis of the testing dataset for TSS[a] collected in 2016 in the lab with the MicroNIR and the MPA and in 2017 in the vineyard and the lab with the MicroNIR only as well as these combined

| Training Statistic | 2016 MicroNIR Laboratory | 2016 MPA Laboratory | 2017 MicroNIR Vineyard | 2017 MicroNIR Laboratory | 2016 MicroNIR combined with 2017 MicroNIR Laboratory |
|---|---|---|---|---|---|
| N | 2078 | 1404 | 251 | 251 | 2371 |
| Mean | 17.53 | 17.29 | 20.76 | 20.73 | 17.93 |
| Median | 17.60 | 17.30 | 20.80 | 20.80 | 17.90 |
| Min[b] | 10.10 | 10.10 | 16.60 | 16.60 | 10.10 |
| Max[c] | 26.70 | 25.30 | 25.50 | 24.80 | 25.50 |
| Range | 16.60 | 15.20 | 8.90 | 8.20 | 15.40 |
| Standard Deviation | 2.43 | 2.55 | 1.45 | 1.41 | 2.57 |
| Coefficient of Variation | 0.14 | 0.15 | 0.07 | 0.07 | 0.14 |

[a]Total soluble solids, [b]Minimum, [c]Maximum

### 5.3.3 Performance of calibration models

Although scanning of both sides of the berries occurred in the laboratory and the vineyard in both years with both instruments, use of the average of the obtained spectra occurred to construct the calibration models for TSS. This was following what Daniels *et al.* (2018) found, that the best calibration models are usually with the average spectra to construct models. Table 5.3 shows the results of the calibration models constructed for TSS for data of 2016 taken with

the MicroNIR and the MPA in the laboratory respectively and of 2017 taken with the MicroNIR in the vineyard and in the laboratory as well as the two datasets (2016 MicroNIR laboratory and 2017 MicroNIR laboratory) combined. The 2016 MicroNIR laboratory dataset also acted as the training dataset to construct a calibration model validated using the 2017 MicroNIR laboratory as a testing dataset. Construction of all the models occurred without using any spectral pre-processing technique or selection of specific wavelengths.

**Table 5.3** Performance of PLS models for table grape quality parameter collected in 2016 with the MicroNIR and the MPA in the laboratory and with the MicroNIR only in 2017 in the vineyard and the laboratory. Construction of a calibration model of the combined MicroNIR data collected in the laboratory for 2016 and 2017 also occurred. The dataset obtained with the MicroNIR in the laboratory in 2016 acted as the training set to construct a calibration model validated using MicroNIR in the laboratory in 2017 dataset as a testing set.

| Statistic | 2016 MicroNIR Laboratory | 2016 MPA | 2017 MicroNIR Vineyard | 2017 MicroNIR Laboratory | 2016 MicoNIR Laboratory combined with 2017 MicroNIR Laboratory | 2016 Laboratory Calibration 2017 Laboratory Validation |
|---|---|---|---|---|---|---|
| LVs[a] | 21.00 | 9.00 | 14.00 | 16.00 | 17.00 | 21.00 |
| $R^2_c$[b] | 0.54 | 0.31 | 0.52 | 0.67 | 0.76 | 0.76 |
| $R^2_{cv}$[c] | 0.49 | 0.26 | 0.28 | 0.53 | 0.76 | 0.75 |
| $R^2_p$[d] | 0.50 | 0.26 | 0.39 | 0.39 | 0.74 | 0.29 |
| Sec[e] | 1.61 | 2.11 | 0.99 | 0.85 | 1.25 | 1.18 |
| Sep[f] | 1.71 | 2.19 | 1.30 | 1.13 | 1.31 | 1.74 |
| LC_Sep[g] | 2.09 | 2.74 | 1.29 | 1.11 | 1.63 | 1.54 |
| LC_bias[h] | 0.97 | 1.26 | 0.60 | 0.51 | 0.75 | 0.71 |
| $RMSE_c$[i] | 1.61 | 2.11 | 0.99 | 0.85 | 1.25 | 1.18 |
| $RMSE_p$[j] | 1.71 | 2.19 | 1.31 | 1.13 | 1.31 | 2.49 |
| $RPD_c$[k] | 1.48 | 1.21 | 1.45 | 1.73 | 2.06 | 2.05 |
| $RPD_p$[l] | 1.39 | 1.17 | 1.10 | 1.31 | 1.97 | 0.97 |

[a]Latent variables, [b]Coefficient of determination for the calibration set, [c]Coefficient of determination for cross-validation, [d]Coefficient of determination for prediction, [e]Standard error of calibration, [f]Standard error of performance, [g]Limit control for SEP (LC_SEP), [h]Limit control for bias, [i]Root mean square error of calibration), [j]Root mean square error for prediction, [k]Residual prediction deviation for calibration, [l]Residual prediction deviation for prediction.

The best predictive results for TSS was with the combined 2016 and 2017 MicroNIR dataset. Although this model did not use the lowest number of latent variables (17) in comparison to the nine, 14 and 16 used for the 2016 MPA, 2017 MicroNIR vineyard and 2017 MicroNIR laboratory datasets did not produce the lowest errors either. The basis for this was the high $R_2$ and RPD values obtained with this model. The $R^2_c$ and $R^2_p$ both equalled 0.76. This was followed closely by the 2016 MicroNIR laboratory cal and 2017 MicroNIR laboratory validation model with R2c = 0.76 and $R^2_p$ = 0.75. The RPD values equalled 2.06 and 1.97 for the calibration ($RPD_c$) and validation ($RPD_p$) sets respectively. This indicated, according to Saeys et al. (2005), that the model could discriminate low from high values of TSS. The 2017 MicroNIR model had the lowest errors with the Sec and $RMSE_c$ = 0.85, $RMSE_p$ = 1.13. This was also the case for the control limits with the LC_Sep = 1.11 and LC_bias = 0.51. The 2017 MicroNIR

vineyard model had the second-lowest errors with the Sec and $RMSE_c$ = 0.99, $RMSE_p$ = 1.31 except for the Sep = 1.30 that was lower than that of the 2017 MicroNIR model (Sep = 1.31). This was also the case for the control limits with the LC_Sep = 1.29 and LC_bias = 0.60. This was followed by the errors of the 2016 MicroNIR laboratory calibration and 2017 MicroNIR laboratory calibration model with Sec and $RMSE_c$ = 1.18. The Sep = 1.74 and $RMSE_p$ = 2.49 were much higher though for this model but the control limits were lower, 1.54 for LC_Sep and 0.71 for LC_bias. The 2016 MPA model performed poorly in terms of all the statistics except using the lowest number of LVs (9).

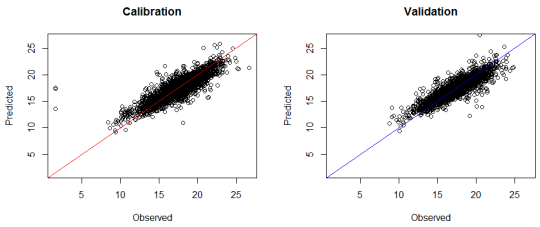
Researchers that also scanned whole table grape berries using NIR spectroscopy found r and RMSEP of 0.91 and 0.96 for SSC (Cao *et al*., 2010), $R^2$ and RMSE of 0.94 and 0.06 (Baiano *et al.*, 2012) and 0.95 and 0.18 (Omar, 2013). The $R^2$ values were 0.93, the RMSEP 0.94, Sep 0.73 and RPD 2.70 for TSS on intact wine grape berries (Barnaba *et al.* (2013). It is immediately clear that the r and $R^2$ values are much higher and the prediction errors much lower than the ones obtained with the different models constructed in this study. It should, however, be noted that the number of samples used in the construction of those TSS models was much lower than the number (N) in this study (Table 5.1 and 5.2).

Another possible reason for the higher r and $R^2$ values and lower RMSEP and RMSE values was because effective wavelengths for calibration development were chosen in the other studies, whereas the whole spectrum was used for all the instruments in this study. Omar (2013) especially found that NIR wavelengths that contained the carbohydrate C-H and O-H bands (910 nm and 950 nm respectively) were the most significant wavelengths when it comes to predicting TSS and that the visible wavelength at 605 nm could vastly improve TSS prediction. This was close to the 633 nm and 643 nm, as Cao *et al*. (2010) also indicated. Baiano *et al.* (2012) used the 840 nm band because in their case the 660-700 nm bands were too strongly associated with the reflection bands of the anthocyanin pigments in the red and black grapes scanned and the 500 nm the green-yellow colour of the chlorophyll pigments in the white grapes scanned. Donis-González *et al.* (2020) built their models in the 740–1070 nm region of two commercially available portable spectrometers to determine non-invasively table grape and peach quality attributes. They found the best model for TSS to be with the F-750 handheld spectrometer when utilising standard normal variate (SNV) as a spectral pre-processing technique. The $R^2_c$ was 0.99 and 0.97, the $R^2_p$ 0.98 and 0.97, the RMSEC 0.36 and 0.55, the RMSEP 0.39 and 0.58, the $RPD_c$ 8.96 and 5.87, the $RPD_p$ 8.03 and 5.43 and the bias 0.00 and -0.01 for the F-750 and SCiO spectrometers respectively. All the statistics for both spectrometers were equally good, unlike in this study where the models constructed with the MicroNIR were much better than the ones constructed on the MPA. Models constructed with the MicroNIR in the laboratory were also better than the ones constructed in the vineyard. This was different from results obtained by Kanchanomai *et al*. (2020) when they used a visible/NIR spectrometer in the 400–1000 nm range to determine SSC, pH, frmness, and seedlessness of

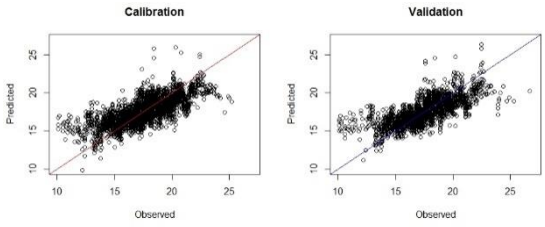
'Kyoho' table grapes. PLS models for SSC in the field were better than those in the laboratory. The $R^2_p$ was 0.6926, SEP = 0.8129, $R^2_c$ = 0.6944 and SEC = 0.7938 for models constructed in the laboratory using multiplicative scatter correction as spectral pre-processing technique. For prediction models constructed in the field $R^2_p$ was 0.8052, SEP = 0.6452, and the $R^2_c$ was 0.7613 and SEC = 0.7016 using Savitzsky-Golay second derivative as spectral pre-processing technique.

The results from this study also highlighted that with the proper spectral pre-processing techniques better quality models are obtainable, as was the case in all the other studies. This, especially since the ability to make coarse to excellent predictions that are still lacking with the models created here. Data collection, however, was over two harvests and years rather than from a single harvest and year. This had a great influence on the quality of the models.

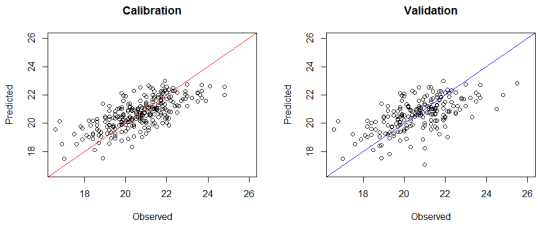
Figure 5.3 shows the calibration and validation plots of the models obtained for TSS, with (A) the 2016 MicroNIR laboratory data; (B) 2016 MPA data; (C) 2017 MicroNIR vineyard data; (D) 2017 MicroNIR laboratory data; (E) 2016 MicoNIR combined laboratory 2017 MicroNIR laboratory data and (F) 2016 MicroNIR laboratory calibration and 2017 MicroNIR laboratory validation data. Figure 5.4 shows the error distribution bars of the models obtained for TSS, with (A) 2016 MicroNIR laboratory data; (B) 2016 MPA data (C); 2017 MicroNIR vineyard data; (D) 2017 MicroNIR laboratory data; (E) 2016 MicoNIR combined laboratory 2017 MicroNIR laboratory data and (F) 2016 MicroNIR laboratory calibration and 2017 MicroNIR laboratory validation data. Figure 5.3 illustrates how the use of more samples can lead to a more even spread of the samples along the calibration line (F), while Figure 5.4 illustrates how even (calibration) and uneven (validation) the spread of the errors can be around zero.



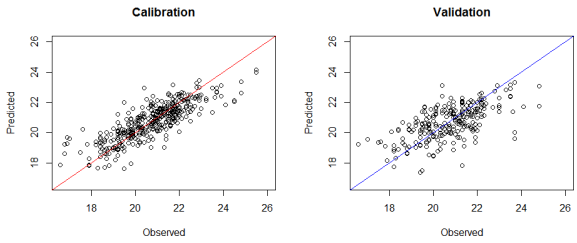

(A) 2016 MicroNIR lab



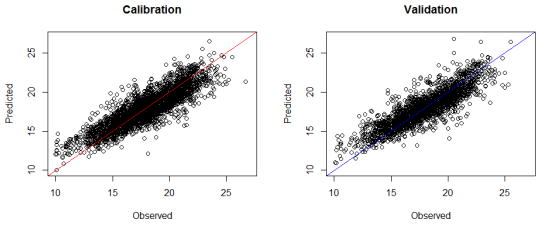

(B) 2016 MPA



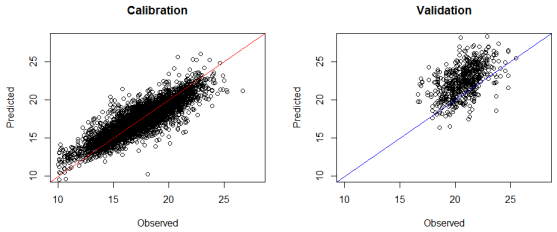

(C) 2017 MicroNIR vineyard




(D) 2017 MicroNIR lab

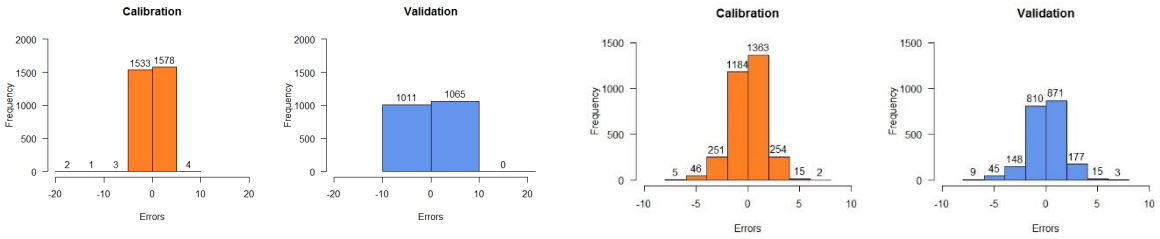



(E) 2016 lab combined with 2017 lab




(F) 2016 lab cal 2017 lab val

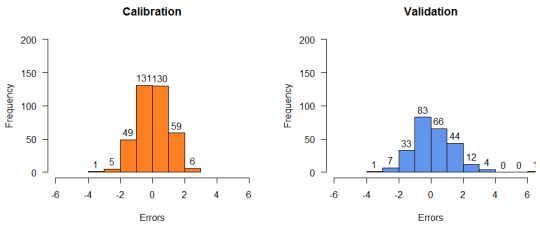
**Figure 5.3** Calibration and validation plots of the models obtained for TSS, (A) 2016 MicroNIR Laboratory (B) 2016 MPA (C) 2017 MicroNIR Vineyard (D) 2017 MicroNIR Laboratory, (E) 2016 MicoNIR combined Laboratory 2017 MicroNIR Laboratory, (F) 2016 Laboratory Calibration 2017 Laboratory Validation.



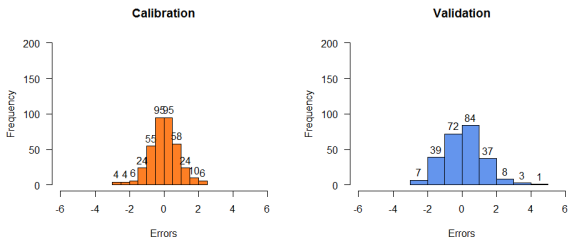

(A) 2016 MicroNIR lab errors



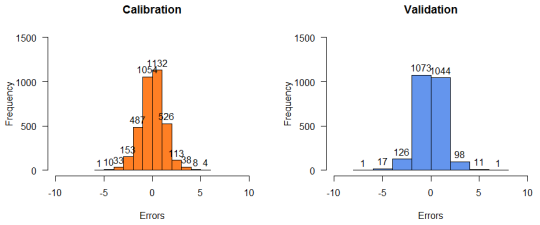

(B) 2016 MPA errors



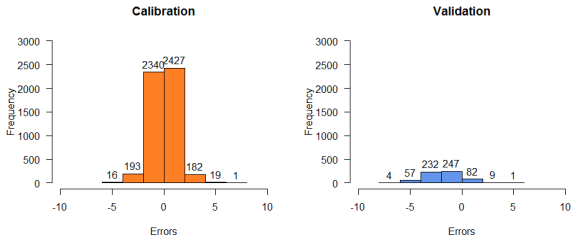

(C) 2017 MicroNIR vineyard errors




(D) 2017 MicroNIR lab errors




(E) 2016 lab combined with 2017 lab errors




(F) 2016 lab cal 2017 lab val errors

**Figure 5.4** Error distribution bars of the models obtained for TSS of whole berries measured (A) 2016 MicroNIR Laboratory (B) 2016 MPA (C) 2017 MicroNIR Vineyard (D) 2017 MicroNIR Laboratory, (E) 2016 MicoNIR combined Laboratory 2017 MicroNIR Laboratory, (F) 2016 Laboratory Calibration 2017 Laboratory Validation.

## 5.4 CONCLUSION

These results illustrated that although obtaining good quality spectra with both the MicroNIR and the MPA spectrometers, the ability to measure the TSS content of whole table grape berries accurately in the laboratory and/or the vineyard was better with the MicroNIR than with the MPA. The application of spectral pre-processing techniques as well as the selection of specific wavelengths strongly associated with TSS should occur during model building to obtain higher accuracy of prediction models. Also in terms of practicability, the MicroNIR is the better instrument because of its ease of use in both the vineyard and the laboratory.

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# Chapter 6: Classification of browning on intact table grape bunches using near infrared spectroscopy coupled with partial least squares-discriminant analysis and artificial neural networks


## ABSTRACT

Table grape browning is a complex physiological disorder that affects the quality of the fruit during cold storage. Several browning phenotypes have been described of which some may be present on the fruit at harvest, albeit not visible to the naked eye. The inability for correct and accurate classification of which grapes will eventually turn brown remains a problem. Novel and innovative ways needs investigation to manage the problem that hampers progressive and sustainable growth of the table grape industry. Given the complex nature of the browning phenomenon, a technique such as near infrared spectroscopy (NIR) can be utilised for non-destructive classification of each browning phenotype. In this study, NIR coupled with partial least squares discriminant analysis (PLS-DA) and artificial neural networks (ANN) are used to classify bunches as either clear, or as having chocolate browning and friction browning based on the spectra obtained of intact table grape bunches. Bunches of Regal Seedless grapes cold stored over different periods were used for this objective. The classification error rate (CER), specificity and sensitivity were used to evaluate the models constructed using PLS-DA and the kappa score was used for ANN. The CER for chocolate browning (25%) was better than that of friction browning (46%) for Week 3 and 4 for both class 0 (absence of browning) and class 1 (presence of browning). Both the specificity and sensitivity of class 0 and class 1 for friction browning were not as good as for chocolate browning. With ANN the testing kappa score to classify table grape bunches as clear or having chocolate browning or friction browning, showed that chocolate browning could be classified with strong agreement during Week 3 and 4 and Week 5 and 6 and that friction browning could be classified with moderate agreement during Week 3 and 4. These results open up new possibilities for the development for quality checks of packed table grape bunches prior to export. This has a significant impact for the table grape industries for it will now be possible to evaluate bunches non-destructively during packaging to determine the possibility of these browning types being present when reaching the export market.


## 6.1 INTRODUCTION

Exported grapes should remain intact and free of damage or defect when they reach the consumer market. Table grape browning can appear as a discolouration of the pulp (flesh or internal browning) and berry skin (skin or external browning) (Vial *et al.*, 2005). This is due to a

dysfunction or disruption of cellular membranes, which allows the mixing of the enzyme polyphenol oxidase (PPO) with phenolic substrates or compounds occurring naturally in the fruit (Ferreira, 1997; Golding *et al*., 1998; Kruger *et al*., 1999). Several different phenotypes of browning have been identified by Fourie (2009). These are external, internal, low temperature, chemical, physical and pathogenic browning. External browning is subdivided into net-like, mottled, friction and contact browning types. Internal browning is expressed as chocolate-, water- and glassy berry. Post-harvest treatment of grapes with methyl bromide and carbon dioxide ($CO_2$) causes damage that is known as chemical browning, while abrasions and bruises are known as physical browning and fungal infection as pathogenic browning (Fourie, 2009).

Ever since the browning phenomenon was first reported in 1989 (Wolf, 1996), it has only become more severe. Numerous research has been conducted to try and find out what exactly is the cause of it on table grapes, but to date, nothing has shown that there is a single dominant factor that can be repeatedly linked to either internal or external browning development (Moelich, 2010). The cultivar, seasonal variations and relative amounts of individual phenolic compounds in grapes, and phenolic distribution in the flesh and skin (Lee and Jaworski, 1989) are just some of the factors that may influence browning while the grapes are still hanging in the vineyard. Zapata *et al.* (1995) also could not find a correlation of real browning in red and white grapes based on the level of peroxidase activity in the grapes.

Specific macro and/or micro-elements and post-harvest factors, like moisture-modifying packaging material, sulphur dioxide ($SO_2$), and modified atmosphere packaging (MAP) possibly influenced the occurrence of external and/or internal browning, but whether a possible relationship existed between them could not be discovered by Burger *et al.* (2005). A correlation between internal browning and post-harvest treatments like methyl bromide fumigation, a toxic odourless gas used to control pests of quarantine significance, in both grapes and apples was found but a clear role that glutathione, an antioxidant, preventing damage to important cellular components, played in it could not be found (Liyanage *et al*., 1993). Even Gonzáles-Barrio *et al*. (2005), after thoroughly explaining the chemical process of the occurrence of browning, could not show that a post-harvest treatment (UV-C light treatment) was the single cause for the browning observed on the white seedless table grape cultivar Superior.

Figure 6.1a and b show this complexity with the two adjacent berries in the peach tray coming from the same bunch harvested from the same vineyard in the same year. One is still healthy and clear and the other has turned completely brown with the possible cause of this browning being a fungal infection (b). Figure 6.2 shows the same berry in Figure 6.1 (a and b) with chocolate browning symptoms on the outside of the berry (a) and when it is cut open (b). Figure 6.3 shows internal browning symptoms from the outside (a) as well as on the inside when the berry is cut open (b). Figure 6.4 shows the gradual development of internal browning from completely clear (a), to symptoms starting to manifest (b). These variations in phenotypic manifestation of grape berry browning pose challenges for the implementation of automated

and non-destructive methods for its detection and management seeing that currently they can only be visually detected through vigorous inspection of the bunches.

(a) (b)

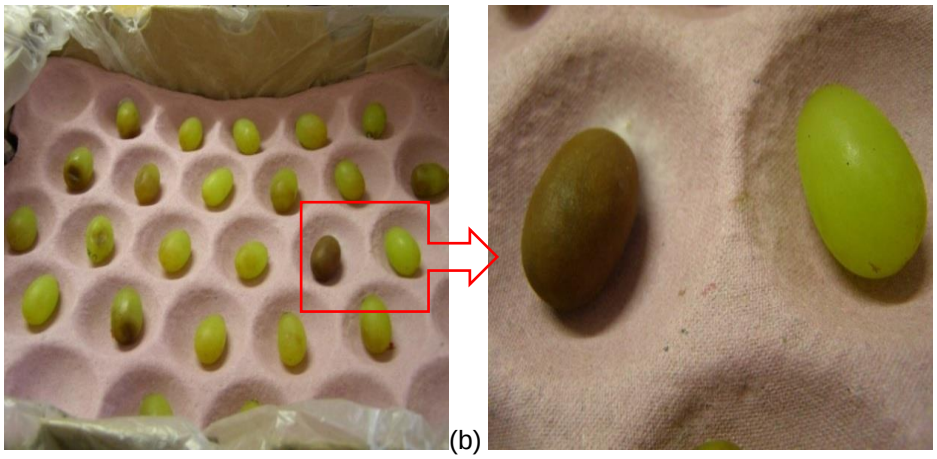

**Figure 6.1** (a) The browning stage of berries in a peach tray after five weeks of cold storage and (b) a berry showing chocolate browning (caused by a fungal infection) and a berry not showing any browning

(a) (b)

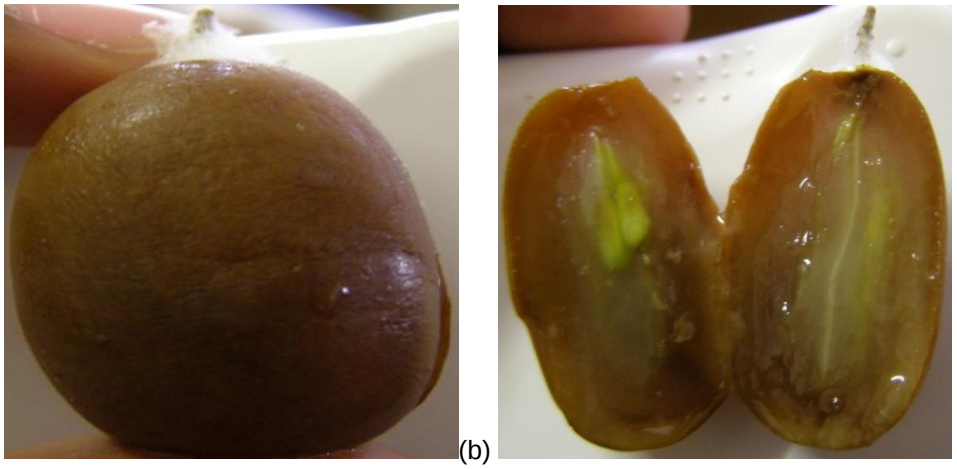

**Figure 6.2** A Regal berry showing chocolate browning on the outside (a) and (b) on the inside caused by a fungal infection

(a) (b)

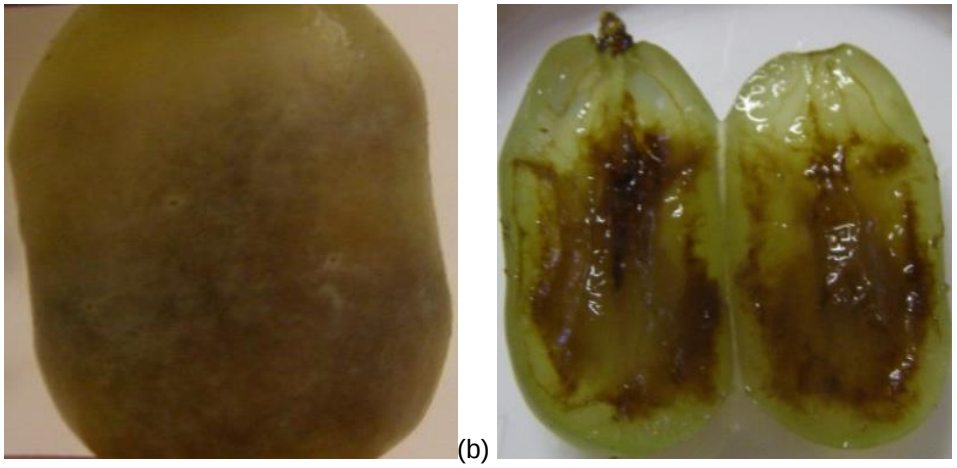

**Figure 6.3** Internal browning as seen (a) from the outside and (b) on the inside of a Thompson seedless berry

(a)  (b) 

**Figure 6.4** A Thompson seedless berry is showing (a) no signs of browning on the inside and (b) onset of internal browning around the vascular tissue.

Several reports have shown that NIR spectroscopy coupled with chemometric techniques such as partial least squares (PLS) and partial least squares-discriminant analysis (PLS-DA) proved to be valuable and versatile tools for the simultaneous determination of an array of quantitative and qualitative parameters on the same sample (Perez-Enciso and Tenehaus, 2003). This includes analysing and classifying a variety of fruit defects and diseases. Kavdir *et al*. (2007) used NIR spectroscopy to assess firmness, skin and flesh colour, as well as dry matter content of pickling cucumbers. Fu *et al.* (2007) demonstrated the utility of visible (VIS)-NIR spectroscopy for discriminating between pear fruit with internal brown heart defects and clear ones. NIR spectroscopy was also utilised successfully to measure microstructure-related changes that occurred because of the internal damage of apples (Clark *et al*., 2003). Ozanich (2001) detected moderate to severe internal disorders in apples such as water-core, internal browning and rot also using NIR spectroscopy. Stemming from these successful applications of NIR spectroscopy, the next logical step was to explore this technology on table grapes. This would also lay the groundwork to also pursue vision-based systems or techniques in the vineyard to evaluate the quality of grapes similar to what Pothen and Nuske (2016) have already done with evaluating the colour development and harvest-readiness of intact table grapes.

PLS-DA is a derivative of the standard PLS regression algorithm that uses class variables instead of numeric variables (Barker and Rayens, 2003). PLS-DA have been used in previous studies to assess which genes are useful to discriminate between different statuses of cancer (Perez-Enciso and Tenenhaus, 2003). Folch-Fortuny *et al.* (2016) used it successfully to make a distinction between healthy and infected citrus fruit. Artificial neural networks (ANN) is a machine-learning framework that attempts to mimic the learning pattern of natural biological neural networks and is based on their ability to “learn” throughout a training procedure exactly where inputs and a set of anticipated results are given. ANN is typically organised in layers, and these layers are made up of interconnected nodes that contain an activation function (Ramadan *et al*., 2005). ANN is a well-established analytical tool and has been used successfully in

combination with other techniques such as principal component analyses (PCA) and PLS. Baldwin *et al*. (2011) used it to help analyse the data obtained by electronic noses and electronic tongues for various parameters from different products. Rodríguez *et al.* (2010) again determined the quality control of Colombian coffee qualities and Cajka *et al.* (2009) confirmed the origin of honey, while Pérez-Magariño *et al*. (2004) classified Spanish denomination of origin rosé wines.

The aim of this study was, therefore, to scan table grape bunches of the cultivar Regal Seedless non-destructively, cold-store the grapes from 0 weeks to six weeks at 0°C and visually assess them at each week after cold storage for the presence of the two different browning phenotypes, chocolate browning and friction browning. Since individual berries behave like individual experimental units although they are part of a bunch, it was an important objective that whole bunches be investigated (table grapes are exported as whole bunches and not individual berries). The presence (1) or absence (0) of the browning phenotypes were noted and then combined with the spectra of the grapes as well as the column that indicated if there was a defect or not into one large dataset. The data was then analysed using PLS-DA and ANN.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Experimental design

Regal Seedless bunches were harvested from two different vineyard blocks—one in the Hex River Valley and one in Wellington, Western Cape, South Africa during 2016. The GPS coordinates for the Wellington vineyard is 33°38'22,0"S,10°50'47,6"E and that of the Hex River Valley 33°27'53,9"S, 19°39'43,7"E.

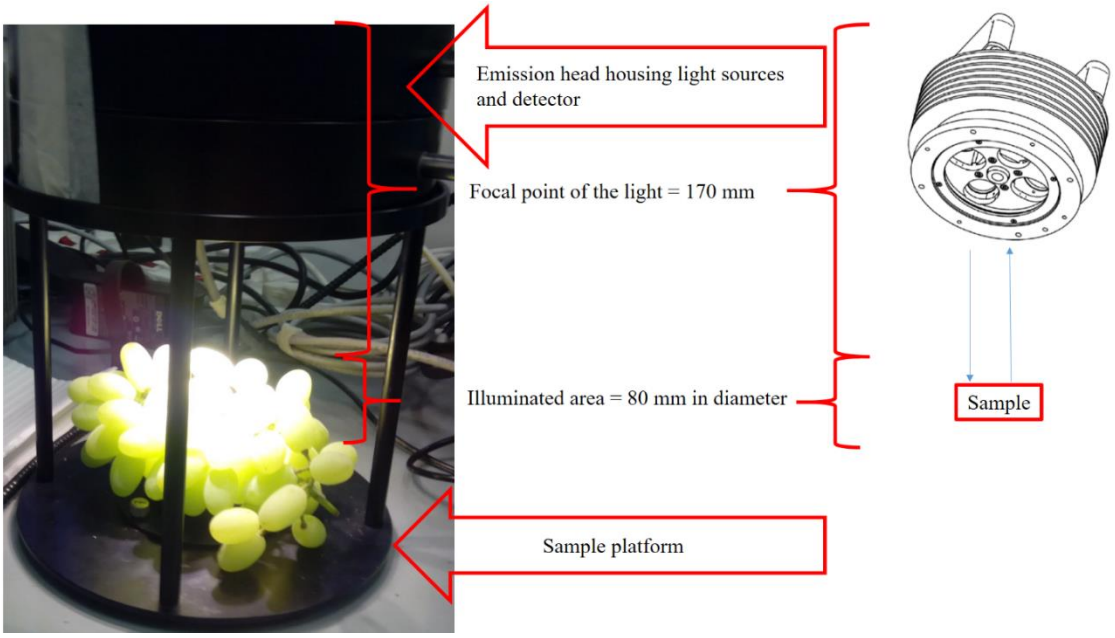


**Figure 6.5** Scanning of an intact Thompson Seedless table grape bunch contactless with the MATRIX-F NIR spectrometer (adapted from Daniels *et al*., 2019).

### 6.2.2 Vineyard treatments and harvesting of bunches

The grapes were prepared according to the standard protocol for export table grapes (Van der Merwe, 2012) that involves shortening, thinning of bunches, followed by the application of gibberellic acid to bunches. Physical removal of some of the berries and laterals on bunches occurred when the berries were 8 to 10 mm in diameter so that bunches would not be too compact at ripening and harvesting. The grapes were harvested between 9 and 10 am and packed in 4.5 kg closed-top, corrugated fibreboard cartons used in the table grape industry. Each bunch was placed in an individual plastic carry bag. The entire carton content was enclosed in a 2-mm perforated, low-density polyethene (LDPE) liner bag that contained a corrugated cardboard sheet at the bottom to reduce abrasion damage. An Uvasys® sulphur dioxide ($SO_2$) generator sheet (http://www.uvasys.com/) covered the grapes to control decay. Decay, also known as grey mould or rot, is caused by an array of fungi like *Botrytis cinerea*, *Aspergillus niger*, *Rhizopus stolonifera* and *Penicillium* species and is responsible for a large part of the postharvest problems experienced with table grapes when they reach the overseas markets (Castillo *et al*., 2010; Gabler *et al*., 2010). The LDPE liner bag containing the grapes and $SO_2$ sheet was folded, the boxes closed, carried out of the vineyard to the end of each row and placed in the shade until all the other grapes were harvested. Packed boxes were loaded into an air-conditioned vehicle and transported by road to the chemical analytical laboratory of the Institute for Wine Biotechnology, Stellenbosch University.

### 6.2.3 Near-infrared (NIR) spectroscopic measurements

NIR spectra of intact table grape bunches were acquired with the diffuse reflectance MATRIX-F FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) (Figure 6.5) that was operated with a contactless measurement head coupled to the spectrometer with a cable for power supply and lamp switching. Samples were illuminated by four air-cooled tungsten NIR light sources (12 V, 5 W) mounted in the measurement head. The measurement area on the sample was 250 mm and the working distance between the emission head and the samples was 170 mm. The light scattered by the sample was collected and guided to the spectrometer with an optic fibre of 1 m. The standard viticultural practice of thinning table grape bunches ensured that the bunches were not too compact and as much as possible of the surface of as many as possible berries were exposed so that proper information collection of as many of the berries on the intact bunch was collected. It is important to keep in mind that a grape bunch is not a uniform sample with many edges increasing the signal-to-noise ratio and that information collected during the scans is of all these different parts of the bunch. The focal point of the lights was 17 cm. The detecting emission head also housed a very sensitive, thermoelectric-cooled and temperature-controlled InGaAs diode detector. The scanning of each bunch was for 60

seconds—once in the middle of the one side and once in the middle on the other side. During each scanning procedure, 32 scans took place per side averaged into a single spectrum. Spectral data were collected in the range 12500 to 4000 $cm^{-1}$ (resolution, 2 $cm^{-1}$; scanner velocity, 10 kHz; background, 32 scans; sample, 32 scans). The number of data points collected during each scan was 1801. The Log (1/R) transformed absorbance spectra were processed using OPUS version 7.2 (Bruker Optics, Ettlingen, Germany) for Windows, and saved after the spectral acquisition. All the boxes were scanned immediately upon arrival in the laboratory (Week 0) and then again at the start of each week of cold storage (Week 1 to Week 6).

### 6.2.4 Visual assessment of browning phenotypes

Visual evaluation of all the boxes was done immediately on the day of harvest (Week 0) and after every week of cold storage (Week 1-Week 6). Berries were removed from the bunch with scissors and individually evaluated for one type of defect only, chocolate browning or friction browning, whichever defect was the most pronounced on it. In the data collection, the status of each grape berry was not recorded, instead, the weight, in grams, of all the grape berries with the same specific defect was recorded. The total weight of each bunch as well as the total weight of the box containing all the grapes were also recorded. For ease of analysis, all bunches were assigned a value 0 when no defect was present at all and a value of 1 when any defect was present. The incidence of chocolate browning was either absent in the other weeks of coldstorage or too low in weeks 3 to 6. Data for week 3 and week 4 and week 5 and week was, therefore, combined for data analysis. Similarly, for friction browning where data of week 3 and 4 was combined.

### 6.2.5 Data analysis

#### 6.2.5.1 Partial least squares discriminant analysis (PLS-DA)

In PLS, the dummy variable Y is used as a response variable, and it is set to 1 if the sample is one of either class and 0 if not. In this study, the defects were scored as 0 = no defect and 1 = defect present. The cut-off value was set at 0.5, above which the sample was predicted as 1 and below at which it was predicted as 0. In this study, the optimal number of latent variables (LV) was chosen based on the minimum root mean square error of cross-validation (RMSECV). The model was cross-validated by Venetian blinds of 10 data splits with 10 samples in each split. Also, specificity (True Negatives/(True Negatives + False Positive)), sensitivity (True Positives/(True Positives + False Negatives)) and classification error rate (CER) for calibration and cross-validation were also used to evaluate the model's performance (Ballabio and

Consonni, 2013; Nicolaï *et al*., 2007). All calculations were performed by PLS-Toolbox for MATLAB (version 8.6.1, Eigenvector Research Inc., USA).

#### 6.2.5.2 Artificial neural networks (ANN)

To determine the relationship between the spectral information of the studied bunches and the presence or absence of different browning phenotypes using ANN, the following procedures were followed. The relevant entries were selected (e.g. weeks in cold storage 3 and 4 from the "No defects - REGAL Week 3 and Week 4, Week 5 and Week 6" dataset). The data was normalised and labelled (0 for no defect and 1 for defect). The two combined datasets had a total of 192 samples, which were divided into four sets. That is 96 training samples (~1/2), 32 validations 1 sample (~1/6), 32 validations 2 samples (~1/6) and 32 testing samples (~1/6). A maximum of four hidden layers was selected and the number of nodes (with a max of 25) in each layer and alpha was set independently. The optimal parameter combination was selected via a grid search (running the model for each set of parameters). The dataset dimensions (number of wavenumbers) were reduced so that the number of dimensions was less than the number of samples, using principal component analysis (PCA).

Cohen's kappa is a statistic (Cohen, 1960; Cohen, 1968) that indicates how well a classification model does in comparison to predicting just the average (McHuhg, 2012). The interpretation of the Kappa score that was used is displayed in Table 6.1. If the kappa score was between 0 and 0.2, there was no agreement between the measured and predicted label, while a score between 0.21 and 0.39 showed minimal agreement and between 0.4 and 0.59 showed weak agreement. There was moderate agreement when the kappa score was between 0.6 and 0.79. A kappa score between 0.8 and 0.9 corresponded to strong agreement between the measured and predicted labels while above 0.9 showed almost perfect agreement. In this study, a kappa score indicating strong was considered 'good' and almost perfect was considered to be 'great'.

**Table 6.1** Kappa score interpretation guide.

| **Kappa** | **Interpretation** |
|---|---|
| 0.0 – 0.20 | No agreement |
| 0.21 – 0.39 | Minimal agreement |
| 0.40 – 0.59 | Weak agreement |
| 0.60 – 0.79 | Moderate agreement |
| 0.80 – 0.90 | Strong agreement |
| >0.90 | Almost perfect agreement |

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 PLS-DA

The CER of chocolate browning for Week 5 and Week 6 was lower (22%) than that of Week 3 and Week 4 for both class 0 and class 1 (Table 6.2) This means that the chocolate browning could be predicted with an accuracy of 75% for Week 3 and Week 4 and 78% for Week 5 and Week 6. This might be attributed to the longer times that the samples of Week 5 and Week 6 were in cold storage and the defect, therefore, developed and/or appeared more pronounced on the bunches. The incidence could also have been more (more chocolate brown berries) in Week 5 and Week 6 than in Week 3 and Week 4. This is also observed where the specificity is concerned, since it was better for class 0 for Week 3 and Week 4 (81%) and better for class 1 in Week 5 and Week 6 (82%). The sensitivity, on the other hand, was higher for class 0 of Week 5 and Week 6 (82%) and for class1 for Week 3 and Week 4 (81%) (Figure 6.6).

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 class 1 of chocolate browning for Week 3 and Week 4 (25%) (Table 6.2). Both the specificity and sensitivity of class 0 and class 1 for friction browning were not as good as for chocolate browning.

The number of flowers on a bunch determines the number of berries on a bunch, so in years that many flowers dropped, fewer berries will develop into berries on bunches and vice versa (Vasconcelos *et al*., 2009). The implication of this for this study is that in years that many flowers were dropped, bunches would have been straggly, allowing more light to interact with the other parts of the bunch and the background and not many berries during scanning. In years when little flowers were dropped, bunches would have been compact and a lot of light would have been reflected from many more berries, but some berries would have had less surface area available for the light to interact with. In both scenarios, fewer berries and more berries on a bunch would also have played a role in the number of specific browning phenotypes that would develop, for example in the event of fewer berries for friction browning.

Also, a table grape bunch consist of berries attached to pedicles/stems, in turn, attached to a central axis (Chervin *et al.,* 2012). Each berry, however, acts as an individual fruit on the bunch. Different cultivars have a different number of berries on a bunch (May, 2000; Vasconcelos *et al*., 2009) and the size and the weight of these berries differ. Regal Seedless can have bunches weighing up to 870 grams, 780 grams and 915 grams respectively if they contain 150 berries that each weight 5.2 grams (Van der Merwe, 2012). The application of PGRs (amount, concentration and combination as well as the time of application) would also have played a major role in the size of the berries (Raban *et al*., 2013). Enlargement sprays are usually applied when berries are 4 to 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 was done when the berries were 8 to 10 mm in diameter to ensure a looser bunch. It is also important that proper bunch thinning be done so that the bunch is not too compact so that as much as possible of the surface of as many berries are exposed. This will ensure that as much possible information on the berries and the bunch can be collected. Friction browning is mostly concentrated at the pedicel part of the berries and the light did not always fall completely on those parts and since they were obscured by the other berries. It is also possible that the spectra would 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 6.7).

**Table 6.2** The classification error rate, specificity and sensitivity of the PLS-DA models constructed for chocolate browning (CB) and friction browning (FB) of Regal Seedless grapes

| Condition | Sample set | Class 0 | | | Class 1 | | |
|---|---|---|---|---|---|---|---|
| | | CER[a] | Spec[b] | Sen[c] | CER | Spec | Sen |
| CB: W3and4[d] | Calibration | 0.15 | 0.865 | 0.815 | 0.15 | 0.815 | 0.865 |
| CB:W3and4 | CV | 0.25 | 0.808 | 0.692 | 0.25 | 0.692 | 0.808 |
| CB: W5and6[e] | Calibration | 0.13 | 0.875 | 0.864 | 0.13 | 0.864 | 0.875 |
| CB: W5and6 | CV | 0.22 | 0.722 | 0.818 | 0.22 | 0.818 | 0.722 |
| FB: W3and4 | Calibration | 0.41 | 0.412 | 0.757 | 0.41 | 0.757 | 0.412 |
| FB: W3and4 | CV | 0.46 | 0.353 | 0.714 | 0.26 | 0.714 | 0.353 |

[a]Class error rate defined as the mean of the false positive and false positive rates; [b]Specificity; [c]Sensitivity. [d]Weeks 3 and 4; [e]Weeks 5 and 6

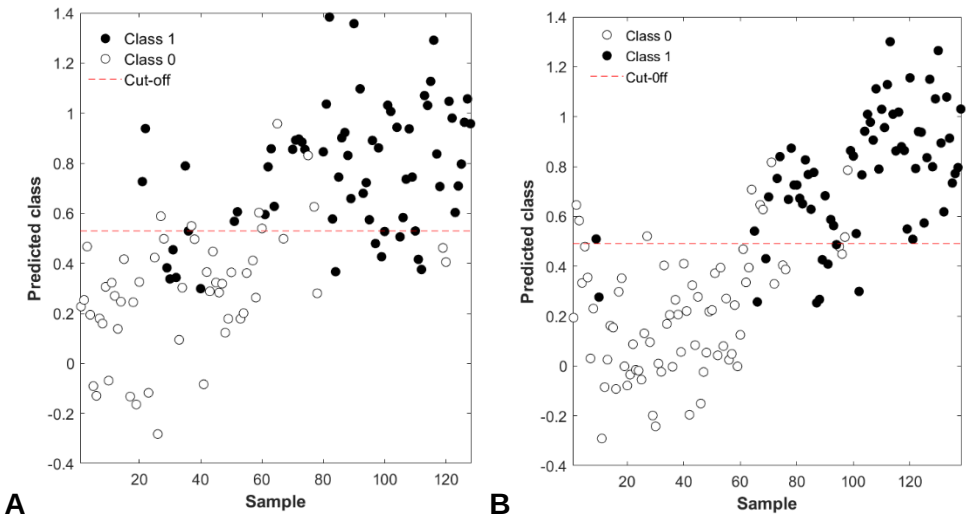


**Figure 6.6** Absence (Class 0, open circle) or presence (Class 1, close circle) **chocolate browning** performed by PLS-DA model, based on NIR spectral data. A. Weeks 3 and 4 and B. Weeks 5 and 6.

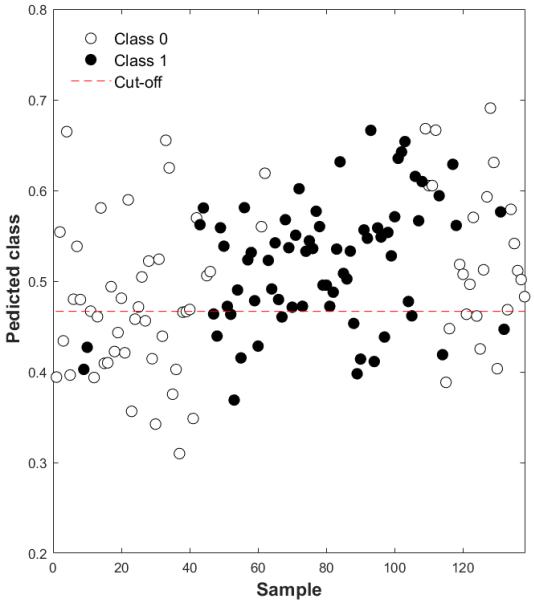


**Figure 6.7** Absence (Class 0, open circle) or presence (Class 1, close circle) **friction browning** performed by PLS-DA model, based on NIR spectral data. A. Weeks 3 and 4.

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. The information that is thus collected during the scans is of all these different parts of the bunch (berries and stems). All this played a role in the CER as well as the specificity and sensitivity of class 0 and class 1 obtained for friction and chocolate browning. The error rates in Haff *et al*. (2013)'s study when they developed an algorithm to identify spots generated in hyperspectral images of mangoes infested with fruit fly larvae were much lower than the ones in this study. Similarly, in the studies of Leemans *et al*. (2002) when they classified two different apple cultivars using machine vision and that of Li *et al*. (2016) when they looked at skin defects of bi-coloured peaches. This could be due to the larger size of mangoes, apples and peaches that they used and, therefore, the larger surface area that was available to the NIR light than the surface area of the single berries that was affected with the browning disorder in this study.

### 6.3.2 ANN

In Table 6.3, the chocolate browning vs 'no defects' for Regal Weeks 3 and 4 showed there was strong agreement (test kappa = 0.88) between the measured and predicted labels for the data when PCA was performed and the number of features (wavenumbers) was reduced to 50 (line 3). From lines 2 and 4 it can be seen that accurate predictions can be done (moderate agreement), but not necessarily consistently (test kappa = 0.71 in line and 0.67 in line 4). This is due to a lack of data causing an insufficient representation of healthy and damaged spectra variation in the training sample set. Chocolate browning vs no defects for Regal Seedless in Week 5 and Week 6 showed a strong agreement between the measured and predicted labels

for the data when PCA was performed and the number of features (wavenumbers) was reduced to 30 (line 2) and 15 (line 4) respectively. Friction vs no defects for Regal in Week 3 and Week 4 showed there is a moderate agreement between the measured and predicted labels for the data when PCA is performed and the number of features is reduced to 80 (line 2).

In other studies where ANN was utilised as analysis technique Zarifneshat *et al*. (2012) successfully evaluated it as an alternative technique to predict the bruise volume of apples. Binetti et al. (2017) used it create models to classify olive oil cultivars based on multiple types of information, standard merceological parameters, NIR data, and nuclear magnetic resonance (NMR) fingerprints. NMR data, however, showed the highest capability (in some cases, accuracy > 99%) to classify cultivars.

**Table 6.3** The number of runs done for each cold storage condition (Week 3 and Week 4 and Week 5 and Week 6) and browning defect, chocolate browning (CB) and friction browning (FB) of Regal Seedless grapes. The number of feature reduction done per PCA and the kappa scores for the first and second validations as well as the test validation are also shown.

| Condition | Runs | PCA | Alpha (α) | Validation kappa score | 2nd Validation score | Test kappa score |
|---|---|---|---|---|---|---|
| **CB: W3and4[a]** | 1 | n/a | 0.01 | 1.0 | 0.65 | 0.59 |
| | 2 | 80 | 0.1 | 1.0 | n/a | 0.71 |
| | 3 | 50 | 1e-5 | 1.0 | 0.656 | 0.88 |
| | 4 | 50 | 1e-6 | 0.93 | n/a | 0.69 |
| **CB: W5and6[b]** | 1 | n/a | 1e-3 | 1.0 | 0.47 | 0.68 |
| | 2 | 30 | 1e-5 | 1.0 | 1.0 | 0.83 |
| | 3 | 30 | 1e-7 | 1.0 | 1.0 | 0.80 |
| | 4 | 15 | 1e-6 | 1.0 | 1.0 | 0.83 |
| **FB: W3and4[c]** | 1 | n/a | 1e-2 | 0.78 | n/a | 0.37 |
| | 2 | 80 | 1e-2 | 1.0 | n/a | 0.73 |
| | 3 | 50 | 1e-5 | 1.0 | 0.49 | 0.51 |
| | 4 | 65 | 1e-9 | 0.92 | 0.77 | 0.47 |

**[a]Weeks 3 and 4; [b]Weeks 5 and 6**

## 6.4 CONCLUSIONS

This study shows the possibility of detecting the presence or absence of different browning phenotypes during different cold storage periods on intact table grape bunches through contactless and non-destructive scanning using NIR spectroscopy. This coupled with PLS-DA analysis and the machine learning technique ANN showed that chocolate browning classification is better than friction browning with both techniques. Table grape bunches are very complex and heterogeneous samples and the difficulty of building accurate classification models for the different browning phenotypes are also highlighted here. In the global context where consumer preference dictates the market, it is paramount that producers have an idea of what the postharvest quality of their produce is going to be. Real-time objective measurements, such as the contactless measurement of intact table grape bunches in the pack-house with NIR

spectroscopy using classification models such as those constructed in this study, present a feasible alternative to exporting table grapes that might turn brown before or when they reach the intended export market. Such measures may help producers to make appropriate marketing decisions, especially for developing countries that operate in a highly competitive niche market and cannot afford any postharvest losses.

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# Chapter 7: General discussions and conclusion

## 7.1 INTRODUCTION

Table grape quality is a very complex concept since it is both of quantitative and qualitative nature. Quantitative attributes are those measured in the vineyard and the packhouse such as total soluble solids (TSS), titratable acidity (TA), and pH of grapes, while qualitative characteristics are the physical aspects such as the shape, colour and size of bunches (Fahmi *et al*., 2012). Both quantitative and qualitative aspects influence the sensory characteristics of table grapes (Sabir and Sabir, 2013) and determine the consumers' preference and buying decision. The quality should thus be perfect on the retail shelves. Table grapes, however, are non-climacteric fruit and maintenance of quality after harvest is, therefore, key throughout the value chain. This chain is also very complex since it not only involves the multiple packaging levels but also the cold storage and transport period during which defects such as rot, sulphur dioxide ($SO_2$) damage, berry crack and one or more of the 17 different browning phenotypes on white seedless grapes can usually occur. The exploration of technology such as near-infrared (NIR) spectroscopy to try to circumvent some of the problems experienced with table grape quality was thus explored during the different stages (vineyard, packhouse and cold storage) during which quality can be monitored in this study. Different chemometric techniques such as partial least squares (PLS) regression, partial least squares discriminant analysis (PLS-DA) and artificial neural networks (ANN) were used to extract information from near-infrared spectra.

## 7.2 PROJECT AIMS AND OBJECTIVES ACHIEVED

### 7.2.1 Identifying the incidence and intensity of table grape defects

Visual perception of fruit is a very important aspect to consumers. Table grapes thus need to be free of any defects once available on retail shelves. Maintaining grape quality throughout the cold storage period is, therefore, of utmost importance. The current practice is a visual inspection of bunches before packaging to assess the presence of defects before cold storage and transportation to export markets. Many previous studies have focused on one defect, e.g. rachis browning on one or more cultivars and the effect that plant growth regulators and packaging has on them (Raban *et al*., 2013); also on preharvest treatments to improve postharvest quality (Champa *et al*., 2014; Sabir and Sabir, 2019), postharvest treatments (Peyro *et al*., 2017; Sabir and Sabir, 2019) as well as a humidification system to control moisture loss and quality of table grapes during cold storage (Ngcobo *et al.*, 2013). To bring novelty to this continuous cycle of evaluation of single or multiple aspects of table grape postharvest quality, it was important to

investigate the incidence and intensity of as many as possible of these different defects and incorporating as much variability as possible. Three different cultivars (Regal Seedless, Thompson Seedless and Prime) were harvested from three different locations (Wellington, the Hex River Valley and Kakamas) over two years (2016 and 2017). The results showed $SO_2$ damage prevalence on all three white seedless table grape cultivars investigated. The incidences of fungal infection, sunburn and abrasion damage were high on Regal Seedless and Thompson Seedless in 2016. Contact, mottled and friction browning as well as bruising damage had higher incidences in 2017 than in 2016. Overall, the intensity of defects was very high in 2016 except on Regal Seedless from the Hex River Valley. Prime from Kakamas and Wellington had the highest intensity of defects in 2017. These results are in agreement with the findings of Zahedipour *et al.* (2019), namely that table grapes from different growing systems (vineyards) show different postharvest behaviours. Moggia *et al*. (2015) also found that radial and mixed internal browning of apples after storage were highly influenced by preharvest conditions (geographic/climatic and maturity variables). The inclusion of such parameters and the effect thereof is thus important. It is, therefore, important to include these quality defects in efforts to develop rapid and non-destructive methods for onsite and online prediction of table grape quality.

### 7.2.2 Single berry quality prediction

Determining the correct harvest maturity parameters of table grapes is one of the most essential steps before harvesting. Measuring a parameter such as TSS is, however, very time-consuming and destructive. Developing faster, non-destructive and more accurate ways of determining TSS in the vineyard or during any of the postharvest stages can lead to a decrease in losses suffered by industry at the postharvest stage. Development of a non-destructive way of determining harvest maturity and quality of table grapes in the vineyard before and after harvesting over two years using handheld and benchtop spectrometers on intact table grape berries was, therefore, investigated. Spectra that were taken in the laboratory with the handheld instrument were more homogenous than the ones taken in the vineyard with the same spectrometer. The results obtained with the benchtop instrument were not as good as the results obtained with the handheld instrument in the laboratory. Both instruments delivered spectra that have the potential to predict TSS in the laboratory and the advantages associated with using handheld instruments in the vineyard were evident as they showed higher accuracy in terms of prediction. Highlighted, however, was the need for exploring the effects of spectral pre-processing and selection of wavelengths. When Cao *et al.* (2010) and Omar (2013) investigated the use of NIR spectroscopy to determine pH and SSC contents of whole table grape berries, Cao *et al.* (2010) and Omar (2013) found that the selection of the most effective wavelengths for the determination of a specific parameter is essential. In a similar experiment by Kanchanomai *et al.* (2020) where a NIR portable instrument was also used for assessing the quality of ‘Kyoho’ table grapes, as a

non-destructive means of analysis under laboratory and field conditions, the importance of pre-processing and selection of wavelengths also became evident. The number of samples in Kanchanomai *et al*. (2020) as in other studies relating to measuring TSS on table grapes with handheld instruments (Donis-González *et al*., 2020) was, however, very low and from a single harvest year. It was, therefore, important that this study measured as many berries as possible as not all berries on all bunches, and not all bunches in a row or block get tested before harvesting. This study lays the foundation for corroborating results of experiments such as those conducted by Font *et al.* (2014) where red grapes were counted from high-resolution images of vineyards taken under artificial lighting at night. TSS can, for instance, also be included as a parameter to assist not only with predicting the harvest readiness state of the colour development of an entire vineyard block, in high resolution as Pothen and Nuske (2016) did, but also based on TSS. Amoriello *et al.* (2018) used a hand-held instrument developed from Vis/NIR spectroscopy to classify the maturity stage of six early-to-late apricot varieties at the wholesale market. The possibility of using this strategy for table grapes should be investigated.

### 7.2.3 Whole bunch internal quality prediction

The determination of internal maturity parameters of table grapes is usually done destructively using time-consuming manual methods. The possibility was investigated to determine whether key fruit attributes, TSS, TA, sugar:acid ratio (TSS:TA ratio), pH, and BrimA (TSS – k x TA), a new parameter that was added, could be determined on intact table grape bunches using Fourier transform near-infrared (FT-NIR) spectroscopy in a contactless measurement mode. The novelty of these experiments, therefore, did not just reside in that whole bunches and not single berries were scanned intact and completely contactless, but in that PLS regression models that were developed for the five maturity and sensory quality parameters using grapes obtained from two consecutive harvest seasons (2016 and 2017); from different origins (the Hex River Valley, Wellington and Kakamas). Results from these experiments thus not only provide the first steps towards a completely non-destructive and contactless determination of internal maturity parameters of intact table grape bunches, on a multi-cultivar level (Li et al., 2019) but opens up the multi-year and multi-origin level as well. Rungpichayapichet *et al.* (2016) for instance, found that model robustness was influenced by harvest year when they evaluated the effect of the harvest year on NIR spectroscopy prediction models to determine the postharvest quality of mango fruit. They found high prediction errors when models from single harvest years were used to predict the data of other years, whereas using combined data from two or three years for calibration greatly enhanced the prediction accuracy. The effect of a whole range of spectral pre-processing techniques, baseline correction, multiplicative scattering correction (MSC), standard normal variate (SNV) moving window smoothing (MWS), Savitzky-Golay first derivative (SG1d)

and different combinations of these, were also explored. TSS performed best when (MSW) + MSC was used as a spectral pre-processing technique, TA with SNV, TSS:TA ratio with SG1d, pH with SG1d and BrimA with MSC. A similar study by Parpinello et al. (2013) using spectral pre-processing techniques to investigate the relationship between sensory and NIR spectroscopy in consumer preference of table grape (cv Italia) showed that different comibinations of spectral pre-processing techniques had different effects on the resultant PLS models.

### 7.2.4 Whole bunch external quality prediction

Table grape browning is a complex physiological disorder that affects the quality of the fruit during cold storage. Several browning phenotypes have been described of which some may be present on the fruit at harvest, albeit not visible to the naked eye. The inability to correctly and accurately classify which grapes will eventually turn brown remains a problem. Novel and innovative ways need an investigation to manage the problem that hampers the progressive and sustainable growth of the table grape industry. Given the complex nature of the browning phenomenon, a technique such as NIR for non-destructive classification of each browning phenotype is necessary. NIR coupled with PLS-DA and ANN were, therefore, used to classify bunches as either clear, or as having chocolate browning or friction browning based on the spectra obtained of intact table grape bunches. Bunches of Regal Seedless grapes cold-stored over different periods were used for this objective. The results obtained in this experiment thus open up new possibilities for the development of quality checks of table grape bunches before packaging and export not just in terms of quantitative parameters, but qualitative ones as well. Cubero *et al.* (2012) for instance used image analysis to develop a new method for detecting pedicels/peduncles and assess the size of grapevine berries. Based on their results this can be easily implemented to accurately estimate the weight of a wide range of fruits including table grapes in an automated inspection system. A non-invasive, objective and quantitative way to determine bunch compactness based on image analysis methodology was also develop by Cubero *et al.* (2015). Siedliska *et al.* (2014) evaluated and created supervised classification models of bruise detection and cultivar detection for five apple cultivars with the use of a hyperspectral imaging system in the VNIR (Visible and Near-Infrared) and SWIR (short wavelength infrared) spectral regions. Yamamoto *et al.* (2015) developed a new image analysis system that can simultaneously evaluate multiple appearance characteristics such as the colour, shape and size, of agricultural products such as strawberries in detail. This has significant implications for the table grape industries; it will now be possible to non-destructively evaluate bunches during the packaging process using these technologies and to determine the probability of defects such as browning types being present when grapes reach the export markets..

## 7.3 CONCLUDING REMARKS

This study has demonstrated considerable variability in the external and internal berry quality on whole table grape bunches. The influence of variation of spectrum measurement position in apple SSC analysis using NIR spectroscopy was studied by Fan *et al.* (2016). The results indicated that the influence of measurement position on the spectra affected the prediction accuracy of SSC. Table grape bunches should, therefore, be scanned from as many as possible positions, since model accuracy is dependent on many factors.

In practical terms, the handheld spectrometers can be used in the vineyard before harvest to test as many as possible berries non-destructively for their sugar content and, therefore, ripeness level, to prevent grapes being harvested that are not at the correct maturity level or harvest readiness. Producers will have a decrease in the postharvest damages suffered due to the incorrect determination and classification of grapes for the export market based on their TSS, TA and TSS:TA ratio.

Another practical implication of the results for the table grape industry is that much quicker decisions can be taken regarding the grape quality, either using one of the parameters measured on whole table grape bunches, or all of them collectively, to determine which class and which export market is suitable for table grapes. Mounting an instrument like the MATRIX-F spectrometer used in this study over the packing line is easy for use after harvest in the packing shed to test the TSS, TA and TSS:TA ratio contactless, simultaneously and non-destructively. With the inclusion of BrimA producers can market grapes accordingly, e.g. low sweetness-high acidity, neutral, high sweetness-low acidity tasting grapes, etc.

Future work aimed at improving calibration models for especially pH and TA, should include wavelength selection. Exploration of other techniques such as ANN, machine-learning algorithms such as super vector machines is essential to improve current results. The accuracy of the prediction models obtained for classifying the different browning defects was excellent, and this shows good promise for practical applications during quality control on-farm (packing shed) and in the packhouse (sorting, grading and packaging) to classify bunches based on probability of developing certain browning phenotypes. It can be concluded that this is a viable method for investigating the automated identification of selected defects in whole table grape bunches, provided the careful selection of the samples in the healthy spectral database.

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