

Development of infrared spectroscopic methods to assess table grape quality

by

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Declaration

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Summary

The two white seedless table grape cultivars, Regal Seedless and Thompson Seedless fulfil a very important role in securing foreign income not only for the South African table grape industry, but the South African economy as a whole. These two cultivars, however, are like so many other white table grape cultivars, also prone to browning, especially netlike browning on Regal Seedless and internal browning on Thompson Seedless grapes. This leads to huge financial losses every year, since there is no established way to assess at harvest, during storage or during packaging, whether the grapes will eventually turn brown. In other words, there is no well-known protocol of assessing the browning risk of a particular batch of grapes prior to export. Numerous studies have been undertaken to determine the exact cause of browning and how it should be managed, but to date, no chemical or physical parameter has been firmly associated with the phenomenon.

The overall aim of this study was thus to find an alternative way to deal with the problem by investigating the potential of near infrared (NIR) spectroscopy as a fast, non-destructive measurement technique to determine the browning potential of whole white seedless table grapes. A secondary aim was the determination of optimal ripeness of table grapes. In this way harvest maturity and quality indicative parameters namely total soluble solids (TSS), titratable acidity (TA), pH, glucose and fructose, also associated with the browning phenomenon, was quantified using models based on infrared spectra.

Three different techniques (a) Fourier transform Near Infrared (FT-NIR), (b) Fourier transform – Mid Infrared (FT-MIR) and (c) Fourier transform – Mid Infrared Attenuated Total Reflectance (FT-MIR ATR) spectroscopy were investigated to determine these parameters. This was done so that a platform of different technologies would be available to the table grape industry.

The grapes used in this study were harvested over two years (2008 and 2009) and were sourced from two different commercial vineyards in the Hex River valley, Western Cape, South Africa. Different crop loads (the total amount of bunches on the vines per hectare) were left for Regal Seedless (75 000, 50 000 and 35 000) and for Thompson Seedless (75 000 and 50 000). Three rows were used for Regal Seedless and two rows for Thompson Seedless. Each row had six sections which each represented a repetition for each crop load. In 2008 these cultivars were harvested early at 16°Brix, at optimum ripeness (18°Brix) and late at 20°Brix. In 2009 they were harvested twice at the optimum ripeness level.

Berries from harvested bunches were crushed and the juice was used to determine the reference values for the different parameters in the laboratory according to their specific methods. The obtained juice was also scanned on the three different instruments. Different software (OPUS 6.5 for the FT-NIR and FT-MIR ATR instruments and Unscrambler version 9.2 for the FT-MIR instrument) as well as different spectral pre-processing techniques were also evaluated before construction of the models for all the instruments.

Partial least squares (PLS) regression was used for the construction of the different calibration models. Different regression statistics, that included the root mean square error for prediction (RMSEP); the coefficient of determination (R^2); the residual prediction deviation (RPD) and the bias were used to evaluate the performance of the developed calibration models. Calibration models which are fit for screening purposes were obtained on the FT-NIR and FT-MIR ATR instruments for TSS (11.40 - 21.80°Brix) ($R^2 = 85.92\%$, RMSEP = 0.71 °Brix RPD = 2.67 and bias = 0.03°Brix), pH (2.94 - 3.9) ($R^2 = 85.00\%$, RMSEP = 0.08 RPD = 2.59 and bias = -0.01) and TA (4.3 - 13.1 g/L), ($R^2 = 90.77\%$, RMSEP = 0.48 g/L RPD = 3.30 and bias = -0.03 g/L). Models for fructose (46.70 – 176.82 g/L) ($R^2 = 74.66\%$, RMSEP = 9.28 g/L RPD = 2.00 and bias = 1.10 g/L) and glucose (20.36 – 386.67 g/L) ($R^2 = 70.71\%$, RMSEP = 11.10 g/L RPD =

1.87 and bias = 1.64 g/L) were obtained with the FT-NIR and FT-MIR ATR instruments that were in some instances fit for screening purposes and in some instances unsuitable for quantification purposes. The FT-MIR instrument gave models for all the parameters that were not yet suitable for quantification purposes.

Combined spectral ranges used for calibration were often similar for some parameters, namely 12 493 - 5 446.2 for TSS and pH, 6 101.9 - 5 446.2 for TSS, TA and fructose and 4 601.5 - 4 246.7 for pH and fructose on the FT-NIR instrument, 2 993.2 - 2 322.3 for pH, TA and glucose and 1 654.3 - 649.4 for pH and glucose on the FT-MIR ATR instrument and sometimes they were adjacent (3 996.6 - 3 661.2, 3 663.5 - 3 327.7 and 3 327.2 - 2 322.3 for TSS and glucose, 1 988.3 - 1 652.8 and 1 654.3 - 649.4 for TSS, pH and TA. Other times they were overlapping (1 654.3 - 649.4 and 1 318.8 - 649.4) for pH, TA and fructose on the FT-MIR ATR instrument. This is a very good sign for transfer of this technology to a handheld device, where adjacent and/ or overlapping wavenumbers are crucial. Instruments which have to determine different parameters over large spectral ranges are not only impractical, because the instrument has to be big, but because it is also very expensive.

Another advantage of implementing especially FT-NIR spectroscopy as a fast, accurate and inexpensive technique for determining harvest maturity and quality parameters is because no sample preparation is necessary and very little waste (few single berries tested) is produced. This is a pre-requisite which is highly recommended in the green era that we are currently living in and will do so for aeons to come. A platform of technologies has now been made available through this study for the determination of the respective parameters in future table grape samples by just taking their spectra on one of the instruments. Indeed something that has not been possible or available for the South African table grape industry before.

Berries for the browning experiments were scanned on a FT-NIR instrument immediately after harvest (before cold storage) and again after cold storage. Before cold storage they were scanned on each side of the berry and after cold storage they were scanned twice on a brown spot if browning was present and twice on a clear spot, irrespective of whether browning was present or not. Inspection of the berries for the incidence of browning after cold storage revealed that Regal Seedless had a higher incidence of browning (68% in 2008 and 66% in 2009) than Thompson Seedless (21% in 2008 and 25% in 2009). Regal Seedless was also more prone to external browning, specifically netlike browning, whereas Thompson Seedless was more prone to internal browning, despite the different phenotypes of browning that were present on both.

Principal component analysis (PCA) done on the spectra obtained before and after cold storage revealed that NIR can capture the changes related to cold storage with the first principal components explaining almost 100% of the variation in the spectra. Classification models also build using PCA was based on spectra of berries that remained clear before and after cold storage and those that turned brown after cold storage. Classification models of berries based on spectra obtained after cold storage (browning present) had a better total accuracy (94% for training- and 87% for test datasets), than the classification models based on spectra obtained before cold storage (79% for training- and 64% for test datasets). The implication of this is that the current models will be able to classify berries in terms of those which have turned brown already and those that remained clear better after cold storage than before cold storage, which is the critical stage where we want to actually know whether the berries will turn brown or not. The potential, however, to use NIR spectroscopy to detect browning before harvest already on white seedless grapes is still present, since all these models were built using the whole NIR spectrum. No variable selection was thus done and all the different browning phenotypes were also used together. Further analysis of the data will thus be based on using variable selection

techniques like particle swarm optimization (PSO) to select certain wavelengths strongly associated with the browning phenomenon and only on the main types of browning (netlike on Regal Seedless and internal browning on Thompson Seedless). This study has major implications for the table grape industry, since it is the first time that the possibility to predict browning with other methods than visual inspection, especially before cold storage, is shown.

Opsomming

Die twee wit pitlose tafeldruif kultivars, Regal Seedless en Thompson Seedless onderskeidelik, speel 'n baie belangrike rol in die verkryging van buitelandse inkomste, nie net vir die Suid-Afrikaanse tafeldruif industrie nie, maar ook vir die Suid-Afrikaanse ekonomie as 'n geheel. Hierdie twee kultivars is egter, soos baie ander wit kultivars, ook geneig tot verbruining. Dit is veral netagtige verbruining op Regal Seedless en interne verbruining op Thompson Seedless wat pertinent is. Hierdie belangrike kwaliteitsprobleme lei jaarliks tot groot finansiële verliese, aangesien daar huidiglik geen gevestigde prosedure is om voor oes, tydens opberging of tydens verpakking te bepaal of die druiwe uiteindelik gaan verbruin nie. Met ander woorde, daar is geen gevestigde protokol vir die beoordeling van die verbruinings risiko van 'n bepaalde groep druiwe voor dit uitgevoer word nie. Talle studies is alreeds onderneem om vas te stel wat die presiese oorsaak van hierdie verskynsel is en hoe dit bestuur moet word, maar geen enkele aspek wat bestudeer is kon tot op hede, herhaaldelik ge-assosieer word met die presiese oorsaak van verbruining nie.

Die oorkoepelende doel van hierdie studie was dus om 'n alternatiewe manier te kry om hierdie probleem aan te spreek. 'n Ondersoek na die potensiaal van naby infrarooi (NIR) spektroskopie as 'n vinnige en nie-vernietigende metings tegniek om die verbruinings potensiaal van 'n wit pitlose tafeldruifkorrel wat nog heel is te bepaal, is onderneem. 'n Sekondêre doel was om die bepaling van optimale rypheid van tafeldruiwe te onderosek. Op hierdie manier is oesrypheid, en die kwaliteitsfaktore, naamlik totale oplosbare vastestowwe (TOVS), titreerbare suur (TS), pH, glukose en fruktose, wat ook gekoppel word aan die voorkoms van verbruining, deur middel van infrarooi (IR) spektroskopie modelle gekwantifiseer. Drie verskillende infrarooi metodes naamlik (a) die Fourier transform naby infrarooi (FT-NIR), (b) Fourier transform - Mid Infrarooi (FT-MIR) en (c) Fourier transform - Mid Infrarooi Verswakte Totale Refleksie (FT-MIR VTR) spektroskopie is gebruik om die aspekte te bepaal. Dis gedoen sodat 'n platform van tegnologie beskikbaar sou wees vir die tafeldruif industrie.

Die druiwe wat in hierdie studie gebruik is, is oor twee jaar (2008 en 2009) en van twee verskillende kommersiële wingerde in die Hexriviervallei, Wes-Kaap, Suid-Afrika ge-oes. Verskillende oesladings (die totale aantal trosse op die wingerdstokke per hektaar) is vir Regal Seedless (75 000, 50 000 en 35 000) en Thompson Seedless (75 000 en 50 000) gelaat. Daar is drie rye gebruik Regal Seedless en twee vir Thompson Seedless. Elke ry het ses vakkies gehad wat dan verteenwoordigend was van 'n herhaling vir elke oeslading. In 2008 is hierdie kultivars by vroeë rypwording (16°Brix), by optimale rypheid (18°Brix) en by laat rypheid (20°Brix) geoes. In 2009 is dit twee keer by die optimale rypheidsgraad geoes. Vir die bepaling van oesrypheid, en die kwaliteitsaspekte is verskillende sagteware (OPUS 6.5 op die FT-NIR en FT-MIR VTR instrumente en Unscrambler weergawe 9.2 vir die FT-MIR instrument) sowel as verskillende spektrale voor-verwerking tegnieke geëvalueer voor die konstruksie van die kalibrasie modelle op die verskillende instrumente.

Parsiële kleinste kwadraat (PKK) regressie is gebruik vir die opstel van kalibrasie modelle vir die bepaling van laasgenoemde aspekte. Verskillende statistieke gegewens is gebruik om die kalibrasie modelle te evalueer, naamlik die bepalingseffisiënt (R^2), die vierkantwortel-gemiddelde-kwadraat fout vir voorspelling (VGKV), relatiewe voorspellingsafwyking (RVA) en sydigheid. Kalibrasie modelle wat geskik is vir keuring is verkry op die FT-NIR en FT-MIR VTR instrumente vir TOVS (11.40 – 21.80°Brix) ($R^2 = 85.92\%$, VGKV = 0.71°Brix, RVA = 2.67 en sydigheid = 0.03°Brix), pH (2.94 – 3.9) ($R^2 = 85.00\%$, VGKV = 0.08 g/L, RVA = 2.59 en sydigheid = -0.01 g/L), en TS (4.3 – 13.1 g/L), ($R^2 = 90.77\%$, VGKV = 0.48 g/L RVA = 3.30 en sydigheid = -0.03 g/L). Modelle vir fruktose (46.70-176.82 g/L) ($R^2 = 74.66\%$, VGKV = 9.28 g/L RVA = 2.00 en sydigheid = 1.10 g/L) en glukose (20.36 – 386.67 g/L) ($R^2 = 70.71\%$, VGKV =

11.10 g/L RVA = 1.87 en sydigheid = 1.64 g/L) is verkry met die FT-NIR en FT-MIR VTR instrumente wat in sommige gevalle gepas was vir keuringsdoeleindes en in sommige gevalle nie geskik was vir kwantifiserings doeleindes nie. Die FT-MIR-instrument het modelle vir al die aspekte gegee wat nog nie vir kwantifiserings doeleindes of vir keuringsdoeleindes geskik was nie.

Gekombineerde spektrale reekse is gebruik vir die kalibrasies wat dikwels soortgelyk was vir sommige aspekte naamlik 12 493 - 5 446.2 vir TOVS en pH, 6 101.9 - 5 446.2 vir TOVS, TS en fruktose en 4 601.5 - 4 246.7 vir pH en fruktose op die FT-NIR instrument, 2 993.2 - 2 322.3 vir pH, TA en glukose en 1 654.3 - 649.4 vir pH en glukose op die FT-MIR VTR instrument. Andersyds, was dit aangrensend (3 996.6 - 3 661.2, 3 663.5 - 3 327.7 en 3 327.2 - 2 322.3) vir TOVS en glukose, 1 988.3 - 1 652.8, 1 654.3 - 649.4 vir TOVS, pH en TS en ander tye was dit weer oorvleuelend 1 654.3 - 649.4 en 1 318.8 - 649.4 vir pH, TS en fruktose op die FT-MIR VTR instrument. Dit is 'n baie goeie teken vir die oordrag van hierdie tegnologie na 'n handgedraagde instrument, waar aanliggende en/of oorvleuelende golfnommers noodsaaklik is. Instrumente wat verskillende aspekte oor groot spektrale reekse moet bepaal is nie net onprakties, omdat die instrument groot moet wees nie, maar dit is ook baie duur.

Nog 'n voordeel van die implementering van veral FT-NIR spektroskopie as 'n vinnige, akkurate en goedkoop tegniek vir die bepaling van oesrypheid, en die kwaliteit aspekte van druiwe is omdat daar geen monster voorbereiding nodig is nie en baie min afval (paar enkele korrels word gemonster) geproduseer word. 'n Voorvereiste wat sterk aanbeveel kom in die groen era waarin ons tans leef en nog vir eeue van nou af gaan doen. 'n Platform van tegnologie is nou beskikbaar gestel deur middel van hierdie studie vir die bepaling van die onderskeie aspekte in toekomstige tafeldruif monsters deur net op een van die instrumente hulle spektra te neem. Inderdaad iets wat nie voorheen moontlik of beskikbaar was vir die Suid-Afrikaanse tafeldruif industrie nie.

Korrels vir die verbruiningseksperimente is geskandeer direk na oes (voor koelopberging) en weer na koelopberging. Dit was voor koelopberging op elke kant van die korrel skandeer en na koelopberging was dit twee maal skandeer op 'n bruin vlek indien verbruining teenwoordig was en twee keer op 'n helder plek, ongeag of verbruining teenwoordig was of nie. Inspeksie van die korrels vir die voorkoms van verbruining na koelopberging het aan die lig gebring dat Regal Seedless 'n hoër voorkoms van verbruining (68% in 2008 en 66% in 2009) as Thompson Seedless (21% in 2008 en 25% in 2009) gehad het. Regal Seedless was ook meer geneig om eksterne verbruining, spesifiek netagtige verbruining te vertoon, terwyl Thompson Seedless meer geneig was om interne verbruining te vertoon, ten spyte van die verskillende fenotipes van verbruining wat teenwoordig was op beide kultivars.

Hoofkomponente analise (HKA) is op die spektra gedoen voor en na koelopberging en naby infrarooi spektroskopie het aan die lig gebring dat die veranderinge wat verband hou met koelopberging met die eerste hoofkomponent (HK) verduidelik kan word met byna 100% van die variasie in die spektra wat daarin vasgevang is. Klassifikasie modelle is ook deur die gebruik van HKA gebou en was gebaseer op die spektra van korrels wat verkry is voor en na koelopberging asook die wat verkry is nadat korrels verbruin het na koelopberging. Klassifikasie modelle van korrels wat gebaseer was op spektra na koelopberging (verbruining teenwoordig) het 'n beter algehele akkuraatheid (94% vir opleidingsdata en 87% vir toetsdata), getoon as die klassifikasie modelle wat gebaseer was op spektra van korrels voor koelopberging (79% vir opleidings data en 64% vir toetsdata). Die implikasie hiervan is dat die huidige modelle in staat sal wees om korrels beter te klassifiseer in terme van diene wat alreeds verbruin het en die wat nie verbruin het na koelopberging as daardie voor koelopberging, wat juis die kritieke stadium is waar ons wil weet of die korrels wel gaan verbruin of nie. Daar is wel potensiaal wat

verder ontgin kan word, aangesien al hierdie modelle gebou is deur gebruik te maak van die hele NIR spektrum. Geen veranderlike seleksie is dus gedoen nie en al die verskillende verbruiningsfenotipes is ook saam gebruik in die opstel van die modelle. Verdere analise van die data sal dus gebaseer word op die gebruik van veranderlike seleksie tegnieke soos deeltjie swerm optimisasie (DSO) wat sekere golflengtes kies wat sterk verband hou met die verbruining verskynsel en slegs die belangrikste tipes van verbruining (netagtig op Regal Seedless en interne verbruining op Thompson Seedless) sal gebruik word. Hierdie studie het 'n baie belangrike implikasie vir die tafeldruifbedryf, want dit is die eerste keer dat die moontlikheid om verbruining te voorspel met ander metodes as visuele inspeksie, veral voor koelopberging, getoon word.

This thesis is dedicated to
My mother Agnes and my brother Andrew.
My late grandmother Sannah and aunt Louise,
As well as the rest of my family and friends.
Thank you for all your unconditional love and support
Throughout my life.

Biographical sketch

Andries Jerrick Daniels was born in Kimberley, South Africa on the 29th of November 1981. He attended Venus Primary School, then Homevale Senior Secondary No. 2 and matriculated at Adamantia High School in 1999. Andries obtained a BScAgric-degree in Viticulture and Oenology in 2005 and a HonsBScAgric-degree in Viticulture in 2008 at the Stellenbosch University.

In 2006 Andries joined the Table Grape Breeding and Evaluation Division (now Cultivar Development) of the Agricultural Research Council as a Research Technician. In 2009, he enrolled for a MScAgric in Viticulture at the Stellenbosch University.

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Preface

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately. Chapter 3 is written according to the style of the South African Journal of Enology and Viticulture and Chapter 4 is written according to the style of the Journal of the Science and Food Agriculture. Each Chapter is introduced separately.

Chapter 1 **General Introduction and project aims**

Chapter 2 **Literature review**

Chapter 3 **Research results**

Quantification of sugar, pH and titratable acidity in table grapes using near-, mid- and attenuated total reflectance mid-infrared spectroscopy

Chapter 4 **Research results**

Preliminary evaluation of monitoring and detection of browning of white seedless table grapes with near-infrared spectroscopy

Chapter 5 **General discussion and conclusions**

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Chapter 1

Introduction and project aims

Chapter 1: Introduction and project aims

1.1 INTRODUCTION

Fresh table grapes are the single agricultural product that contributes most to the total agricultural export revenue of the South African economy. It contributed in total 23% during the first quarter of 2012. This amounted to more than R 2.5 billion (BFAP, 2012). Due to their delicateness and extreme perishability, the losses suffered during the preparation, harvest, packing, storing, transport and distribution of table grapes can also be very high (Mencarelli *et al.*, 2005). For this reason, extra care has to be taken during all these processes, especially while the fruit is still in the vineyard and attached to the vine, since this is where the final quality of the fruit is influenced most. Table grape cultivars' quality can, however, only be characterized by physical evaluation (berry texture, colour and taste) (Cliff *et al.*, 1996) and by analysis of chemical compounds related to quality, namely sugar and acidity. Physical maturity of grapes is still defined as the stage when the fruit reaches its largest diameter (berry size) and cluster weight (Fahmi *et al.*, 2012) and chemical maturity is still measured based on the values of total soluble solids (TSS), titratable acidity (TA) and pH (Viti-Notes, 2005). Šuklje *et al.* (2012), however, found that although a close correlation between berry diameter (physical) and TSS concentration (chemical) was observed in their experiments, berries of the same diameter had different TSS concentrations. This provides a severe challenge for table grape producers who assume that all berries in a bunch due to their uniformity in diameter contain the same sugar content, or is at the same harvest maturity.

Of the various postharvest quality defects like berry split, SO₂ damage, decay and stem desiccation that table grapes suffer from, berry browning is the most serious, especially in white seedless table grape cultivars, like Thompson Seedless and Regal Seedless (Fourie, 2010). Browning of berries in white seedless table grapes often only occurs in specific berries in a bunch and in no particular pattern in terms of position (top, middle or bottom) of the bunch. It can, therefore, be speculated that browning of berries, or the potential thereof, is related to specific characteristics located in the berry itself and not in the bunch as a whole.

Furthermore, since browning of white grapes commonly becomes visible in the later stages of cold storage, the potential of berries to discolour is not observable at harvest or during packing (Vial *et al.*, 2005). This lack of information at harvest, or during grape packing on the browning risk associated with a particular batch of white seedless varieties destined for the export market makes this a very serious problem. Consumers reject grapes that have turned brown, because it is perceived as a loss of quality. Producers thus continue to suffer huge financial losses due to this quality deterioration problem, since table grapes with visible browning gather lower prices than unaffected grapes (Vial *et al.*, 2005).

In our continuously changing economic environment, where competition in the markets is becoming more aggressive, and the mounting consumer preferences for the quality of the products they buy, this cannot be afforded. The South African table grape industry is therefore in dire need to keep abreast with the new technologies and to evaluate the usefulness of these when applied to the pressing issues of the industry. Based on the economic importance of the SA table grape industry, it is imperative to ensure that the same high quality product is delivered year after year, by monitoring table grape quality both quantitatively and qualitatively, using advanced monitoring techniques.

1.1.1 BROWNING IN TABLE GRAPES

Browning of fruit, including table grapes, is a complex biological phenomenon, in which physiological, physical and pathogenic factors may play a role (Avenant, 2007; Ferreira *et al.*, 2005; Kruger *et al.*, 1999). Recent research on the cellular level of grapes showed no major changes in cell wall polysaccharide composition occurred during softening of ripening grape berries, but that significant modification of specific polysaccharide components, together with large changes in protein composition occurs (Nunan *et al.*, 1998). It is the initial damage to fruit which causes a dis-functioning or disruption of cellular membranes, which allows mixing of the enzyme polyphenol oxidase (PPO) with phenolic substrates or compounds occurring naturally in the fruit (Ferreira, 1997; Golding *et al.*, 1998). The process consists of two phases of which the first is enzymatic and the second not. During the first, monophenols are converted to diphenols located in the vacuoles (Kruger *et al.*, 1999). The diphenols are then oxidised by means of hydroxylation enzymes and then orthoquinone by means of oxidase enzymes in the presence of oxygen, through the action of PPO located in the cytoplasm (Macheix *et al.*, 1991; Liyanage *et al.*, 1993; Nicolas *et al.*, 1994; Zapata *et al.*, 1995;). During the second phase, spontaneous polymerisation takes place during which the initial products of the reaction are quinones, which are then subjected to further reactions (polymerisation) leading to the formation of melanin (brown pigments) which are responsible/characteristic of the brown colour/browning phenomenon (Sapis *et al.*, 1983a).

The browning phenomenon is not a recent occurrence and a lot of work has also been done on browning in white wine (Simpson, 1982; Peng *et al.*, 1998; Clark and Scollary, 2002). Sapis *et al.* (1983a&b) were the first to examine the browning capacity of white grapes. Except for the pamphlet released by Fourie (2009) describing the several different phenotypes of berry browning on table grapes which is discussed in section 2.3 there is very limited published research on the browning phenomenon in SA.

Browning becomes visible only after it has reached the overseas markets. It could therefore have started at any of the stages from packing to storage and transport of grapes to overseas markets. It has, however, come to light recently that some types of browning may even be present in the vineyard (DFPT Researchers, 2009). The table grape industry of South Africa identifies six main groups of browning. These are external, internal, low temperature, chemical, physical and pathogenic browning. External browning can be subdivided into different types, namely net-like, mottled, friction and contact browning. Internal browning is expressed as, chocolate-, water- and glassy berry. Methyl bromide and CO₂ damage is known as chemical browning, while abrasions and bruises are known as physical browning and fungal infection as pathogenic browning (Fourie, 2009). The two most common types of browning that occur on white seedless table grapes though are internal and external browning in their various forms. Various unpublished research have been done in South Africa over the years to determine the exact cause of browning, but to date, no single dominant implicating factor, which can be repeatedly linked to either internal or external browning, has been identified (DFPT Researchers, 2009). On-going research in Australia is taking a biochemical approach to understanding skin browning in white seedless table grapes (Australian Table Grape Annual Industry report 2007/08)

By looking at results obtained from correlation studies, the complexity of the browning phenomenon becomes even more apparent and it is sometimes difficult to find consistency in data and interpretations. For example, the development of browning symptoms on Princess, a Californian white seedless table grape cultivar, showed that the grapes had very high incidences of berry skin browning, but very low incidences of berry flesh browning (Vial *et al.*, 2005). Most of the berry skin browning occurred in grapes that were past optimum ripeness and appeared after three weeks of cold storage. Skin browning was therefore directly related to fruit maturity, while vineyard

location had greater impact on the incidence of skin browning than maturity (Vial *et al.*, 2005). The complexity of this postharvest quality defect, due to its nature (occurs on tissue level), provides serious challenges for the monitoring of fruit quality.

1.1.2 WHY INFRARED (IR) SPECTROSCOPY?

IR spectroscopy, especially near-infrared (NIR) spectroscopy, has already been used with great success for different agricultural applications, like the assessment of soil properties in reflectance mode (Bilgili *et al.*, 2010), the simultaneous prediction of alkaloids and phenolic substances in green tea leaves in reflectance mode (Schulz *et al.*, 1999), the evaluation of firmness of peaches also in reflectance mode (Fu *et al.*, 2008), the quality control of green Rooibos and Honeybush (Manley and Botha, 2006), the detection of brown heart of pear (Fu *et al.*, 2007), the establishment of prediction models for quality parameters in Japanese plums (Louw and Theron, 2010) and the determination of quality parameters in Cavendish banana during ripening (Liew and Lau, 2012). Mid-infrared (MIR) spectroscopy has been used to evaluate assimilable nitrogen in grape juice (Skoutelas *et al.*, 2011) and it is especially widely used in wine analysis for quantification of components like tannins in red wine (Fernandez and Agosin (2007) and screening of different parameters (alcoholic degree, volumic mass, total acidity, pH, volatile acidity, glycerol, total polyphenol index, reducing sugars, lactic, malic, tartaric and gluconic acids, colour, tonality, total sulphur dioxide and free sulphur dioxide) in wine (Urbano-Cuardado *et al.*, 2004).

Whilst there are other analytical techniques such as time-resolved reflectance spectroscopy for the non-destructive detection of brown heart in pears (Zerbini *et al.*, 2002), the biochemical characterisation of core browning and brown heart disorders in pear by multivariate analysis (Larrigaudière *et al.*, 2004), scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Fanta *et al.*, 2012) as well as HS-SPME and GC-TOFMS (Louw and Theron, 2012) which can be used to analyse and monitor table grape quality qualitatively, they are very expensive, sophisticated and not readily available for routine analysis (refer to section 1.2. and 1.3). Thus, following the successful applications of IR spectroscopy, the obvious analytical strategy followed in this project, was to investigate the usefulness of IR spectroscopy not only to investigate browning in table grapes, but to make available a platform of technologies with fast and high throughput of information regarding harvest maturity and quality determining parameters such as TSS, pH, TA, glucose and fructose.

1.2 PROBLEM STATEMENT AND RESEARCH QUESTIONS

The current study had two focus points for which specific aims have been formulated: one which has a scientific focus area and another with an industry focus area. These project aims have been formulated to effectively guide the decisions made during the experimental design of the project.

1.2.1 SCIENTIFIC PROBLEM STATEMENT

Browning is a complex phenomenon occurring on a cellular level. Knowledge of how and exactly when it is occurring inside or on the berry surface is not known and no technology exist yet to determine its progression from harvest until after cold storage (DFPT Researchers, 2010). Harvest maturity and quality determining parameters such as TSS, pH, TA glucose and fructose, which has vast implications for the table grape industry; had to be determined separately to obtain an indication if the ripeness levels of table grapes can be determined accurately before harvesting. Non-destructive analysis of different quality parameters like firmness and soluble solids content for apple fruit using hyper spectral imaging (Lu, 2007), firmness and yellowness of mango during

growth and storage using visual spectroscopy (Jha *et al.*, 2006) and even spatial characterisation of wine grape clusters in terms of sugar content and distribution of berry volumes within clusters using magnetic resonance imaging (Andaur *et al.*, 2004) have been done. The research conducted in this study was not entirely non-destructive (bunches not measured *in situ* on the vine), but destructive (bunches removed from the vine). Berries were also removed from bunches (destructive) and some were kept whole (non-destructive) to evaluate browning (qualitative analysis) and some mashed (destructive) to determine the maturity parameters TSS, pH, TA, glucose and fructose (quantitative analysis). The main aim was thus to first obtain the wavelengths that were strongly associated with browning in the qualitative experiments and those that could be used to develop calibration models for the maturity parameters in the quantitative experiments. Destructive measurements, therefore, had to be conducted for the interim before *in situ* measurements could be attempted.

1.2.2 INDUSTRIAL PROBLEM STATEMENT

The first obvious question for the industry is whether or not the possibility of browning occurring on South African table grapes can be classified before harvesting already? The pressing question for the industry is if yes, whether they should start marketing SA table grapes locally instead of sending them to overseas markets? The problem, however, would be with the income received due to a shift in the markets. Grapes that are exported garner much higher prices than those sold locally. Producers, therefore, have to know the potential incidence of browning on table grapes before harvest.

1.3 PROJECT AIMS

The overall aim of this project was to investigate whether IR spectroscopy can be used to generate spectra of whole table grape berries to evaluate the browning potential of white seedless table grapes and a secondary aim was to determine harvest and quality determining parameters, also using IR spectroscopy:

1.3.1 QUANTITATIVE CALIBRATION FOR HARVEST MATURITY AND QUALITY DETERMINING PARAMETERS

Based on the preliminary indications that maturity (Vial *et al.*, 2005) could play a role in browning potential, it was also an important objective of this study to quantify TSS, pH, TA, glucose and fructose. The development of calibration models for the harvest maturity and quality determining parameters TSS, TA, pH, glucose and fructose of table grapes was initiated to lay a platform for investigating the correlation of these parameters with the occurrence of browning.

Different techniques, namely Fourier transform Near Infrared (FT-NIR), Fourier transform – Mid Infrared Attenuated Total Reflectance (FT-MIR ATR) and Fourier transform – Mid Infrared (FT-MIR) spectroscopy in different scanning modes, were evaluated to assess the transfer of this technology to the industry.

1.3.2 QUANTITATIVE CALIBRATIONS TO DETECT BROWNING

An important milestone of this project was to see whether the generated spectra could capture any time related changes associated with storage and/or browning and then setup a qualitative

calibration model to ultimately detect the browning potential of white seedless table grapes, at the earliest possible stage before the occurrence of browning.

1.4 EXPERIMENTAL DESIGN SUMMARY

The entire experimental design for this study is illustrated in Figure 1. In total, two different experiments were conducted. The order in which the tests took place was as follows: Firstly the grapes were harvested, berries were removed from bunches and assigned in different lots for the certain experiment i.e. those that were used for the harvest maturity and quality parameter determinations and those that were used for the browning experiment. The berries selected for the harvest maturity and quality parameter determinations were prepared for reference value determinations and scanning on the different IR instruments (left hand side of figure). Quantitative determinations were performed on the juice of the berries on the three different instruments for determination of the harvest maturity and quality parameters (Chapter 3). Berries selected for the browning experiments were scanned (right hand side in the figure) and qualitative determinations were performed on the NIR instrument to determine browning potential by building classification models based on spectra obtained before and after cold storage (Chapter 4).

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Chapter 2

Literature review

Chapter 2: Literature review

2.1 INTRODUCTION

The table grape industry is of huge economic importance to South Africa (SA). The country is the third largest producer of table grapes (1.7 million tonnes in 2010) in the Southern hemisphere after Chile and Argentina (<http://www.nda.agric.za/docs/AMCP/Tablegrapemvcp2011-12>). SA's main grape cultivars are Thompson Seedless, Crimson Seedless, Red Globe, Prime Seedless and Sugaone (<http://www.nda.agric.za/docs/AMCP/Tablegrapemvcp2011-12>). According to recent statistics, the European Union (EU) is the leading export market for South African grapes, accounting for 58% of all exports. The United Kingdom is the second most important partner, accounting for 22% of exports, followed by the Far East (9%), Middle Europe (6%), and Russia and Eastern Europe at 2% (Siphugu, 2011). Data released by the Bureau for Food and Agricultural Policy (BFAP, 2012), showed that exports reached a record peak of 245 780 tonnes (or 55 million cartons) in the 2011/2012 season, which represents a 22 % increase from the 201 500 tonnes exported in 2010/2011. Table grapes clearly earn SA valuable foreign exchange when considering that prices between R51 and R73 per 4.5 kg box were obtained during the 2009/2010 season (Van der Merwe, 2011). It is therefore essential that optimum grape quality is obtained in the vineyard and maintained during cold storage, transport, on the retail shelves and until the produce is in the hand of the consumer.

The best possible quality of any fresh commodity exists at the moment of harvest and the challenge is therefore to deliver a product to the consumer with the same level of freshness that it had at harvest (Bachmann and Earles, 2000). The South African Table Grape Industry (SATI) is under continuous pressure to deliver produce of superior quality, not only to keep a competitive edge in the international market, but also to meet the continually changing demands and preferences of a heterogeneous international consumer base. Quality is perceived through visual and organoleptic means by the consumer. These are the attributes that initially attract the consumer and, therefore, have a big impact on purchase decisions. Any defect that negatively affects the appearance of the grapes will ultimately reduce the product's market value, the consumer's confidence in the cultivar or the producer and render the fruit unmarketable. This begs the question as to how science can support the table grape industry in the challenging task of successfully monitoring table grape quality, both qualitatively (visual appearance and taste) and quantitatively (chemical composition) throughout the whole value chain.

This literature review takes a critical look at published research on the assessment of table grape quality (section 2.2), with specific focus on the browning disorder (discussed in section 2.3). In section 2.4 the theory of infrared spectroscopy and an exploration of the possibilities that this technique offer to monitor table grape quality, are discussed. Some examples of chemometric techniques that are useful to extract relevant data from infrared spectra are discussed in section 2.5.

2.2 TABLE GRAPE QUALITY

2.2.1 CONSUMERS' EVALUATION OF QUALITY IN TABLE GRAPES: IMPLICATIONS FOR MONITORING

Factors that have been shown to influence consumers' preferences for table grapes, include taste and flavour (Mencarelli, *et al.*, 2005; Cliff *et al.*, 1996), berry colour (Deng *et al.*, 2005) and other attributes such as visual appearance of the fruit, including berry shape and size, bunch shape and size, appearance of the stems, skin and flesh firmness, (Deng *et al.*, 2005). This clearly implies that table grape's acceptance by the consumer as having the *right* quality, is reliant on some measurable qualitative properties such as firmness and taste, as well as quantitative properties such as sugar and acid content (Rolle *et al.*, 2012). Table grape quality, however, is majorly dependent on the maturity level at which grapes were harvested (Jayasena and Cameron, 2008). It is, therefore, very important to harvest table grapes at the right time, but since there is no standard manner in which the right harvest time can be determined, certain factors have to be taken into consideration. These include the soluble solid concentration (SSC) of the grapes, as well as the titratable acidity (TA) and the °Brix/acid ratio (Baiano *et al.*, 2012). SSC in grapes refers to the amount of sugars (glucose and fructose), frequently measured in °Brix and the organic acid composition, which is measured as TA (expressed as g/L tartaric acid) and pH (Shiraishi *et al.*, 2010; Fahmi *et al.*, 2012). In SA, SSC is referred to as total soluble solids (TSS) and is also measured in °Brix. In order to monitor table grape quality, a broad spectrum of components has to be determined. The concentration of organic acids tends to be less in comparison to that of sugar, but if it is too high it leads to a negative impact on the taste of the grapes (Liu *et al.*, 2006). According to Dokoozlian (2000), the juice pH is a measure of the hydrogen ion concentration in the berry and is generally related to juice acidity. Considering that quality determining parameters like firmness and soluble solids content in other fruit types has been determined successfully using fast and economical measuring techniques (Nicolai *et al.*, 2008; Penchaiya *et al.*, 2009), it was deemed feasible to also investigate such techniques for monitoring table grape quality.

2.2.2 CHALLENGES RELATED TO MAINTAINING QUALITY IN TABLE GRAPES

Possibly, the greatest challenges in keeping table grapes fresh are related to the delay between harvests and until the fruit reaches the consumer and the temperature fluctuations experienced during all these stages. Table grapes are non-climacteric fruit, which start to lose water immediately after harvest and subsequently during handling and transportation (Crisosto *et al.*, 2001; Candir *et al.*, 2012). This poses serious challenges during the long storage and transport periods that table grapes have to endure when the export market is far from the country of origin, as in the case of SA that export table grapes to, amongst other countries, the UK. To maintain high quality throughout all these different stages, the appropriate postharvest strategies like the right cold storage and controlled atmospheric (CA) conditions (which are 2% oxygen (O₂) with or without 5% CO₂) have to be followed (Balic *et al.*, 2012).

SA benefits from a shorter shipping distance to Europe than other Southern hemisphere competitors like Chile and Argentina. Pre-shipment storage (3 to 12 days) and shipment by sea (approximately 14 days to the Middle East, 16 days to the European markets and 22 days to the Far East and United States of America) is supposed to occur at -0.5°C (Burger *et al.*, 2005). Adequate cooling is, therefore, the most critical phase in the postharvest handling procedure, in order to maintain quality. However, upon arrival in the European markets where the daily winter temperatures are very low (frequently below 10°C in United Kingdom), transport of grapes

sometimes occurs without cooling. When the grapes are displayed in the supermarkets, they are exposed to higher temperatures, mostly above 15°C. Display can be 7 to 18 days later, depending on how the market situation is at that point in time. This time lapse is in addition to the ~ 3 to 12 days required to load the grapes onto the ship and then another 16 days shipment time (Burger *et al.*, 2005). Typically, a total of 26 to 36 days since harvest can elapse before the produce is on retail shelves.

Despite these time lapses and temperature fluctuations that pose a risk to fruit quality, consumer preference dictates that the fruit looks as good and fresh as at harvest. This means the grapes should be as free as possible from skin breaks, bruises, spots, rots, decay and other deterioration (Wilson *et al.*, 1995). Export table grape cultivars must therefore, not only have a good storage life, but a very good shelf life as well, to meet all these high expectations. The quality defects that typically occur in table grapes include several conditions, as outlined below. Berry browning, discussed in detail in the following section, manifests with several different phenotypes, while berry crack refers to a condition where the berry splits open, either along the longitudinal side of the berry or around the berry stem, or sometimes at both positions (Zoffoli *et al.*, 2008). Gray mold is a type of berry rot caused by the fungus *Botrytis cinerea* during storage for long periods of time (Retamales *et al.*, 2003). SO₂ damage is caused by fumigation of grapes directly with the gas, or the use of SO₂ generating pads that are placed on top of grapes packed in cartons to control postharvest diseases such as gray mold (Crisosto *et al.*, 1994). This practise, however, can lead to bleaching of the berry skin, as well as the stems turning brown prematurely (Marois *et al.*, 1986). Zoffoli *et al.* (2008) discovered that SO₂ treatment also causes hairline cracks on table grape berries. These cracks are very fine in comparison to normal berry cracks and are thus not visible to the naked eye.

Due to these problems experienced with SO₂, alternative treatments have been investigated. These include the use of carbon dioxide (CO₂) alone or in combination with CA (Yahia *et al.*, 1983), fumigation with methyl bromide (MB), a toxic odourless gas used to control pests on fruits such as grapes and apples (Liyanage *et al.*, 1993) and modified atmosphere packaging (MAP) (Mahajan *et al.*, 2007). Continuous research has been undertaken on these aspects, like determining optimum CO₂ and O₂ levels during CA (Crisosto *et al.*, 2002), finding alternatives to the use of MB (Schneider *et al.*, 2008) and controlling its release into the atmosphere (Leesch *et al.*, 2000) as well as the use of chlorine gas generators in MAP to prevent gray mold (Zoffoli *et al.*, 1999). Karabulut *et al.* (2004) also investigated postharvest ethanol and hot water treatments to control gray mold and most recently, the use of essential oils from sweet basil, fennel, summer savory and thyme (Abdollahi *et al.*, 2012), as well as ethanol vapour-generating sachets (Candir *et al.*, 2012) were tested to control gray mold.

2.3 TABLE GRAPE BERRY BROWNING

The occurrence of postharvest browning in SA of export table grapes was first reported in 1989 for the Waltham Cross cultivar. Since then, browning has not only increased for this cultivar and Regal Seedless over the last two decades, but was also reported for several other white cultivars, such as Thompson Seedless and Victoria (Wolf, 1996; Avenant, 2007). Browning is a significant problem in the production of white table grapes worldwide, leading to a large-scale on-going project in Australia to investigate the biochemical basis of this quality defect (Australian Table Grape Annual Industry report 2007/08). Despite our incomplete understanding of the causes of browning, it will for obvious reasons, be extremely valuable to have some assessment of the risk of browning in a batch of grapes, already at harvest, before grapes are packed and exported, which served as the basis why this present study was undertaken.

2.3.1 FRUIT BROWNING

Fruit browning is a widespread problem and disorders usually classified as browning of the tissues, show variations in the size and the location of the affected area (Burzo *et al.*, 2001). Kruger *et al.* (1999) gave a detailed description of browning problems experienced with papayas, litchis, mangos and pineapples. Other fruit types also prone to browning are plums (Kapp and Jooste, 2006), apples (East *et al.*, 2005), pears (Fu *et al.*, 2007), bananas (Friedman, 1996), peaches and nectarines (Crisosto *et al.*, 1993). In table grapes, either internal tissue browning, external skin browning or both, are present (Vial *et al.*, 2005). Stem browning (Figure 2.1) can also occur on white (Thompson Seedless) and red cultivars like Redglobe (Crisosto *et al.*, 2001) and Flame Seedless (Carvajal-Millán *et al.*, 2001).



Figure 2.1 Picture showing stem browning on a red and a white table grape cultivar (Photo obtained from: http://www.redorbit.com/news/science/1483439/getting_fresher_grapes_to_the_table/).

2.3.2 POSSIBLE CAUSES FOR THE OCCURRENCE OF BROWNING IN TABLE GRAPES

The hypothesis is that browning is a brown discoloration of the berry flesh (Pool and Weaver, 1970) and/or berry skin (Wolf, 1996) due to a dis-functioning or disruption of cellular membranes, which allows mixing of the enzyme polyphenol oxidase (PPO), mainly located in the cytoplasm of grape skin cells (Rathjen and Robinson 1992), with phenolic substrates or compounds occurring naturally in the vacuoles of the fruit (Ferreira, 1997; Golding *et al.*, 1998; Kruger *et al.*, 1999). Quinones are formed, which, through polymerization reactions, leads to the formation of brown pigments that are characteristic of the browning phenomenon (Sapis *et al.*, 1983a). It would seem as if there are three factors that have a strong positive influence on the occurrence of browning and the rate at which it appears in grapes and grape juice, namely cell wall and cell membrane integrity, the phenolic substrates in the vacuoles of cells that can be oxidised, the PPO activities and oxygen availability (Macheix *et al.*, 1991). Non-enzymatic reactions, in which compounds other

than phenolics are involved, such as lipids, are also known to be involved in browning reactions (Hidalgo and Zamora, 2000). There is indeed a pressing need for research to identify possible biomarker molecules that are positively associated with browning, with the aim of using this information in a predictive manner. 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 (Lee and Jaworski, 1989). 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) that may be responsible for accelerating or slowing the process (Macheix *et al.*, 1991).

Ever since Sapis *et al.* (1983a&b) first examined the browning capacity of white grapes, there has been very limited published research on the table grape browning phenomenon especially in SA. Only Fourie (2009) released a pamphlet describing the several different phenotypes of berry browning on table grapes, which is discussed in section 2.3, and Moelich (2010) conducted an investigation to establish the possible role of forced air cooling in berry browning development of table grapes. This is despite the serious nature of this postharvest quality defect, which due to its nature and occurrence on tissue level, is very complex to monitor.

2.3.3 BROWNING PHENOTYPES AND THEIR MANIFESTATION IN TABLE GRAPES

SATI identifies six main groups of browning (Table 2.1), of which the two major types that occur on white seedless table grapes are respectively, external and internal browning in their various forms. These different types of browning manifest with different development profiles. External browning in the form of netlike browning, may already be present on berries of a lot of bunches prior to harvest, in particular on Regal Seedless (DFPT Researchers, 2009).

Table 2.1 The six main groups of browning found on table grapes and the different forms they manifest in (adapted from Fourie, 2009).

External browning	Internal browning	Physical browning	Chemical browning	Low temperature browning	Pathogenic browning
Netlike browning	Chocolate browning	Bruising	Methyl bromide damage	Freezing damage	Fungal infection
Mottled browning	Water berry	Abrasions	CO ₂ damage	Cold damage	
Friction browning	Glassy berry				
Stylar-end russet spots					
Stylar-end necrotic spots					
Contact browning					
Peacock spot					
Sunburn					

Monitoring physical browning such as abrasions (brown, scar-like tissue on the berry surface) and bruising (flattened areas on berry surface) is easy. This is because the physical damage which has been inflicted, often by rubbing of stems or shoots against the berries, or bunches pressing against each other within packed cartons, can be reduced or completely eliminated. The same can be said for chemical browning (yellow-brown discolouration of damaged areas), low temperature browning (berries are a brown, milky colour, while the rachis and laterals of the bunch appear olive brown) and pathogenic browning (brown lesions and/ or blemishes on the berry surface due to fungal

infections associated with *Botrytis cinerea*). These are all caused by problems in the procedures followed to keep grapes healthy and fresh during the shipping, transport and storage of table grapes. The effects of either too high CO₂ gas modification or modified atmosphere treatments, too much methyl bromide fumigations, as well as improper cooling methods (freezing/ chilling damage) can also all be addressed.

The problem, however, lies in monitoring external browning, which can manifest in so many different phenotypes. This can prove to be quite challenging since netlike browning, for example, appears as dashed-like brown necrotic streaks, progressing from the bottom (stylar-end) towards the top (stem-end) of the berry, whereas mottled browning appears as brown blotches on the berry surface. Friction browning on the other hand is expressed as circular spots close to the pedicel area and is associated with rolling of berries against each other. Then there are stylar-end russet spots which refer to brown russet-like damage at the stylar end of the berry, characterised by irregular shaped spots that exhibit a circular damaged area. Stylar-end necrotic spots appear as brown spots, characterised by slightly sunken necrotic tissue, often associated with secondary pathogenic infection. Where berries touch each other, square-like flattened areas at the stem-end of the berry can occur, which appears as brown marks on the berry surface. This is referred to as contact browning. Almost similar to contact browning, where adjacent berries also touch, brown circles, or half-circles, with a clear centre, on the surface of berries can occur. This is then referred to as peacock spot browning and symptoms can already be present in the vineyard. Sunburn appears as a brownish colouration of the berry surface. This is as a result of direct exposure to and damage by the sun, often characterised by a leathery, rough touch (Fourie, 2009).

Monitoring or detecting internal browning types like chocolate berry and water berry, will prove challenging, since different cultivars do not express the symptoms in the same way. Internal browning on Thompson Seedless, for instance, starts in the middle of the berry parallel to the vascular system (Figure 2.2b) and never develops further on the skin, as in another white cultivar, Princess (Vial *et al.*, 2005). The monitoring device will therefore have to provide a signal that will be able to penetrate deep into the fruit to collect and capture the physical and chemical information that will allow discrimination between a berry that will remain healthy (Figure 2.2a) and one that will eventually turn brown after storage (Figure 2.2b and Figure 2.3a&b). Chocolate berry internal symptoms show a brown discolouration, which originates mostly from the stylar-end of the berry, while in severe instances the whole berry may appear brown as can be seen Figure 2.3 (a), where it was caused by a fungal infection. Water berry refers to browning of berries, associated with dehydration, often related to damage to the stem starting at the stem-end, extending towards the stylar-end of the berry as the disorder progresses. Symptoms include berries exhibiting a dull, translucent brown appearance, with browning progressing from the inside, outwards (Fourie, 2009).

Clearly, the complex and huge variations in phenotypic characteristics of grape berry browning pose challenges for implementation of rapid non-destructive monitoring methods. Not only is it important to know which of these different browning phenotypes are prevalent on which cultivar, but also how to monitor it. This will help with applying the correct postharvest management practices and making the right marketing decisions to ensure optimal quality of grapes once it reaches the consumer.

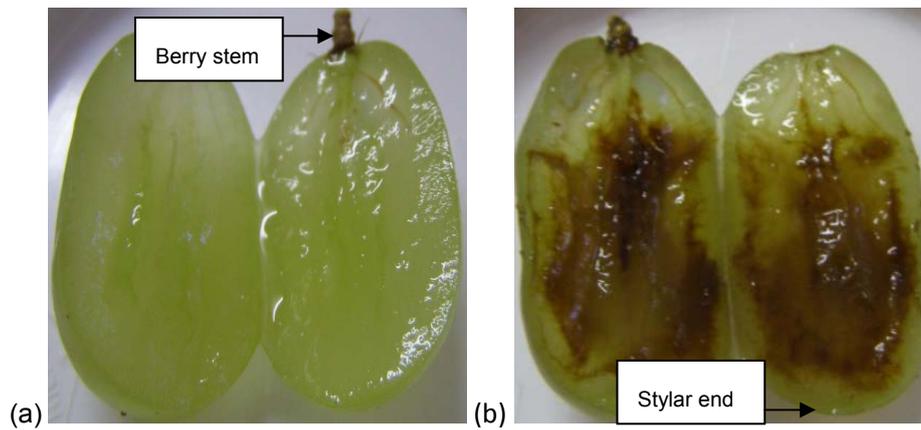


Figure 2.2 (a) Appearance of a healthy Thompson Seedless table grape berry after five weeks of cold storage; and (b) symptoms of internal browning of a Thompson Seedless berry where browning is starting parallel to the vascular system in the centre of the berry (own data).

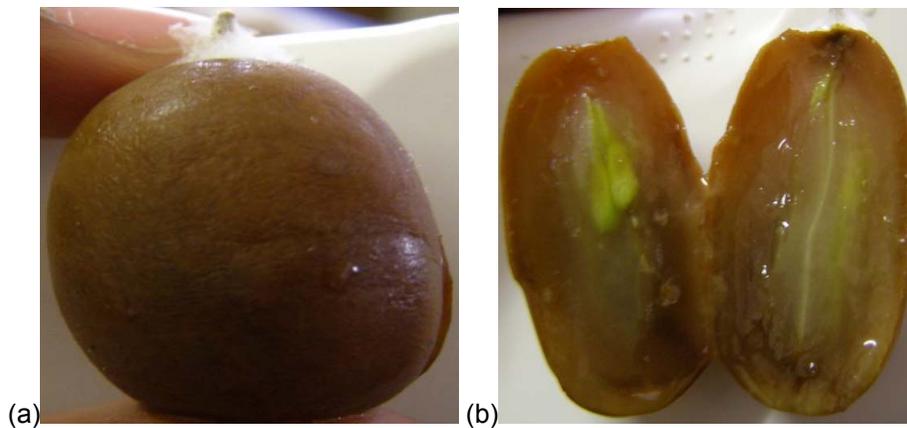


Figure 2.3 (a) Chocolate browning of a Regal Seedless table grape berry caused by fungal infection (white growth at pedicel end of berry); and (b) the same berry cut in half (own data).

A delay in transport and temperature fluctuations are inevitable and will always play a role in the postharvest quality of table grapes. Indeed there seems to be a limit as to what can be done to maintain postharvest quality during this time. Methods to monitor the qualitative and quantitative aspects of the grapes, however, will greatly help with making informed marketing decisions as early as possible, preferably prior to harvesting. Ideally, non-destructive methods to monitor quality in the vineyard will be of great help towards this end. Although the berries used for qualitative analysis in this study were scanned whole, it was still done destructively, since the berries were removed from bunches and bunches were removed from the vine. This however, had to be done for the first stages of the research, to test the concept that infrared spectroscopy-based calibration models could be constructed that can capture the information related to browning, so that these methods can eventually be used in the vineyards to monitor quality non-destructively.

2.4 INFRA-RED SPECTROSCOPY

2.4.1 INTRODUCTION

Infrared (IR) spectroscopy deals with the interaction of matter with the infrared region of the electromagnetic spectrum, which is usually divided into three regions; near-, mid- and far-infrared, named for their relation to the red region of the visible spectrum (Skoog *et al.*, 1997). The mid-infrared (MIR) region is usually defined as ranging from 4 000 to 400 wavenumbers (cm^{-1}), or in terms of nanometres (nm), from 2 500 to 2.5×10^4 nm. The near-infrared (NIR) region of the electromagnetic spectrum lies between the visible and infrared regions and spans the range of wavelengths between 780 and 2 500 nm ($12\,800 - 4\,000 \text{ cm}^{-1}$). Wavenumbers can easily be converted to wavelengths and *vice versa*, through the conversion factor ($\text{cm}^{-1} = 1/\text{nm} \times 10^6$ and $\text{nm} = 1 \text{ cm}^{-1} \times 10^6$). It is, however, convention to use nanometres in NIR and wavenumbers in MIR. A diagrammatic representation is shown in Figure 2.4 where, however, the wavelength is shown in metres and the wavelength in micrometres (μm).

In practice, the ranges on infrared spectrometers of different suppliers may differ from those mentioned above, mainly because different instruments are designed for different applications. It is, therefore, possible to find combinations of the visible plus NIR regions on a single instrument, or NIR plus MIR regions. In the following sections the different modes of spectral acquisition and the main differences between NIR and MIR are discussed, as well as their potential applications. A brief look is taken at the theory and principles and advantages of FT-MIR and FT-NIR spectroscopy, as well as the application it has for detection of browning in fruit.

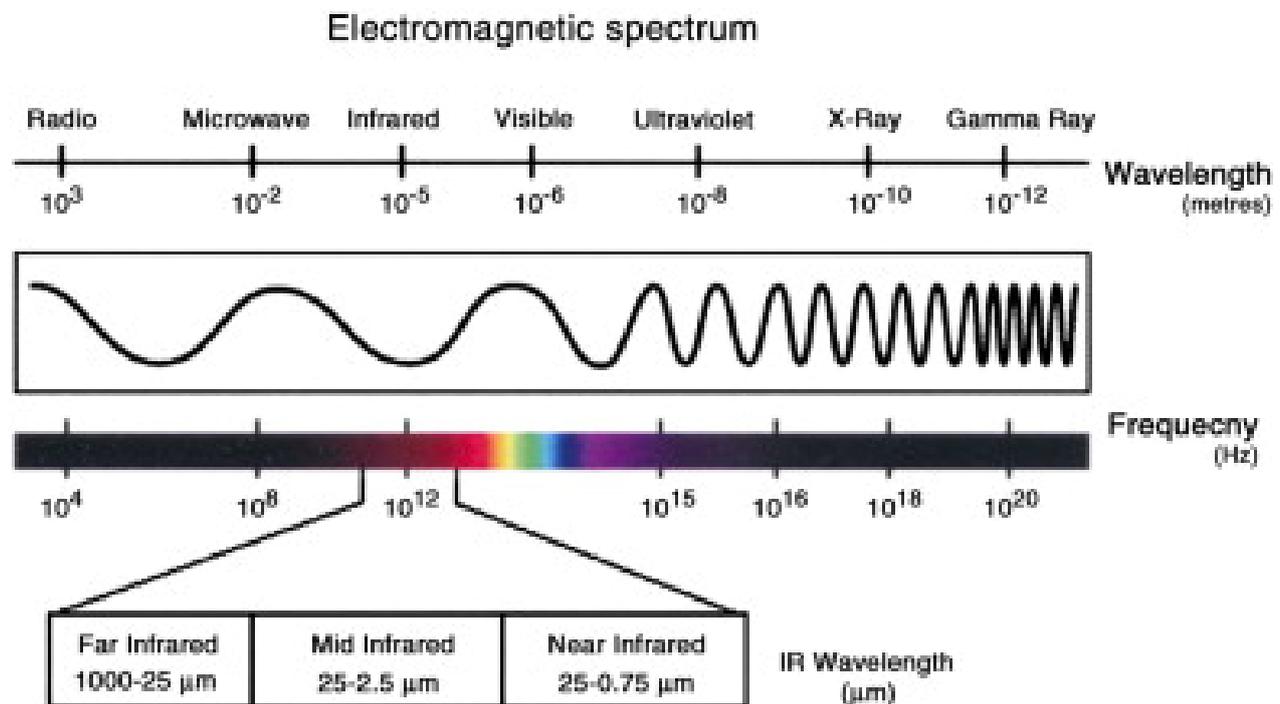


Figure 2.4 Diagrammatic illustration of the electromagnetic spectrum showing the near- mid- and far infrared regions relevant for this study, with their relation to the red region of the visible spectrum. The different wavelengths and frequency over which they span are also shown (taken from Noreen *et al.*, 2012).

2.4.2 MODES OF SPECTRAL ACQUISITION

There are several modes for acquisition of the infrared spectra. One or more of these are usually available on one spectrometer, for example transmission, absorbance and reflectance mode. All these different combinations provide the user with a wide range of options, with which to develop a portfolio of methods suitable for different sample matrices, such as liquids, semi-solids (pastes) and solids. Liquids and semi-solids are usually measured in transmission mode and solids in reflectance mode (Antal and Dávid, 2007). The same sample can also be measured in both modes (Manley *et al.*, 1994).

With transmission mode, the light that passes straight through the sample is measured, while absorbance mode measures the light that is absorbed by the sample (Figure 2.5). With the first two modes, the light source and detector are on opposite sides of the sample. With reflectance mode, the light beam is guided through the sample and the light source and detector are on the same side of the sample, as shown in Figure 2.5. In one version of reflectance, referred to as attenuated total reflectance (ATR), a transparent crystal with high refractive index, such as a diamond for instance, guides the light to be totally internally reflected in the crystal. The IR radiation follows the shape as determined by the cut of the diamond and protrudes a few micrometres (0.5 – 5 μm) from the surface of the crystal into the sample (low refractive index). In those infrared regions where the sample absorbs energy, the total energy of the infrared wave is attenuated and measured at the detector (Figure 2.6) (Smith, 2011; Goormaghtigh *et al.*, 1999).

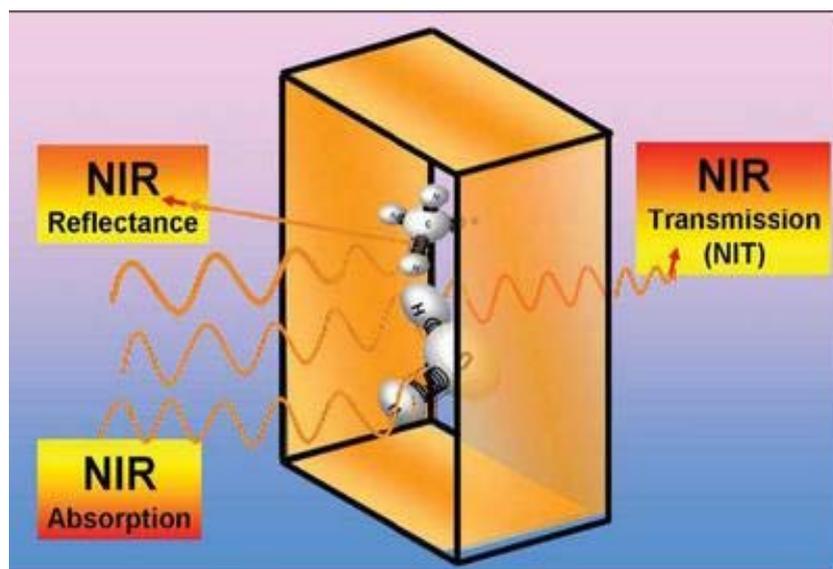


Figure 2.5 Diagram explaining transmission, reflectance and absorbance modes in NIR spectroscopy (Source: <http://en.engormix.com/MA-pig-industry/articles/near-infrared-nir-spectroscopy-t136/p0.htm>)

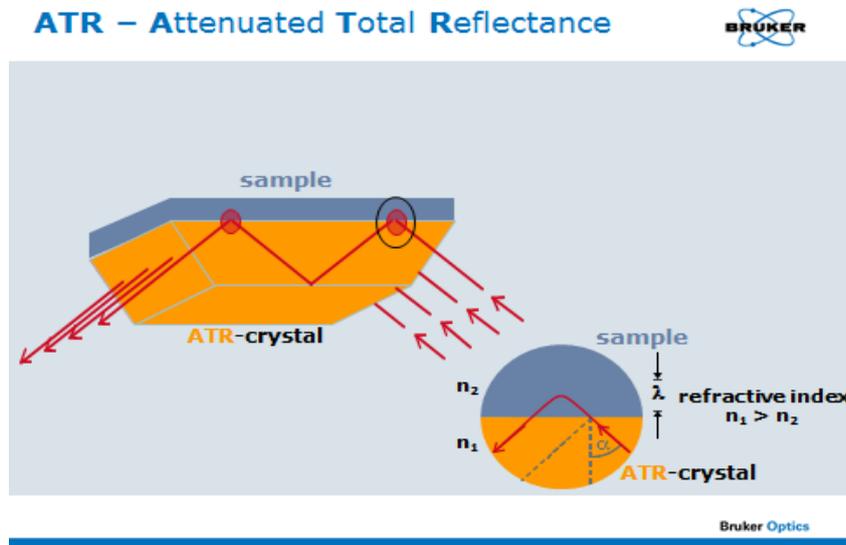


Figure 2.6 Diagram explaining FT-MIR ATR spectroscopy (Used with permission from Bruker Optics, Ettlingen, Germany, <http://www.bruker.co.za>)

2.4.3 MAIN DIFFERENCES BETWEEN MIR AND NIR

MIR spectra contain the measurements of fundamental vibrations of diatomic molecules in the mid-IR region (Barton II, 2002), while NIR spectra contain the intensities of overtones (780 - 1 800 nm) and combinations bands resulting from fundamental vibrations (1 800 – 2 500 nm) of the mid-IR region (Arikan, 2008).

MIR spectroscopy is used to measure the chemical composition of substances like solids, liquids and gases. Absorbance by organic molecules is very specific and the structure of the molecules can be deduced from the absorbance pattern at the various wavenumbers (Skoog *et al.*, 1997). MIR light is absorbed more strongly by biological molecules compared with NIR light (Magwaza *et al.*, 2012), which makes NIR more suitable for utilisation in acquiring information about the internal structure of products, since the light can penetrate deeper into the object irradiated. Infrared irradiation of an object, like a grape berry for instance, will result in the light being reflected, absorbed or transmitted due to the numerous phase changes caused, for instance by cell walls, that the infrared light encounters (Nicolai *et al.*, 2007). How much of which of the three occurs, however, is depended upon the chemical and physical properties of the sample (Lu, 2007). Hence, the external reflectance off a fruit surface for instance, gives information about the surface of the sample as well as the internal structure and chemistry scattering which is caused by numerous alterations at phase changes inside the material (Nicolai *et al.*, 2007). NIR will, therefore, be good to determine whether any change in the chemical (TSS, TA, pH glucose and fructose) or physical aspects (browning), due to cell wall degradation or cell death of the grapes, has occurred. Ultimately external browning (due to reflectance) and internal browning (due to scattering) can then be measured.

With NIR there is also no need for any or further sample preparation and a measurement can be taken anywhere (in the laboratory as was done in this study or in the vineyard later on when this technology will be transferred to the industry), where-as with MIR sample preparation might be necessary (filtration of juice samples in this study) (Bellon-Maurel and McBratney, 2011).

Fourier transform spectrometers use an interferometer to generate the light and the first signal is generated in the time domain (Skoog *et al.*, 1997). The Fourier transformation is responsible to convert the time domain to the wavelength, or wavenumber domain, as is

applicable. FT-IR spectrometers provide the infrared spectrum much more rapidly than the dispersive instruments (Pavia *et al.*, 2009).

2.4.4 THEORY AND PRINCIPLES

Biological substances are made out of molecules that have covalent bonds which can absorb a number of frequencies in the infrared region (Caplan *et al.*, 2006). As with other types of radiation, molecules are excited to a higher energy state when they absorb infrared radiation. This causes them to go into vibrational and rotational states. The frequencies at which they vibrate can be calculated, since a specific covalent bond will only absorb unique frequencies and at intensities characteristic for the bond in question (Antal and Dávid, 2007). No two molecules of different structure have exactly the same infrared absorption pattern, also referred to as infrared spectrum. Thus, the infrared spectrum of a substance can be considered as a fingerprint of that particular substance (Kümmerle *et al.*, 1998). By comparing the infrared spectra of two substances thought to be identical, one can establish whether they are, in fact, identical. In most cases the two substances will be identical if their infrared spectra coincides peak for peak (absorption for absorption) and statistical indices such as the chi-squared value, often used to evaluate the degree of similarity, are the same. Spectral libraries that show the wavelengths at which the most abundant covalent bonds in inorganic substances absorbs are available and can be referred to in order to obtain information on their structure from infrared spectra (Pavia *et al.*, 2009).

An example of a FT-NIR spectrum taken of a whole table grape berry is shown in Figure 2.7 (a) and one taken of a table grape juice sample on the FT-MIR ATR instrument is shown in Figure 2.7 (b). Water is the most abundant chemical constituent of most fruit and vegetables and is a highly polar molecule. This is due to the weak van der Waals forces that exist between the millions of separate water molecules. Water is, therefore, highly polar and absorbs infrared radiation over hundreds of adjacent wavenumbers, thereby dominating the infrared spectrum as can be seen in Figure 2.7 (a&b). These water absorption bands are fairly broad with centers at approximately $10\,309\text{ cm}^{-1}$, $8\,333\text{ cm}^{-1}$ and $6\,897\text{ cm}^{-1}$ (Sims and Gamon, 2003) and at $3\,400\text{ cm}^{-1}$ and $1\,650\text{ cm}^{-1}$ (Brubach *et al.*, 2005). The absorption range ($6\,301\text{ cm}^{-1}$ – $4\,403\text{ cm}^{-1}$) for glucose and fructose in aqueous solutions is also shown (Rambla *et al.*, 1997) in Figure 2.7 (b).

The IR spectrum is essentially composed of a large set of overtones and combination bands. This, in combination with the complex chemical composition of a typical fruit or vegetables, causes the NIR spectrum to be highly intricate. Since grape berries are made out of different tissues and some of the light with which the berries are scanned with can be reflected off different surfaces or objects within close proximity of the berries, it will, therefore, be difficult to tell which band in the spectrum should be assigned to which compound of which tissue (clear part or brown part). Special mathematical methods known as chemometrics are, therefore, needed to retrieve the important information that is contained within the IR spectrum (Nicolai *et al.*, 2007).

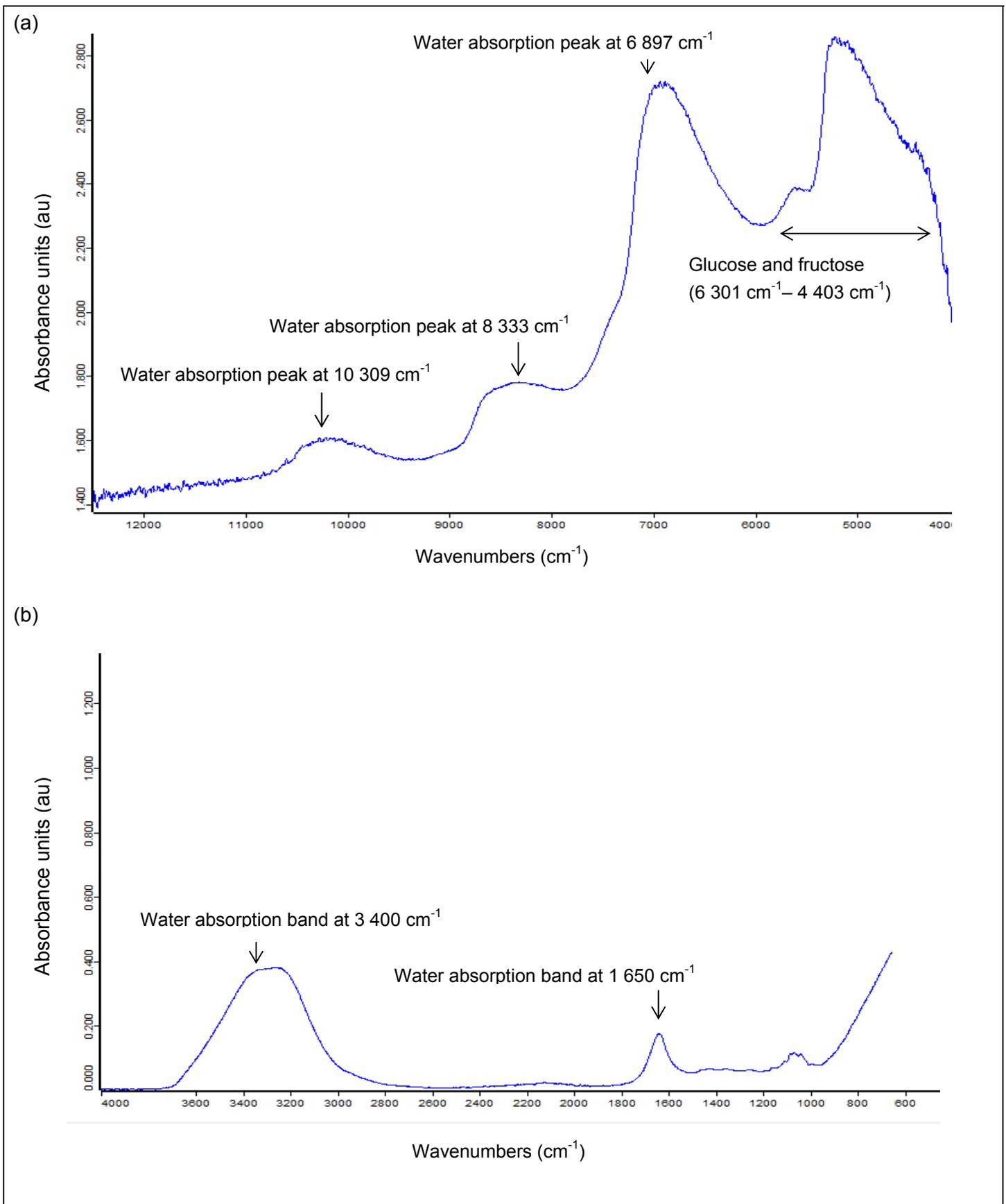


Figure 2.7 (a) Example of FT-NIR spectrum taken of a whole table grape berry indicating water absorption peaks with centres at 10 309 cm⁻¹, 8 333 cm⁻¹ and 6 897 cm⁻¹, and absorption range for glucose and fructose in aqueous solutions (6 301 cm⁻¹ – 4 403 cm⁻¹); and (b) FT-MIR ATR spectrum taken of a table grape juice sample with strong water absorption bands at 3 400 cm⁻¹ and 1 650 cm⁻¹.

2.4.5 ADVANTAGES OF FT-MIR AND FT-NIR SPECTROSCOPY

Most analytical techniques require sample preparation to separate the components of interest so that those can be determined individually. After such treatments the product no longer has its original shape and constitution, since both these are destroyed during sample preparation and analysis. This sample destruction presents a problem if there is a need for the original sample for further utilisation as in the case of table grapes. These two requirements, maintenance of physical and chemical integrity and rapid analysis time, therefore, provides the motivation for using non-destructive analytical methods like NIR to measure fresh produce quality attributes. An idea penned three decades ago by Polesello and Giangiaco (1981), but which has not yet been implemented in the table grape industry of SA to monitor postharvest quality of fruit.

Clément *et al.* (2008) determined the lycopene content of intact tomato fruit non-destructively by using NIR in reflectance mode. It, therefore, seemed quite feasible to determine browning of whole white seedless table grape berries non-destructively also using NIR in reflectance mode. The determination of the maturity parameters, TSS, pH, TA, glucose and fructose were however, done destructively (berries were crushed to obtain juice for measurements), but no further sample preparation was necessary to extract and measure the desired components.

Another disadvantage of the traditional analytical methods is the lengthy time needed for each individual analysis. A total of 327 typical German wines and must were analysed by Patz *et al.* (2004) by means of liquid FT-MIR spectroscopy. Analysis of each sample only took 90s to complete, but results for 20 important parameters in wine were obtained simultaneously. Measurement of the maturity and quality parameters in this study were also fast and results were obtained immediately.

FT-MIR and FT-NIR spectroscopy can also either be used on their own or together as in the following examples where FT-MIR and FT-NIR spectroscopy was used to assess the quality of Chinese preparation oil (Wu *et al.*, 2008) and carbon and nitrogen originating from char and forest-floor material in soils was distinguished (Michel *et al.*, 2009).

The versatility of FT-NIR spectroscopy to determine an array of different parameters on the same sample was displayed when used to determine firmness, skin and flesh colour, and dry matter content of pickling cucumbers (Kavdir *et al.*, 2007) and to measure firmness and SSC of bell pepper (Penchaiya *et al.*, 2009).

There is also limited or no need for sample preparation as shown when whole apples bought from a supermarket were scanned to measure acidity, SSC and firmness (Lammertyn *et al.*, 1998). It is applicable to a wide variety of different types of samples like dried green Honeybush plant material which was scanned in diffuse reflectance mode for quantification of the phenolic compounds mangeliferin and hesperidin (Joubert *et al.*, 2006), phenolic compounds in wine scanned in transmission mode (Cozzolino *et al.*, 2004) and intact frozen olive fruit samples scanned in reflectance mode to confirm the usefulness of FT-NIR analysis as a selection tool in olive breeding programs (León *et al.*, 2004). Even wheat and barley were investigated through the secondary structure of their storage proteins by means of FT-MIR spectroscopy (Marakenko *et al.*, 2002).

2.4.6 APPLICATIONS TO DETECT BROWNING IN FRUIT

The chemical components or the cellular structure of a berry that has gone brown due to either internal browning or abrasion damage will be different from that of a berry that has remained healthy. This change in chemistry or physical structure can potentially be picked up by a reflectance or absorbance measurement and be revealed in the resulting spectrum (Xing and

Guyer, 2008). Various other studies have already reported on accurate, fast, economical and, most importantly, non-destructive determination of fruit or vegetable flesh qualities such as firmness and colour using NIR spectroscopy (Ozanich, 2001, Kavdir *et al.*, 2007). Fu *et al.* (2007) showed the feasibility of visible near-infrared (VIS-NIR) techniques for classifying pear with brown heart from sound ones. Clark *et al.* (2003) showed that NIR spectroscopy could also be used to measure microstructure-related attributes such as internal damage of apples and Ozanich (2001) detected moderate to severe internal disorders such as water-core, internal browning and rot in apples.

In the same sense NIR spectroscopy could also be implemented in monitoring of these quality defects qualitatively in table grapes. Based on these previous successes obtained with the application of infrared spectroscopy to detect browning in apples and pears, it was considered feasible to investigate the utility of this technology in table grape browning. For technological and economic reasons, the table grape sector will only benefit from non-invasive analytical methods such as this to facilitate quality control of grapes. Also, to make available a portfolio of techniques, not only for external appearance and internal quality, but where grape maturity and quantitative quality parameters such as TSS, pH, TA, glucose and fructose can be determined in reflectance and transmission mode.

2.5 CHEMOMETRICS

2.5.1 INTRODUCTION

Chemometrics is a technique that is applied to extract relevant information from chemical data through the use of statistical and mathematical methods (Hopke, 2003). Paul (2009) discussed the wide use of NIR and MIR spectroscopy and the application of chemometrics in almost all of the different agricultural sectors in SA. These include food science, industrial, environmental, wine and viticulture industries; in the table grape industry neither IR spectroscopy nor chemometric techniques have been applied. This is surprising given the powerful capacity of these techniques to extract relevant information from complex measurements such as IR spectra, as demonstrated in this study. Two techniques that are considered as workhorses in chemometrics are principal component analysis (PCA) and partial least squares (PLS) regression. Due to their prominent role in this thesis, these techniques are discussed in sections 2.5.2 and 2.5.3 respectively.

2.5.2 PRINCIPAL COMPONENT ANALYSIS (PCA)

The underlying principle in chemometrics is the treatment of a dataset as a matrix, which consists of the **X**-matrix (independent variables, such as infrared spectra), a **Y**-matrix (response variables, such as chemical concentrations for TSS, pH, TA) and the **E**-matrix (error, or variance not captured by the chemometric model) (Esbensen, 2006). PCA projects, through graphical displays, an overview of the major variation in the **X**-matrix, as well as the underlying causes for this variation in the sample set (Nieuwoudt *et al.*, 2004). In this way, so-called *outlier* samples that differ largely from the whole sample population can be identified. Two important plots used to project this information are the scores plot and loadings plot, respectively. Figure 2.8 (a) show a score plot of FT-MIR spectra taken of table grape juice. The PCA model of TSS show the variation in the dataset by displaying the spectra in space demarcated by principal components (PC's), also referred to as latent variables. PC's are vectors, therefore, they have length and dimension and are calculated as a combination of the original variables and to contain, in decreasing order, most important variation in the sample set (Esbensen, 2006). The information of most importance can be found in the data (Esbensen, 2006). The first two PC's as can be seen in Figure 2.8 (a) captures

almost a hundred percent of the variance related to TSS. The first PC explains 94% of the variation in the data, the second PC 3% and the third one also 3%. Scores refer to the co-ordinates of each sample (in this case IR spectrum of the sample) in this new space defined by PC's. Each complete spectrum is projected by a single marker (sample number) in the plot.

The loadings plot in Figure 2.8 (b) shows the contribution or “loading” of the original variables (wavenumbers in the example shown) to the new position of the samples in the scores plot (Esbensen, 2006).

Mathematically, the decomposition of the complete dataset into different matrices can be presented as follows:

$$\mathbf{X}(n,m) = \mathbf{T}(n,k)\mathbf{P}(k,m)^T + \mathbf{E}(n,m)$$

Where, \mathbf{X} is the independent variable matrix, \mathbf{T} the scores matrix, \mathbf{P} the loadings matrix, \mathbf{E} the error matrix, n the number of objects/samples, m the number of variables and k the number of PC's used (Esbensen, 2002).

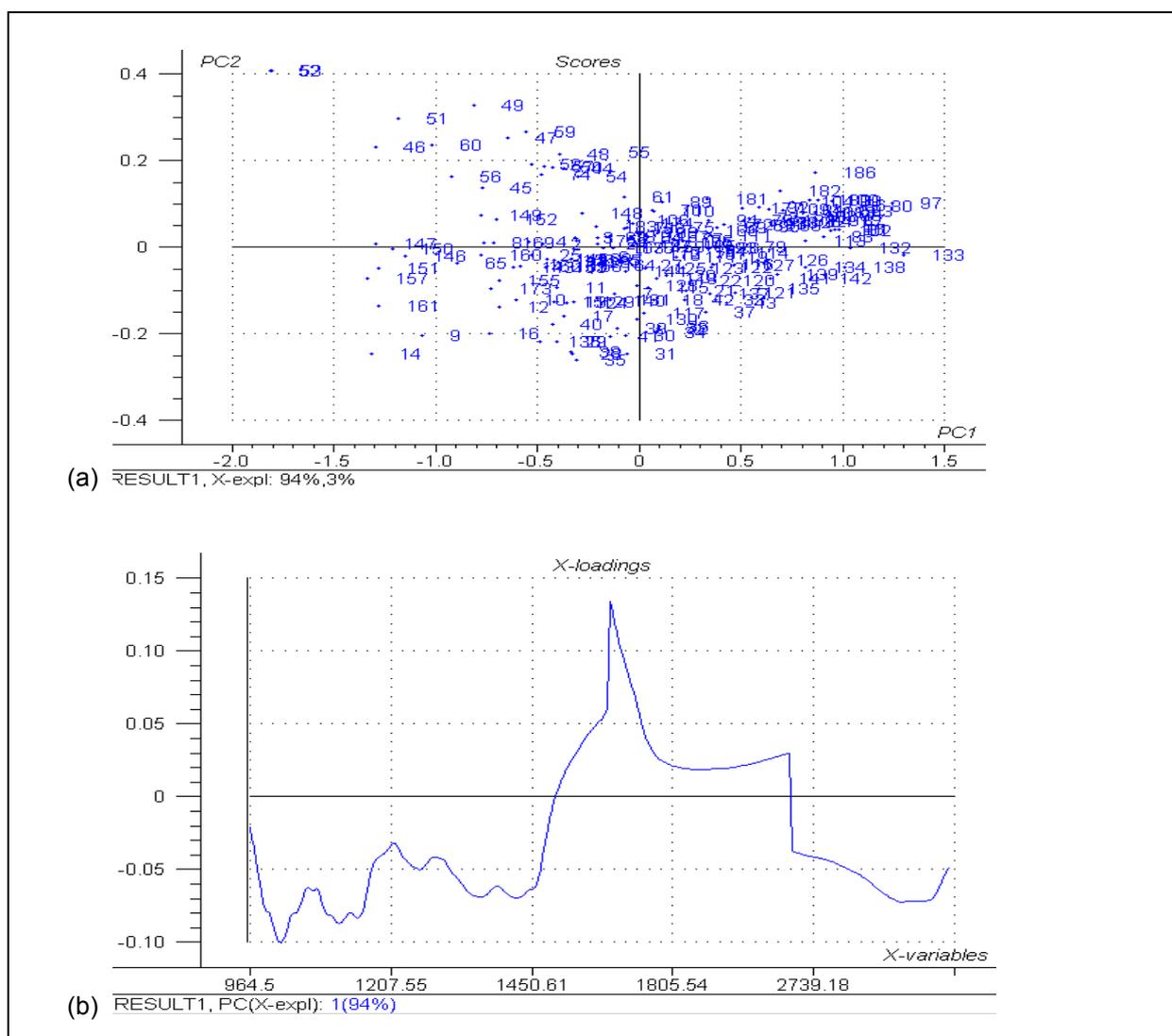


Figure 2.8 (a) PCA score plot showing distribution of the FT-MIR infrared spectra of table grape juice; and (b) Loadings plot showing that the information captured at almost all wavenumbers contributed to the pattern observed in the scores plot (own data).

2.5.3 PARTIAL LEAST SQUARES REGRESSION

Partial least squares (PLS) regression is a bilinear modelling method, which seeks to find a relationship between the **X**-matrix (berry spectra in this case) and the **Y**-matrix (the response variables, maturity parameters TSS, pH, TA, glucose and fructose in this case), that were measured by relevant reference methods in the laboratory (Esbensen, 2006; Saeys *et al.*, 2008). The relationship between the **X** and **Y** matrices is mathematically calculated and referred to as a calibration model.

The performance of PLS models can be evaluated in terms of different statistical indicators that show the model accuracy and prediction error that can be expected with future similar measurements. In this study, the calibration model accuracy was described by the root mean square error of cross validation in calibration data (RMSECV), the root mean square error for prediction (RMSEP) in the validation data, the coefficient of determination (R^2), the residual prediction deviation (RPD) and the bias of the model. The R^2 value represents the proportion of explained variance of the response variable in the calibration or validation set (Saeys *et al.*, 2005; Brown *et al.*, 2005). The RMSECV value gives an indication of the prediction error in the calibration set used for building the model, while RMSEP value gives the average uncertainty that can be expected for predictions of future samples (Esbensen, 2006). The 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 of the validation set and gives some indication of the efficiency of a calibration model (Williams and Norris, 2001). An RPD value of 2.5 to 3 shows that the model is fit for quantification, one of 2 to 2.5 shows that it is fit for screening purposes and one of 1.5 to 2 shows that the model is not suitable for quantification purposes (Saeys *et al.*, 2005). Bias gives an indication of the systematic error in the predicted values and is calculated as the average difference between the reference values and the corresponding predicted values, also referred to as the residual values (Esbensen, 2006). The residual will be equal to zero if there is no difference between predicted vs reference values.

In addition to the abovementioned statistical parameters, the *rank* of a calibration model is also taken into account to evaluate possibilities of overfitting of a model. Rank refers to the number of PLS components used in the construction of the model (Arcenegui *et al.*, 2008). The optimum number of PLS components is usually judged by the RMSECV value which should be as small as possible. A relatively low number of PLS components is generally desirable to avoid modelling noise or information not related to the response variables of interest (Fernández-Novales *et al.*, 2009). All these statistical indicators are shown in Figure 2.9 for a calibration model constructed for TSS on the FT-NIR instrument.

For a calibration model to perform well on future samples it has to be extensively tested by independent test samples that have not been part of the calibration set. In addition, the calibration set should always span the concentration range of the reference values (Nicolai *et al.* 2007). In order to increase the accuracy of the calibration models it is important to have sufficient variability in the dataset on which the model is to be based. Temperature effects (Segtnan *et al.*, 2005), season to season variation (Bobelyn *et al.*, 2009), cultivar effects (Peirs *et al.*, 2003) and batch effects (Sahni *et al.*, 2004) all contribute towards variability in spectra. In this study two cultivars from two different blocks were harvested over two different seasons and at three different crop loads and sugar levels to try and address this requirement.

Multivariate calibration models are referred to as *robust* when the prediction accuracy is relatively insensitive towards unknown changes of external factors (Diezma-Iglesias *et al.*, 2008). This means that the model statistical parameters do not change very much when new samples are added to the calibration set (Geladi and Kowalski, 1986). Calibration sets, therefore, have to be

carefully selected, as was done in this study, in order to design calibration models that find a balance between robustness and accuracy of prediction (Nieuwoudt *et al.*, 2004).

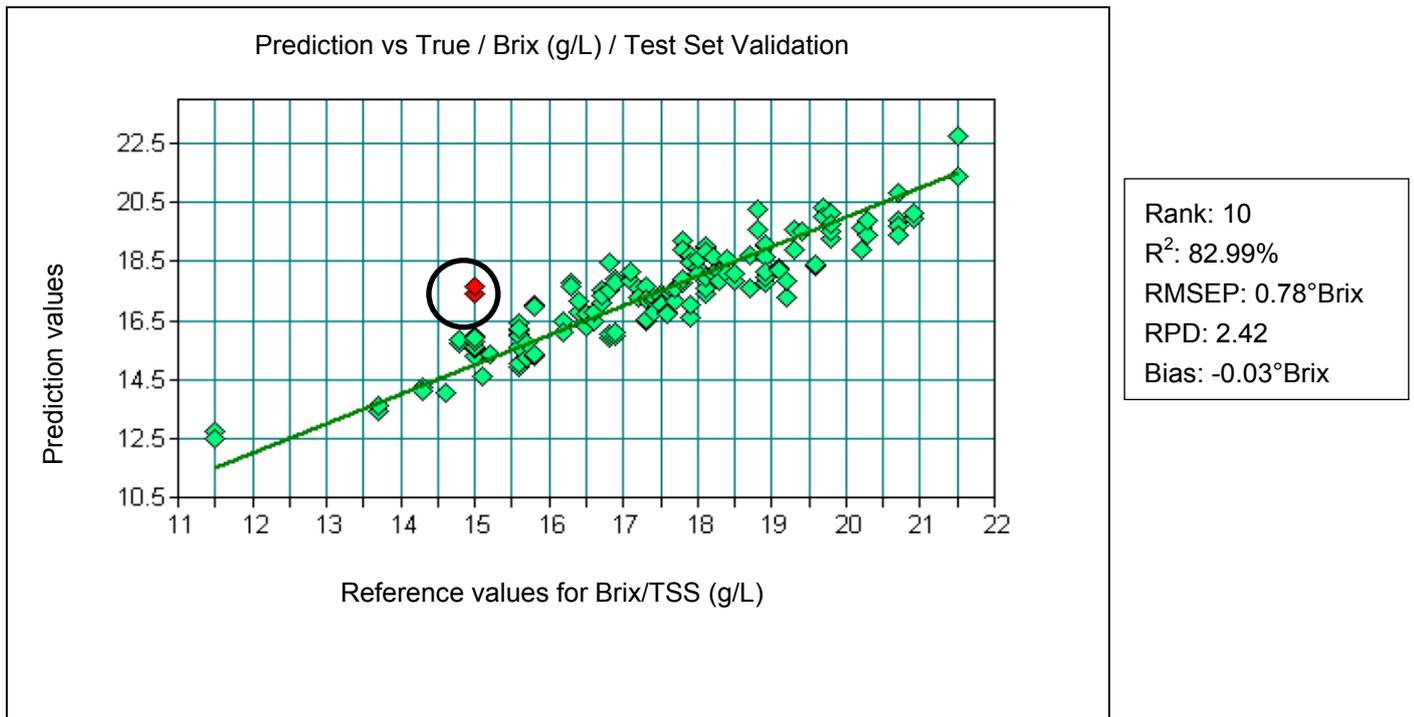


Figure 2.9 Regression statistics for the PLS calibration model constructed for quantification of TSS ($^{\circ}$ Brix/g/L), based on FT-NIR spectra of table grape juice. The different statistical indicators (text box insert) to describe the performance of the model are shown. The outliers are enclosed in a circle in the figure.

2.5.4 DISTRIBUTION OF REFERENCE MEASUREMENTS

The way the values of reference measurements are distributed is very important due to the implications that it has for the construction of the calibration models. In any given data range there is a minimum and a maximum value. In order to establish how the reference values in a dataset is distributed a histogram can be plotted. This is done by plotting the value intervals against the number of observations in each interval (Figure 2.10 (a) and (b)). The histogram is one of the seven basic tools used for quality control of a dataset. Here it gives a good indication of the spread of the reference data used to construct calibration models. Depending on the values in the dataset, the histogram can either follow a normal distribution as can be seen in Figure 2.10 (a) for the combined 2008 and 2009 reference data of Regal Seedless and Thompson Seedless for TSS, or it can be highly or moderately skewed to the left or right. If the histogram is skewed to the right or positively skewed it looks like the one in Figure 2.10 (b) which is the combined 2008 and 2009 reference data of Regal Seedless and Thompson Seedless for TA.

It is not always possible to get a perfect distribution of reference data, due to practical reasons, such as the fact that grape berries are individual biological entities in which biochemical processes such as the accumulation of sugar and the breakdown of acids do not always occur in synch with each other, as well as the element of human error during the conduct of the experiments to determine the reference values. The implication of this for the selection of calibration monsters is the distribution of the samples along the calibration curve. Some of the samples may be grouped at one end of the calibration curve with spaces in between and then one or a few samples at the other end. This is illustrated clearly by the distribution of the data in Figure 2.10 (b). To tackle this issue of skewness in data, proper sampling procedures can be followed during the selection of

calibration samples. The reference measurements should also be refined and be done as accurately as possible. Certain transformations can also be applied to the data if it happens to be highly negatively or positively skewed.

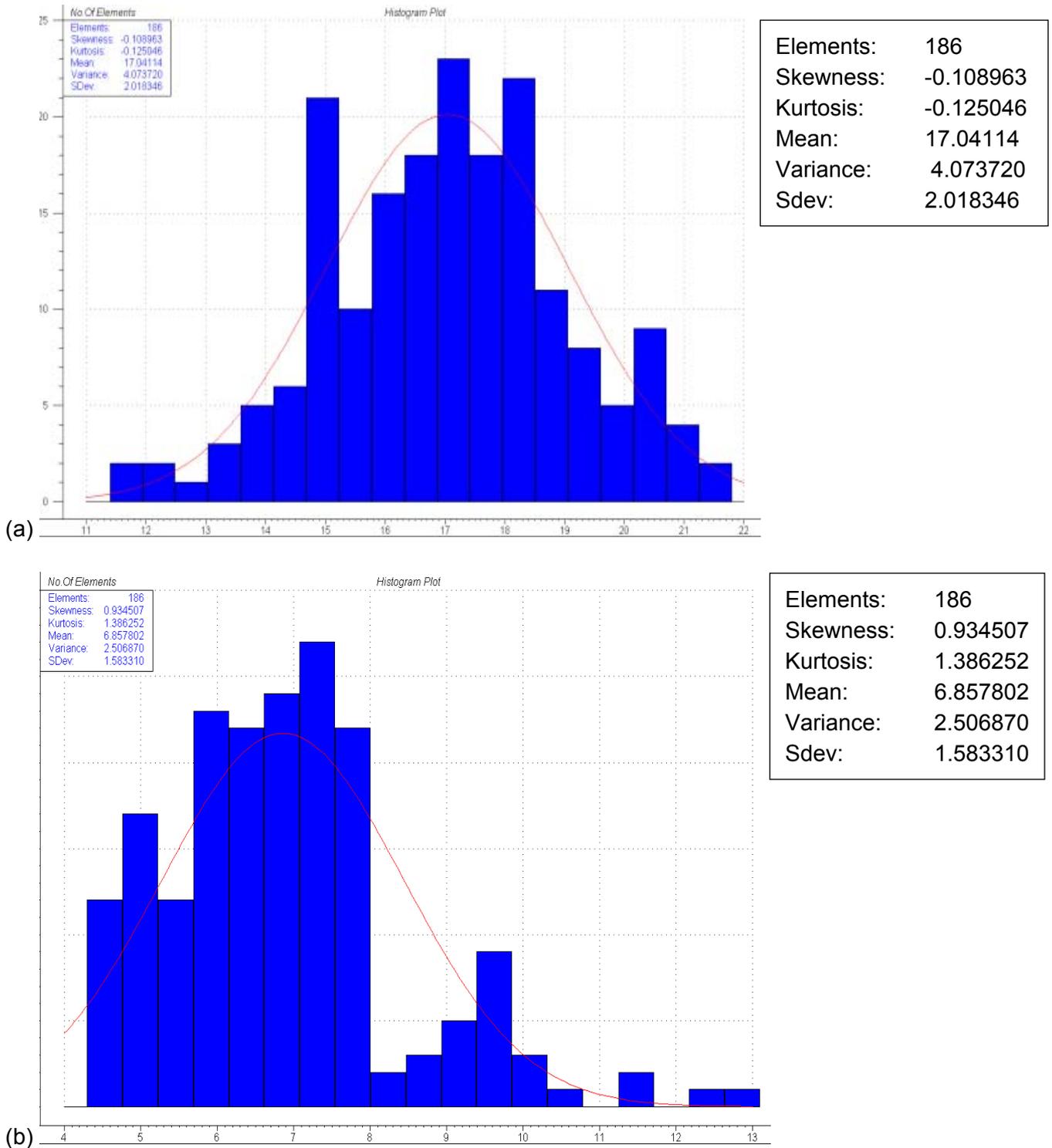


Figure 2.10 Example of a histogram showing combined 2008 and 2009 reference data of Regal Seedless and Thompson Seedless for (a) TSS which follows a normal distribution; and (b) TA which do not follow a normal distribution (Own data).

2.6 SUMMARY

Browning of white seedless grapes remains an economically, serious problem for table grape producers in SA, as is the case all over the world. The problem is clearly of a complex and multivariate nature since the research to date has shown that there is no single dominant factor directly associated with either internal or external browning (DFPT Researchers, 2009).

The table grape industry is currently depended on quality classification systems for fruit, like most other industries, based on the external aspects like colour, size and absence of blemishes of the product. This is currently the only way to evaluate grapes for browning. The focus of this thesis is to investigate new and improved methods, like IR spectroscopy, to try and monitor table grape quality qualitatively and quantitatively throughout the value chain.

IR spectroscopic analysis is fast and multiple parameters can be determined simultaneously. In many examples cited from the literature in this review it is clear that quantitative IR spectroscopic results are comparable, or in some instances better in accuracy than those of conventional analytical techniques. As there is no or very little need for sample preparation or pre-treatment their precision is usually higher (Lu *et al.*, 2008).

This study will, therefore, not only explore the possible future development of detection or prediction systems for browning on white seedless table grapes, but also the creation of a platform of technologies to determine table grape maturity and quality parameters, TSS, pH, TA, glucose and fructose fast and accurately. The importance of expanding the range of metabolites measured for the purposes of identification of biomarker molecules associated with browning has already been stressed earlier in this review. It is also important to keep in mind that supporting analytical techniques, such as mass spectrometry and chromatography will undoubtedly, play an important role in such an endeavour.

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Chapter 3

Research results

Quantification of sugar content, pH and titratable acidity in table grapes using near-, mid- and attenuated total reflectance mid infrared spectroscopy

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Quantification of sugar content, pH and titratable acidity in table grapes using near-, mid- and attenuated total reflectance mid infrared spectroscopy

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3.1 ABSTRACT

Infrared (IR) spectroscopy was investigated as a fast and easy way to determine total soluble solids (TSS, measured as °Brix), pH, titratable acidity (TA, expressed as g/L tartaric acid), glucose (g/L) and fructose (g/L) in the juice of two white seedless table grape cultivars, Regal Seedless and Thompson Seedless, on three different infrared spectrometers. Partial least squares (PLS) regression was used to construct calibration models of the parameters on each instrument. These models were then compared using the prediction error (R^2), the root mean square errors of prediction (RMSEP), the ratio of prediction to deviation (RPD) and the bias to see which instrument delivered the best calibration model for all the parameters. The FT-NIR and FT-MIR ATR instruments gave calibration models that were fit for screening purposes for TSS (11.40 - 21.80°Brix) ($R^2 = 85.92\%$, RMSEP = 0.71 °Brix RPD = 2.67 and bias = 0.03°Brix), pH (2.94 - 3.9) ($R^2 = 85.00\%$, RMSEP = 0.08 RPD = 2.59 and bias = -0.01) and TA (4.3 - 13.1 g/L), ($R^2 = 90.77\%$, RMSEP = 0.48 g/L RPD = 3.30 and bias = -0.03 g/L). Models for fructose (46.70 – 176.82 g/L) ($R^2 = 74.66\%$, RMSEP = 9.28 g/L RPD = 2.00 and bias = 1.10 g/L) and glucose (20.36 – 386.67 g/L) ($R^2 = 70.71\%$, RMSEP = 11.10 g/L RPD = 1.87 and bias = 1.64 g/L) were obtained with FT-NIR and FT-MIR ATR instruments that were in some instances fit for screening purposes and in some instances unsuitable for quantification purposes. The FT-MIR instrument gave models for all the parameters that were not yet suitable for quantification or screening purposes. There are now not only high throughput and powerful analytical methods available for the wine and other fruit industries, but for the table grape industry as well. This grants the opportunity for this technology to be transferred to the table grape industry, where these parameters can be determined in the vineyard, with fast and accurate results possible without any sample preparation.

KEYWORDS: Total soluble solids (TSS), pH, titratable acidity (TA), glucose, fructose, FT-NIR, FT-MIR, FT-MIR ATR, PLS calibration models.

3.2 INTRODUCTION

Table grape ripening is characterised by three different stages during their development, respectively the first stage of fast berry growth, the second is the lag stage and the third stage is again one of fast berry growth (Dokoozlian, 2000). *Véraison*, which refers to berry softening, occurs during the third stage of development of grape berries and signals the onset of ripening (Coombie and McCarthy, 2000). This is accompanied not only by the outward changes in the appearance of the grapes, like the enlargement of berries and the accumulation of colour in pink,

red and black varieties, but by other chemical processes as well, which are involved in the ripening process (Nunan *et al.*, 1998). These are the accumulation of sugar (TSS, glucose and fructose) and flavour compounds, as well as the breakdown of acids (tartaric and malic acid) simultaneously taking place in the grape berries (Dokoozlian, 2002). Evaluation of the outward appearance of table grapes alone is therefore not enough to determine if the grapes are at the correct ripeness stage for harvesting and if they have the right eating and postharvest quality. This especially considering that different table grape varieties have different optimum ripeness levels (Kliewer, 1967). Internal processes are, therefore, also of great importance, because as the TSS increases in the berries, the juice pH rises and the TA declines (Viti-Notes, 2005). Knowledge of whether this is occurring in the correct ratio (sugars accumulating as acidity is declining to ensure correct ratio at harvest for optimum quality) is of great importance. This especially since it is known that grape berry growth and maturity are not always in direct relationship and that variation in maturity between the berries, within a bunch and between bunches on a vine exist (Šuklje *et al.*, 2012). Although the concentrations of organic acids are low compared to that of the sugars in ripe berries, high acidity has a negative influence on the palatability of table grapes (Liu *et al.*, 2006) and this quality parameter should, therefore, be carefully monitored prior to harvest.

The determination of optimum ripeness levels for harvesting of table grapes is, therefore, of critical economic importance to table grape industries. Pioneering research done more than twenty years ago in South Africa (SA), showed that sugar content, TSS, pH and TA are important quantitative chemical parameters on which to evaluate optimum ripeness levels (László and Saayman, 1985), as well as palatability of the grapes (László and Saayman, 1993). Recent research confirmed these early conclusions, and according to Fahmi *et al.* (2012), the physical characteristics (berry size, colour, shape and firmness) and chemical composition (sugar and acidity) of table grapes can be used to benchmark the fruit quality. The decision to harvest has far reaching consequences for fruit quality, because if producers harvest their grapes too early, the fruit may not be palatable when finally available to the consumer. The sugar will be too low and the acid too high, affecting the palatability of the grapes negatively (Liu *et al.*, 2006). Grapes that are harvested late in the season, on the other hand, not only miss the right time in the market to garner favourable prices, but are also exposed to unfavourable environmental factors in the vineyards, like rain, diseases, such as botrytis and insect pests, like fruit flies (Hellman, 2004).

Table grape maturity is, therefore, a very complex but important factor that should be monitored on a constant, and an accurate basis, using rapid, high sample-throughput methods. The quantification of TSS is relatively easy and can be done with a handheld refractometer in the laboratory or in the vineyard. TA, pH, glucose and fructose quantification, however, requires special laboratory equipment and chemicals and can, therefore, not easily be done in the vineyard. Glucose and fructose are the main sugars in grapes and are usually present in almost equal amounts (Liu *et al.*, 2006). Their concentrations, however, may vary during the developmental stages of the grapes leading up to maturity (Muñoz-Robredo *et al.*, 2011). It was, therefore, also important to monitor both as harvest maturity and quality determining parameters.

Non-destructive methods with high throughput of information about the maturity status of table grapes are, therefore, of utmost necessity to the table grape industry of South Africa. This is particularly pressing for the local industry to maintain a competitive edge amongst fast emerging table grape producers in the Southern Hemisphere and the technological advances that are made in the field of postharvest technology. In Italy physico-chemical and sensory characteristics of table grapes are predicted through the application of hyperspectral imaging (Baiano *et al.*, 2012). The time to still rely on laborious and time consuming methods of determining especially pH, TA, glucose and fructose in the laboratory should be over. In order to improve the quality of its product, it is absolutely necessary that new, fast and cost effective techniques with the same accuracy, if

not better, than the current wet chemistry methods are investigated and implemented in the industry. In addition to the important role that TSS, pH and TA play in the assessment of grape maturity, it was also found that a relationship existed between harvest maturity and skin browning of Princess, a white seedless table grape cultivar (Vial *et al.*, 2005) in California and speculation is rife that this is also true for the two table grape cultivars (Regal Seedless and Thompson Seedless) used in this study.

The method of choice for rapid, high sample-throughput quantification of chemical compounds is infrared spectroscopy (De Villiers *et al.*, 2011). Both Fourier transform mid-infrared (FT-MIR) spectroscopy and Fourier transform near infrared (FT-NIR) spectroscopy have been used for the analyses of different parameters in wine-related matrices, but the technology has not yet been used on table grapes. FT-MIR is widely used in wine analysis (Gishen and Holdstock, 2000; Patz *et al.*, 2004; Nieuwoudt *et al.*, 2004a&b; Nieuwoudt and Bauer, 2004, and Fernández and Agosin, (2007). Several compounds are routinely analysed in fermenting must (Dubernet and Dubernet, 1999; Magerman, 2009) and the routine parameters TSS, pH and TA in freshly pressed grape juice (Swanepoel *et al.*, 2007). Nine chemical characteristics of wine grapes have been analysed with a portable, handheld near infrared (NIR) spectrometer (Kaye and Wample, 2005). Grape quality in terms of TSS has been estimated by Diezma-Iglesias *et al.* (2008), while reducing sugar content during grape ripening has been determined by Fernández-Novales *et al.* (2009). Cynkar *et al.* (2007) successfully quantified the concentration of total glycosylated flavour precursor compounds in white grape juice. FT-NIR spectroscopy has also been used for compositional analysis of soluble solids content in agricultural commodities such as apples (Peirs *et al.*, 2003; Lu, 2007) as well as quality defect issues, such as the detection of brown heart of pear (Fu *et al.*, 2007).

Infrared (IR) spectroscopy utilises the infrared region of the electromagnetic spectrum which is usually divided into three regions for practical purposes; near-(12 800-4000 cm^{-1}), mid- (4 000 to 400 cm^{-1}) and far-infrared, named for their relation to the visible spectrum (Skoog *et al.*, 1997). Compounds, both inorganic and organic, that have covalent bonds, absorb specific frequencies of electromagnetic radiation upon interaction with infrared radiation (Pavia, 2009). MIR spectra contain the information related to fundamental vibrations (Skoog *et al.*, 1997) of chemical bonds in functional groups such as C-C, C-H, O-H, C=O and N-H, upon absorption of radiation in the mid infrared (IR) region (Nieuwoudt *et al.*, 2004a; Nieuwoudt and Bauer, 2004). NIR spectra, on the other hand, contain overtones and combination bands that are derived from fundamental vibrations of the mid-infrared region (Skoog *et al.*, 1997). Typical features of NIR spectra of organic matter include the O-H bonds of water, as well as bonds such as C-N, N-H and C=O, characteristic to organic matter (Rinnan and Rinnan, 2007).

The objective of this study was, therefore, to evaluate the quantification of TSS, pH, TA, glucose and fructose with infrared spectroscopy. Three different spectrometers were used to develop a portfolio of methods, suitable for the industry and the research laboratory.

3.3 MATERIALS AND METHODS

3.3.1 EXPERIMENTAL DESIGN

Two commercially important white seedless table grape cultivars, Thompson Seedless and Regal Seedless, respectively, were harvested over two harvest seasons from two different vineyards in the Hex River Valley, Western Cape, South Africa. In the Regal Seedless vineyard a different crop load (the total amount of bunches on the vines per hectare) were left in each row that was used as an experimental unit. In row 1 the total crop load was 75 000 bunches, in row 2, 50 000 bunches and in row 3, 35 000 bunches. Each row had six sections which represented a repetition for each

crop load. Two bunches were harvested from each section every harvest date. The harvest date corresponded to the sugar level on that specific day of harvest. For Thompson Seedless there were only two crop loads left on the vines 75 000 in row 1 and 50 000 in row 2. The same as with Regal Seedless each row also had six sections and each section also presented a repetition of the crop load in that row. Two bunches were also harvested from each section every harvest date. In 2008 the cultivars were harvested three times during the season; early at 16°Brix, at optimum ripeness at 18°Brix and late at 20°Brix. In 2009 the two cultivars were each harvested four times during the season, early at 16°Brix, twice at the optimum (18°Brix) (László and Saayman, 1993; Fraser, 2007) and late at 20°Brix. Since each section represented a repetition, for each harvest date or sugar level 18 samples were obtained for Regal Seedless and 12 samples for Thompson Seedless. This resulted in a total of 210 samples (54 for Regal Seedless in 2008 and 72 in 2009, for Thompson Seedless 36 in 2008 and 48 in 2009).

3.3.2 SAMPLING

In order to ensure that berries were selected to be representative berries were picked at different positions along the longitudinal bunch axis; 70 berries were randomly cut off with a clean, sharp scissor from the top, middle and bottom of each bunch. Fifty berries were put into a plastic bag so that the TSS in °Brix, the titratable acid (TA) in g/L and the pH could be determined. Twenty berries were put into another plastic bag to determine the glucose and fructose content. All the bags were put into separate, clearly marked 4.5 kg boxes for easy transport and storage.

3.3.3 REFERENCE MEASUREMENTS

3.3.3.1 TSS, pH and TA

The total of 210 grape juice samples were prepared by crushing the berries that were removed from bunches in a small sterile plastic bag for 1 minute and then filtered through cheese cloth to obtain clear, free flowing juice. The juice was used immediately to determine the TSS, TA and pH using methods recommended by the *Office International de la Vigne et du Vin* (<http://www.oiv.com>). TSS (measured in °Brix) was measured with a handheld digital refractometer (ATAGA Palette Digital Refractometer PR-32 Alpha, Tokyo, Japan). TA and pH were determined by using an automatic titrator (TIM 865 Titration Manager, Radiometer Analytical, Villeurbanne Cedex, France). To get an indication of the accuracy of the reference methods used, the standard error of laboratory (SEL) was used (Snedecor and Cochran, 1989). For TSS the SEL was 1°Brix, for pH it was ± 0.1 and for TA it was ± 0.2 g/L.

3.3.3.2 Glucose and Fructose

Twenty grape berries were placed into a 500 ml plastic container and homogenised with a Cambrook's Stick Blender for determination of glucose and fructose. It was determined beforehand that 20 berries would be necessary to obtain enough juice to determine glucose and fructose as well as for measurement on the three different IR spectrometers. Unlike the fifty berries which were used for determination of TSS, pH and TA, these grape samples were homogenised. This was because of the laboratory where the samples were analysed for TSS, pH and TA, daily receiving a large number of samples for analysis. Homogenisation of every sample from, a practical perspective would have been time consuming and was just not possible. The pulp was centrifuged at 12 000 rpm for 10 minutes at 16°C in a SORVAL RC 6 Superspeed centrifuge (Thermo Fisher

Scientific, Bath, United Kingdom). Aliquots (1.5 ml) of the clear juice (~100 ml) were frozen in Eppendorff tubes and the rest were used for measurement on the three different IR spectrometers. R-Biopharm enzymatic assay analysis kits (catalogue number 10139106035 AEC Amersham, Sandton, South Africa) were used for analysis of glucose and fructose. The tests were done according to the method specified by the manufacturer; except that the total assay volume (3 ml) was scaled down to 1 ml. Absorbance readings were taken at 340 nm on a Helios β Thermo Spectronic Spectrophotometer (Thermo Fisher Scientific, Bath, United Kingdom). Each juice sample was analysed in duplicate. The coefficient of variance determined for the duplicate test was 5%.

3.3.4 IR SPECTROSCOPY MEASUREMENTS

Three different infrared spectrometers namely (a) the Bruker Multipurpose Analyser (MPA FT-NIR), (b) the Foss (FT-MIR) and (c) the Alpha (FT-MIR ATR) spectrometer were used to determine the different parameters so that a platform of technology would be available to the table grape industry.

3.3.4.1 SAMPLE PREPARATION

Spectra of the juice samples on all three spectrometers were taken shortly after homogenisation of the berry samples in section 3.3.3.2. Before measurements were done in absorbance/transmission mode on the FT-MIR instrument (Foss Analytical, Denmark) as well as on the FT-NIR instrument (Bruker Optic GmbH, Ettlingen, Germany), the juice was first filtered with a Filtration Unit (type 79500, Foss Analytical, Denmark) and filter paper (Lasec, Cape Town, South Africa). This was done to avoid any particles that might still be left in the juice from becoming stuck in the 37 μm optical pathlength of the cuvette of the WineScan. No further sample preparation was, however, done for measurement of the juice samples on the FT-MIR ATR instrument.

3.3.4.2 FT-NIR SPECTROSCOPY

NIR spectral data were collected in transmission mode using a quartz flow cell of 1 mm pathlength on the Multipurpose Analyser (MPA) FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany). The spectral data were collected over the range 12 500 to 4 000 cm^{-1} (resolution 8 cm^{-1} ; scanner velocity 10 kHz; background with air, 16 scans; sample, 16 scans). Two independent aliquots of each juice sample were measured. The ambient temperature of the laboratory was $\pm 22^\circ\text{C}$.

3.3.4.3 FT-MIR ATR SPECTROSCOPY

A small volume (0.5 mL) of unfiltered juice was placed onto the 5 mm diameter sample plate of the Alpha FT-MIR ATR spectrometer (Bruker Optic GmbH, Ettlingen, Germany) for measurement. The spectral data were collected over the range 12 500 to 4 000 cm^{-1} (resolution, 8 cm^{-1} ; scanner velocity 10 kHz; background with air, 16 scans; sample, 16 scans). Two independent aliquots of each juice sample were measured. The sample plate was rinsed with clean water between measurements. The attenuated total reflectance mode only became available recently. It has the huge advantage, in that it is a very small instrument particularly useful for industry laboratories and no sample preparation is necessary.

3.3.4.4 FT-MIR SPECTROSCOPY

With the FT-MIR instrument the spectrum of each juice sample was obtained immediately after filtration in transmission mode. The transmission spectra are, however, converted into linearised absorbance spectra through a series of mathematical procedures (Nieuwoudt *et al.*, 2004a) including Fourier transformation (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001). The FT-MIR instrument is equipped with a Michelson interferometer and CaF₂-lined cuvette with a path length of 37 μm (Foss Analytical, Denmark). Since the WineScan is a specialised instrument designed especially for quantification of compounds in wine- and grape-derived matrices, some instrument settings are pre-set by the manufacturer and cannot be changed by the user. These include the sample temperature, which is set at 40°C, the scanning interval set from 930 to 5 011 cm^{-1} at a resolution of 4 cm^{-1} and the number of repeated scans of each sample, which is set at 20. Zero liquid S-6060 (Foss Analytical, Denmark) was scanned hourly prior to the addition of the samples to obtain a background scan (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001). Processing of the spectra generated on the WineScan instrument was done using the Advanced Performance software module of the WineScan instrument. Repeated scans of each sample were averaged and processed to a single beam transmission spectrum through a series of mathematical procedures, including Fourier transformation. Background absorbance was corrected for by division of the sample spectrum by the zero liquid spectrum at each recorded wavenumber. Finally, the corrected transmission spectra were converted to linearised absorbance spectra. The spectra were exported from the FT-MIR instrument and used in Unscrambler version 9.2 software (CAMO ASA, Oslo, Norway) to construct calibration models for the different parameters using PLS regression, as described in section 2.5.3, Chapter 2.

3.3.5 SPECTRAL PRE-PROCESSING

There were three different types of IR spectra generated for determining harvest maturity and quality parameters. Two of these on the FT-NIR and FT-MIR ATR instruments were generated in OPUS 6.5 and the other one on the FT-MIR instrument using Unscrambler version 9.2.

As part of the construction of the calibration models, spectra were subjected to pre-processing techniques to evaluate the effect of these treatments on the models' predictive abilities. The aim was to reduce optical interference not related to the chemical composition of the samples, like light scattering caused by dust particles or spray residues that might have been present on the surface of the berries (Arcenegui *et al.*, 2008). No spectral pre-processing, first and second derivative, straight line subtraction, vector normalization, multiplicative scattering correction, constant offset elimination and minimum-maximum normalization, as well as a combination of these methods were tested during calibration using OPUS 6.5 spectroscopic software (Bruker Optic GmbH, Ettlingen, Germany) on the FT-NIR and the FT-MIR ATR instruments. On the FT-MIR instrument no spectral pre-processing, first and second derivative, vector normalization, multiplicative scattering correction, minimum-maximum normalization, as well as a combination of first derivative and vector normalization were tested during calibration using Unscrambler version 9.2 software (CAMO ASA, Oslo, Norway).

3.3.6 CALIBRATION

In this study FT-NIR spectra were obtained in transmission mode from 12 500 cm^{-1} – 4 000 cm^{-1} , which resulted in 2 203 wavenumbers in the columns of the dataset. FT-MIR ATR spectra were

obtained in attenuated total reflectance mode from 4 000 cm^{-1} – 600 cm^{-1} which resulted in 2 404 wavenumbers in the columns of the dataset and FT-MIR spectra were obtained in absorbance mode from 930 cm^{-1} – 5 000 cm^{-1} , which resulted in 1 060 wavenumbers in the columns of the dataset. All the samples were measured in duplicate and both spectra were used during the calibration process. The reference values were duplicated for each sample which resulted in 420 observations in the rows of the dataset. The dataset were auto-scaled through mean centering. Principal component analysis (PCA) was performed to obtain an overview of the data and to observe clustering patterns and deviating spectra that do not fit in with the rest of the sample population. The latter group, also referred to as potential outlier samples, can have large negative effects on the performance of calibration models (Nieuwoudt *et al.*, 2004a). The dataset (414 samples) was divided in half and each set used alternatively as a training or calibration set (207 samples) and a test or validation set (207 samples). In all cases the minimum and maximum values for each chemical compounds were placed in the calibration set.

To construct calibration models for each parameter investigated in this study (TSS, pH, TA, glucose and fructose), PLS regression, a bilinear modelling method, which seeks to find a correlation between the spectra taken of the table grape juice samples on the different IR instruments and the reference values that were obtained in the laboratory for the different maturity parameters, was applied. PLS regression is a well known technique that have been described by several authors (Esbensen, 2006; Saeys *et al.*, 2008). If outliers were present these were removed and successive rounds of PLS regression done with the reduced dataset. After all outliers had been removed and a final PLS regression was done, the resultant calibration model was validated with the test dataset.

3.3.7 STATISTICAL INDICATORS

The performance of PLS models were evaluated in terms of different statistical indicators that show the model accuracy or prediction error that can be expected with future unknown samples. In this study the following statistical indicators were used: root mean square error for prediction (RMSEP) value which gives the average uncertainty that can be expected for predictions of future samples (Esbensen, 2006), the coefficient of determination (R^2) value which represents the proportion of explained variance of the response variable in the calibration or validation set (Saeys *et al.*, 2005; Brown *et al.*, 2005); the residual prediction deviation (RPD) value which is defined as the ratio of the standard deviation of the reference data of the validation set, to the RMSEP value and gives some indication of the efficiency of a calibration (Williams, 2001; Saeys *et al.*, 2005), and bias, which gives an indication of the systematic error in the predicted values and is calculated as the average difference between the reference values and their corresponding predicted values, also referred to as the residual values (Esbensen, 2006). A summary of the criteria used to interpret the accuracy of the FT-IR calibrations is given in Table 3.1. The rank of the model is the number of PLS components used in the construction of the model (Esbensen, 2006).

Table 3.1 Summary of criteria used to interpret the accuracy of the FT-IR calibrations (Williams, 2001; Saeys *et al.*, 2005).

Performance criterium	Fit for quantification	Fit for screening	Unsuitable for quantification
R^2	>70%	= 70%	<70%
RPD ^a	>3	2 – 3	1 - 2

^aRPD, Residual predictive deviation (Williams, 2001; Saeys *et al.*, 2005)

3.4 RESULTS AND DISCUSSION

3.4.1 DESCRIPTIVE STATISTICS OF REFERENCE SAMPLES

The statistics used to describe the reference values obtained for each parameter after determination in the laboratory according to their respective methods were the minimum, maximum, mean, coefficient of variation and standard deviation values (Table 3.2). The experiment was designed so that grapes were harvested at an early harvesting stage (16°Brix), optimum harvesting stage (18°Brix) and late harvesting stage (20°Brix). However, due to the variability that can occur within the berries of the same bunch (berries sampled at the top, middle and bottom part along the longitudinal axis of the bunch) and between bunches, the eventual reference values achieved, as in this instance, may be different than the initially measured ones in the vineyard, which was done prior to harvest on a few berries from different bunches with a handheld refractometer.

A large portion of the soluble solids in grapes is sugars and the total sugars account for more than 90% of TSS at harvest (Muñoz-Robredo *et al.*, 2011). This is expressed as °Brix and basically means the grams of sugar per 100 mL of juice. The maximum TSS value in this experiment was 21.08, the minimum was 11.40 and the mean was 17.04 as contained in Table 3.2. Seedless table grape varieties like Regal Seedless and Thompson Seedless are usually harvested at a TSS level of about 17°Brix (Fourie, 2010), but may be harvested at levels as low as 14°Brix (Mencarelli *et al.*, 2005) and as high as 21°Brix (Fourie, 2010). In an earlier report, the range of TSS in mature grapes was found to vary widely from 13.7 to 31.5°Brix (Kliewer, 1967). This accentuates the fact that there is no clear indication whether the grapes are all at the same sugar level when they are harvested. Clearly, the grapes in this experiment was harvested somewhat too soon, since some berries had not even yet reached the acceptable TSS level of 16°Brix deemed acceptable for an early harvest. This can also be clearly seen in the skewness distribution of the data for TSS (Figure 3.1). Very bad skewness can have a definite effect on the selection of calibration samples and this can have a serious effect on future predictions.

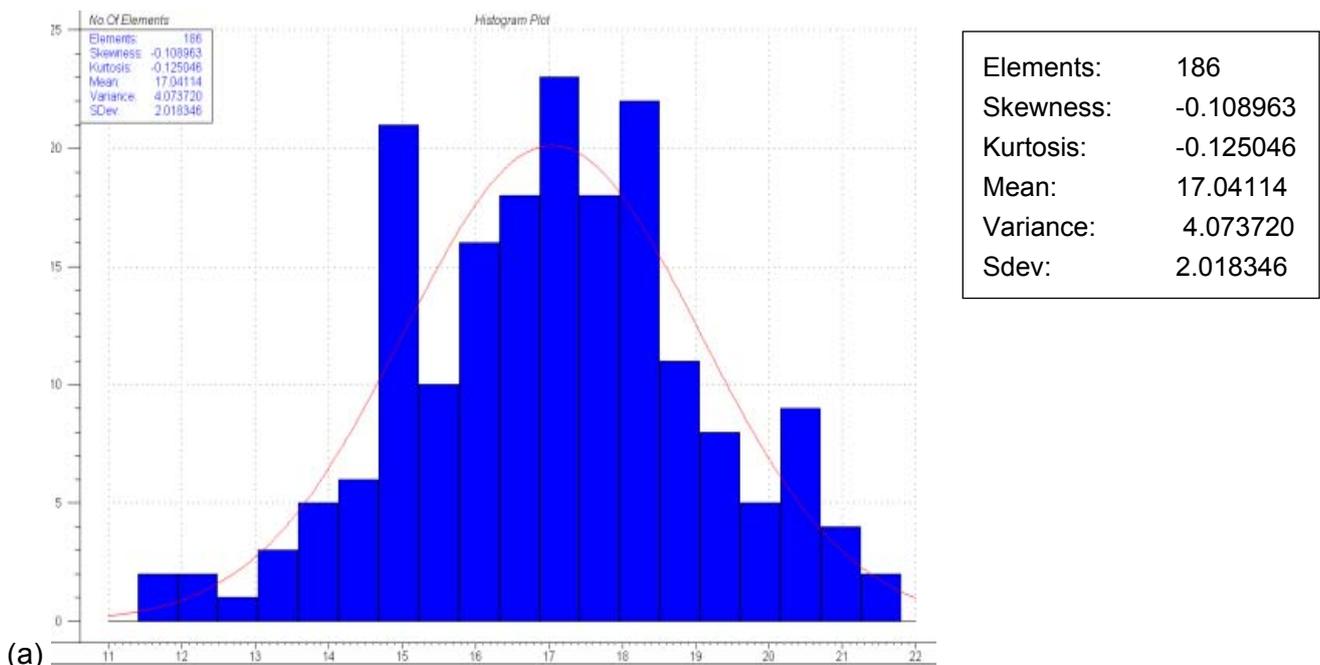


Figure 3.1 Histogram showing the skewness of the combined 2008 and 2009 reference data of Regal Seedless and Thompson Seedless for TSS.

Juice acidity is normally related to the juice pH and is a measure of the hydrogen ion concentration in the berry (Fahmi *et al.*, 2012). During the early stages of berry development, juice pH is reasonably constant, remaining near a value of 2.5, and then rises steadily during ripening as acid anions are formed and the amount of malic acid in the berry decreases (Dokoozlian, 2000). This is evident in the minimum value of 2.94 for pH in this experiment and the maximum value of 3.90. The mean was 3.49. These values are similar to juice pH of Thompson Seedless grapes at commercial maturity which usually ranges between 3.5 and 3.9 at harvest (Dokoozlian, 2000). In the experiments of Vial *et al.* (2005) where they also harvested at TSS levels similar to those in this study, early (16°Brix), optimum ripeness level (18°Brix) and late (>18°Brix) they obtained pH values that ranged between 3.46 and 3.7, which were fairly close to values obtained in this study.

During berry development TA usually decreases as TSS increases (Dokoozlian, 2000). TA values with a maximum of 13.10 g/L, minimum value of 4.30 g/L and a mean value of 6.86 g/L were obtained in this experiment. Grapes contain significant amounts of organic acids. They are a very important component 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). Higher acid levels in fruit are often associated with lower pH values and *vice versa*. However, as can be seen in Table 3.2, a higher acid level seems to be associated with a higher pH level.

Glucose values ranged from a minimum of 20.36 g/L and a maximum of 386.67 g/L with a mean of 81.35 g/L. Fructose had a minimum value of 54.51 g/L, a maximum value of 176.82 g/L and a mean of 94.36 g/L. Glucose and fructose are the predominant sugars in grape berries of most cultivars although they may vary a lot (Manning *et al.*, 2001). Values of 3.80 – 174.20 g/L for glucose and 3.00 – 174.80 g/L for fructose were obtained by Muñoz-Robredo *et al.* (2011) in their studies and in another study by Liu *et al.* (2006) values of 45.86 – 122.89 g/L for glucose and 47.64 – 131.04 g/L for fructose were obtained. The initial values of glucose and fructose in the study of Muñoz-Robredo *et al.* (2011) are much lower than in this present study, since measurements were started two weeks before *veraison*. The maximum values were, however, more closer to those obtained in this study for fructose and so were the minimum values for fructose obtained in the studies of Liu *et al.* (2006). Glucose and fructose were present in almost equal amounts, unlike in this study, where the minimum glucose values were much lower and the maximum levels much higher. It should be kept in mind that samples for glucose and fructose measurements were frozen and contamination might have crept in somehow, obscuring results.

Table 3.2 Descriptive Statistics of reference values for the table grape quality parameters TSS, pH, TA, glucose and fructose. All the samples of the different harvest dates were pooled together.

Parameter	Total number of samples	Min ^a	Max ^b	Mean	CV ^c (%)	SDev ^d
TSS ^e (°Brix)	414	11.40	21.80	17.04	1.25	0.21
pH	414	2.94	3.90	3.49	0.20	0.01
TA ^f (g/L)	414	4.30	13.10	6.86	8.29	0.57
Glucose(g/L)	364	20.36	386.37	81.35	2.44	01.99
Fructose(g/L)	364	54.51	176.82	94.36	5.07	04.78

^aMin, minimum, ^bMax, maximum, ^cCV, coefficient of variation, ^dSDev, standard deviation, ^eTSS, total soluble solids in °Brix, ^fTA, titratable acidity expressed as g/L tartaric acid.

3.4.2 FT-IR SPECTRA

Examples of a raw FT-NIR spectrum (12 800 to 4 000 cm^{-1}) is shown in Figure 3.1, a FT-MIR ATR spectrum (4 000 to 400 cm^{-1}) in Figure 3.2 and a FT-MIR spectrum (930 to 4 942 cm^{-1}) in Figure 3.3. The major features of the FT-NIR spectra are the broad water absorption peak at 6 897 cm^{-1} and the encircled area around 5 000 cm^{-1} , which shows that the detector was saturated. The pathlength could not be reduced, since the smallest pathlength cuvette (1 mm) was already used. The absorption range for glucose and fructose lies at 6 301 cm^{-1} to 4 403 cm^{-1} on this spectrum.

The FT-MIR ATR spectrum show strong water absorption peaks that appears with centers at 3 400 cm^{-1} and 1 650 cm^{-1} . The fingerprint areas for pH, TA, glucose and fructose appears from 1 654.3 cm^{-1} to 649.4 cm^{-1} and for TSS in the regions 3 996.6 cm^{-1} to 3 661.2 cm^{-1} , 3 327.2 cm^{-1} to 2 322.3 cm^{-1} and 1 988.3 to 1 652.8 cm^{-1} .

The FT-MIR spectrum's major features include the encircled areas around 1 600 cm^{-1} and 3 400 cm^{-1} where the centres for water absorption peaks usually appear. Here signs of extreme saturation by the detector can also be noticed. This despite the smallest pathlength (37 μm) that was used which is fixed on the WineScan.

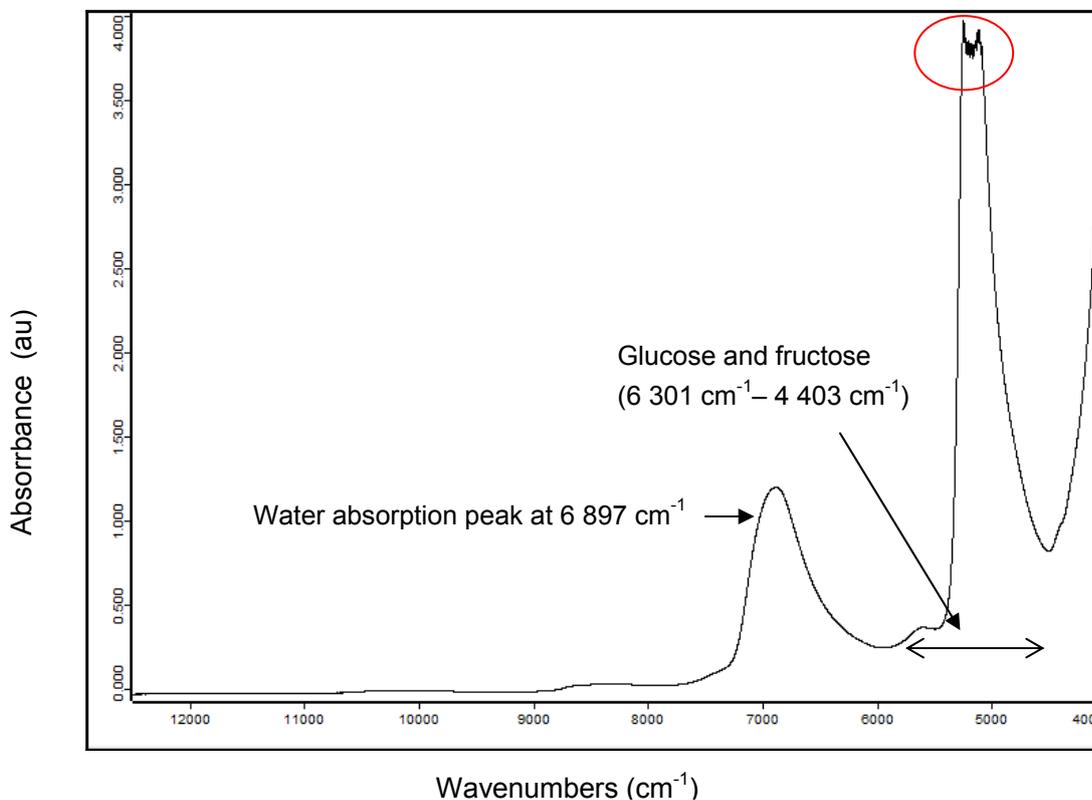


Figure 3.1 FT-NIR spectrum of table grape juice generated in the wavenumber range 12 800 cm^{-1} to 4 000 cm^{-1} . A water absorption peak appears which centres around 6 897 cm^{-1} . The absorption range for glucose and fructose lies at 6 301 cm^{-1} to 4 403 cm^{-1} on this spectrum.

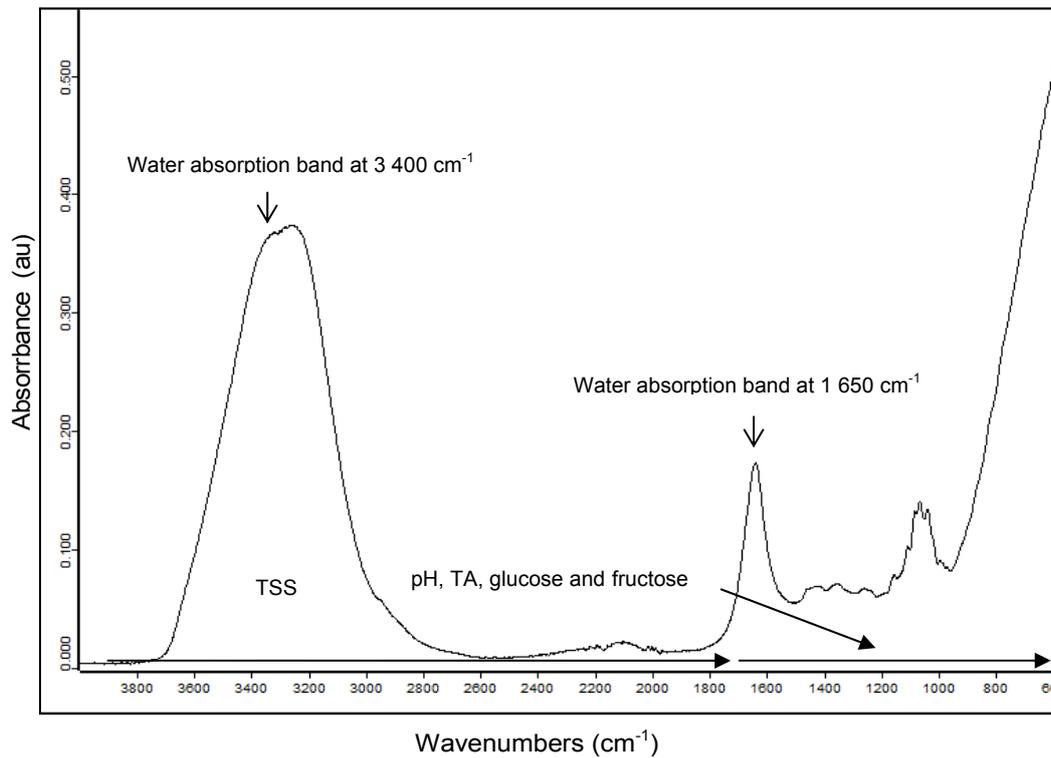


Figure 3.2 FT-MIR ATR spectrum of a table grape juice sample taken from wavenumbers $4\,000\text{ cm}^{-1}$ to 400 cm^{-1} . Strong water absorption peaks appears with centres at $3\,400\text{ cm}^{-1}$ and $1\,650\text{ cm}^{-1}$. The fingerprint areas for pH, TA, glucose and fructose appears from $1\,654.3\text{ cm}^{-1}$ - 649.4 cm^{-1} and for TSS in regions $3\,996.6\text{ cm}^{-1}$ - $3\,661.2\text{ cm}^{-1}$, $3\,327.2\text{ cm}^{-1}$ - $2\,322.3\text{ cm}^{-1}$ and $1\,988.3\text{ cm}^{-1}$ - $1\,652.8\text{ cm}^{-1}$.

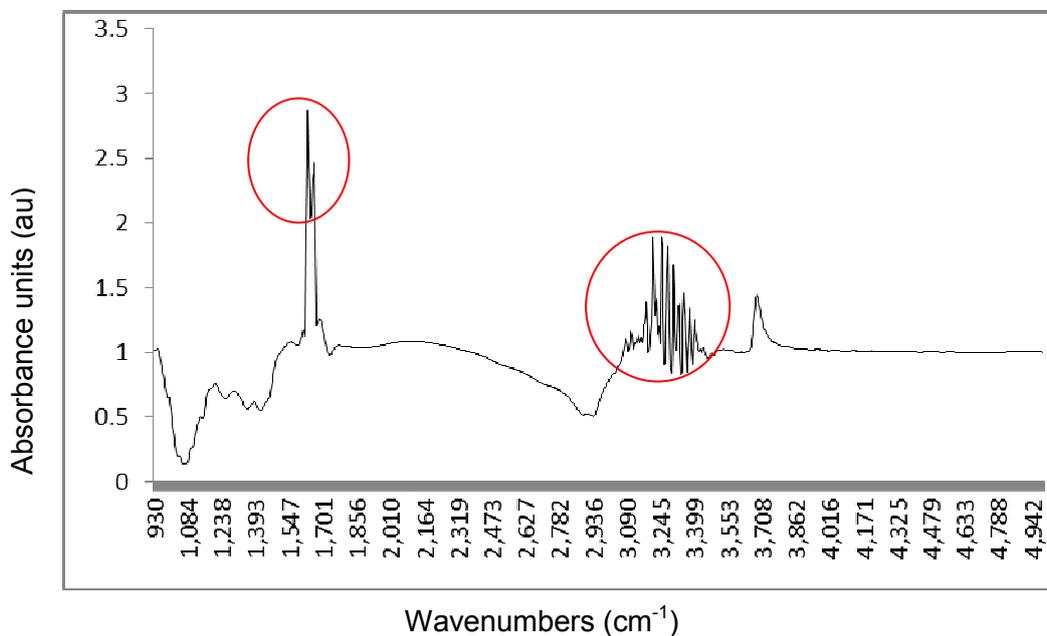


Figure 3.3 Example of a raw table grape juice spectrum taken on the FT-MIR spectrometer from wavenumbers 930 cm^{-1} to $4\,942\text{ cm}^{-1}$. The encircled areas around $1\,600\text{ cm}^{-1}$ and $3\,400\text{ cm}^{-1}$ are where the centres for water absorption peaks usually appear. Here signs of extreme saturation by the detector are shown. This despite the smallest pathlength ($37\text{ }\mu\text{m}$) that was used which is fixed on the WineScan.

3.4.3 PLS CALIBRATION

The ranges, wavenumber regions which correlate the most with the chemistry of the parameters, the rank (number of PLS components used in the construction of the model) and the spectral pre-processing method used for the FT-NIR instrument are shown in Table 3.3 and Table 3.4 for the FT-MIR ATR instrument. For the FT-MIR instrument the whole spectrum range and the same spectral pre-processing methods as those on the FT-MIR ATR instrument were used.

The rank of the model is also showed in Table 3.3 and Table 3.4 for the FT-NIR- and FT-MIR ATR instruments respectively. The optimum number of principal component (latent variables) in case of PLS1 are found to be three at the lowest level of residual validation variance (Jha *et al.*, 2006), since a relatively low number of latent variables (LV) are generally desirable to avoid modelling noise signals (Fernández-Navales *et al.*, 2009). In this exercise the rank varied from 3 (glucose) to 10 (TSS) on the FT-NIR instrument and from 2 (glucose) to 6 (pH) on the FT-MIR ATR instrument.

Table 3.3 The ranges and spectral pre-processing techniques used on the FT-NIR instrument.

Parameter	Spectral pre-processing technique	Combined spectral ranges used for calibration	Rank
TSS	Minimum-maximum normalization	12 493.1 - 5 446.2 6 101.9 - 5 446.2	10
pH	Straight-line subtraction	12 493.1 - 5 446.2 4 601.5 - 4 246.7	8
TA	Multiplicative scattering correction	6 101.9 - 5 449.1 4 100.5 - 4 249.6	9
Glucose	First derivative + Straight-line subtraction with 17 smoothing points	7 500.1 - 6 800.1	3
Fructose	No spectral pre-processing	12 493.1 - 7 498.2 6 101.9 - 5 446.2 4 601.5 - 4 246.7	7

Table 3.4 The ranges and spectral pre-processing techniques used on the FT-MIR ATR instrument.

Parameter	Spectral pre-processing technique	Combined spectral ranges used for calibration	Rank
TSS	Vector Normalization	3 996.6 - 3 661.2 3 327.2 - 2 322.3 1 988.3 - 1 652.8 984.8 - 649.4	3
pH	Constant offset elimination	2 993.2 - 2 322.3 1 654.3 - 649.4	6
TA	Vector Normalization	2 994.8 - 2 323.2 1 654.5 - 984.4	4
Glucose	Second derivative with 17 smoothing points	3 663.5 - 3 327.7 2 994.8 - 2 323.2 1 654.5 - 650	2
Fructose	No spectral pre-processing	3 996.6 - 1 988.3 1 318.8 - 649.4	3

3.4.3 EVALUATION OF QUANTITATIVE CALIBRATION MODELS

The calibration curves and the regression statistics obtained for each parameter on each instrument are shown in Figures 3.4 to 3.8 and a summary is given in Table 3.5. For the new calibration models, the sample set ($n = 414$) was divided in a calibration set ($n = 207$) and an independent validation set ($n = 207$) on the FT-NIR, FT-MIR ATR and FT-MIR instruments.

Table 3.5 Summary of regression statistics obtained for the various table grape quality parameters on the FT-NIR-, FT-MIR ATR- and the FT-MIR instruments.

Instrument	FT-NIR				FT-MIR ATR				FT-MIR			
	Parameter	R^2 ^a	RMSEP ^b	RPD ^c	Bias	R^2	RMSEP	RPD	Bias	R^2	RMSEP	RPD
TSS ^d	85.92	<u>0.71</u>	<u>2.7</u>	0.03	77.07	0.91	2.1	0.14	<u>87.86</u>	2.53	1.3	<u>0</u>
pH	85.00	<u>0.08</u>	<u>2.6</u>	-0.01	84.56	<u>0.08</u>	2.6	-0.01	<u>92.48</u>	0.15	0.7	<u>0</u>
TA ^e	90.77	<u>0.48</u>	<u>3.3</u>	-0.03	86.95	0.56	2.8	0.10	<u>95.28</u>	0.80	0.5	<u>0</u>
Glucose	65.88	13.1	1.8	3.65	70.71	<u>11.10</u>	<u>1.9</u>	1.64	<u>87.58</u>	18.72	0.6	<u>0</u>
Fructose	74.66	<u>9.28</u>	<u>2.0</u>	1.10	58.88	11.60	1.6	3.24	<u>77.78</u>	34.59	1.6	<u>0</u>

^a R^2 , coefficient of determination. ^b RMSEP, root mean square error of prediction. ^c RPD, standard deviation to RMSEP ratio. ^d TSS, total soluble solids. ^e TA, titratable acidity.

3.4.4.1. TSS

A total of 10 factors obtained the lowest RMSEP of 0.71 °Brix on the FT-NIR instrument. The bias of the new calibration model was small (0.03 °Brix). Both these values were lower than the SEL of 1 °Brix. The R^2 was equal to 85.92% and a RPD value of 2.7 was obtained. The value of 2.7 indicates that the model is fit for screening purposes. The R^2 of 85.92% used as a criterion to evaluate the performance of the model, however, proved that the model is suitable for quantification purposes.

A total of 3 factors obtained the lowest RMSEP of 0.91 °Brix on the FT-MIR ATR instrument. The bias of the new calibration model was larger (0.14 °Brix) than on the FT-NIR, but still less than the SEL. The R^2 was equal to 77.07% and a RPD value of 2.1 was obtained. The value of 2.1 indicates that the model is fit for screening purposes. The R^2 of 77.07%, however, indicated that the model is suitable for quantification purposes.

On the FT-MIR instrument a total of 3 factors obtained the lowest RMSEP of 2.53 °Brix. The bias of the new calibration model was 0 °Brix. The bias was lower than the SEL, but the RMSEP value was higher. The R^2 was equal to 87.86% and a RPD value of 1.3 was obtained. The value of 1.3 indicates that the model is not yet suitable for quantification or screening purposes. The R^2 of 87.86%, however, indicated that the model is suitable for quantification purposes.

All three R^2 values were lower than the 99% obtained when FT-MIR spectroscopy was used to quantify TSS by Swanepoel *et al.* (2007). This can be related to the distribution or variation of the lower values as can be seen in Figure 3.4.

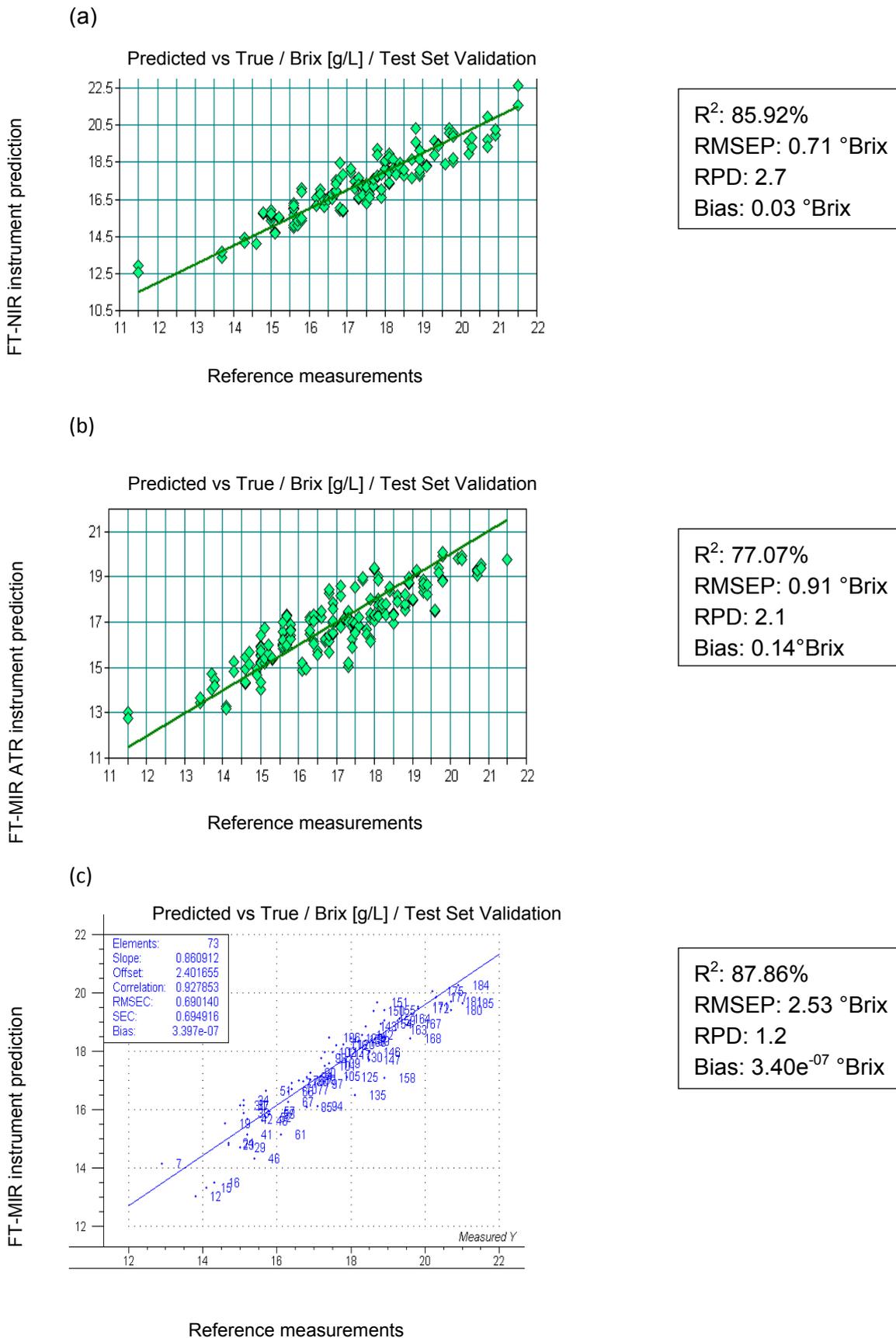


Figure 3.4 Test set validation plots for TSS on the (a) FT-NIR, (b) FT-MIR ATR and (c) FT-MIR instruments with the respective regression statistics obtained in the inserted textbox. Individual samples are represented by the blocks in the FT-NIR- and FT-MIR ATR instruments plots and by numbers in the FT-MIR instrument plot.

3.4.4.2 pH

A total of 8 factors obtained the lowest RMSEP of 0.08 on the FT-NIR instrument. The bias of the new calibration model was small (-0.01). Both these values were lower than the SEL of ± 0.1 . The R^2 was equal to 85.00% and a RPD value of 2.6 was obtained. The value of 2.6 indicates that the model is fit for screening purposes. The R^2 of 85.00% used as a criterion to evaluate the performance of the model proved that the model is suitable for quantification purposes.

The results obtained for the new calibration model on the FT-MIR ATR instrument were almost identical to those obtained on FT-NIR instrument except that a total of 6 factors obtained the lowest RMSEP of 0.08 on the FT-MIR ATR instrument. The bias of the new calibration model was also small (-0.01). Once again both values were lower than the SEL. The R^2 was equal to 84.56% and a RPD value of 2.6 was obtained. The value of 2.6 indicates that the model is fit for screening purposes and the R^2 of 84.56% that the model is suitable for quantification purposes.

On the FT-MIR instrument a total of 3 factors obtained the lowest RMSEP of 0.15. The bias of the new calibration model was 0. Although the bias was less than the SEL, the RMSEP value was way too high. This together with a RPD value of 0.7 indicates that the model is unsuitable for quantification and screening purposes, although the R^2 of 92.48% indicates that it is suitable for quantification purposes.

The R^2 on all three instruments were lower than the 95% obtained when FT-MIR spectroscopy was used to quantify pH in grape juice by Swanepoel *et al.* (2007). Once again the distribution of the lower values also seemed to have played a role in this (Figure 3.5).

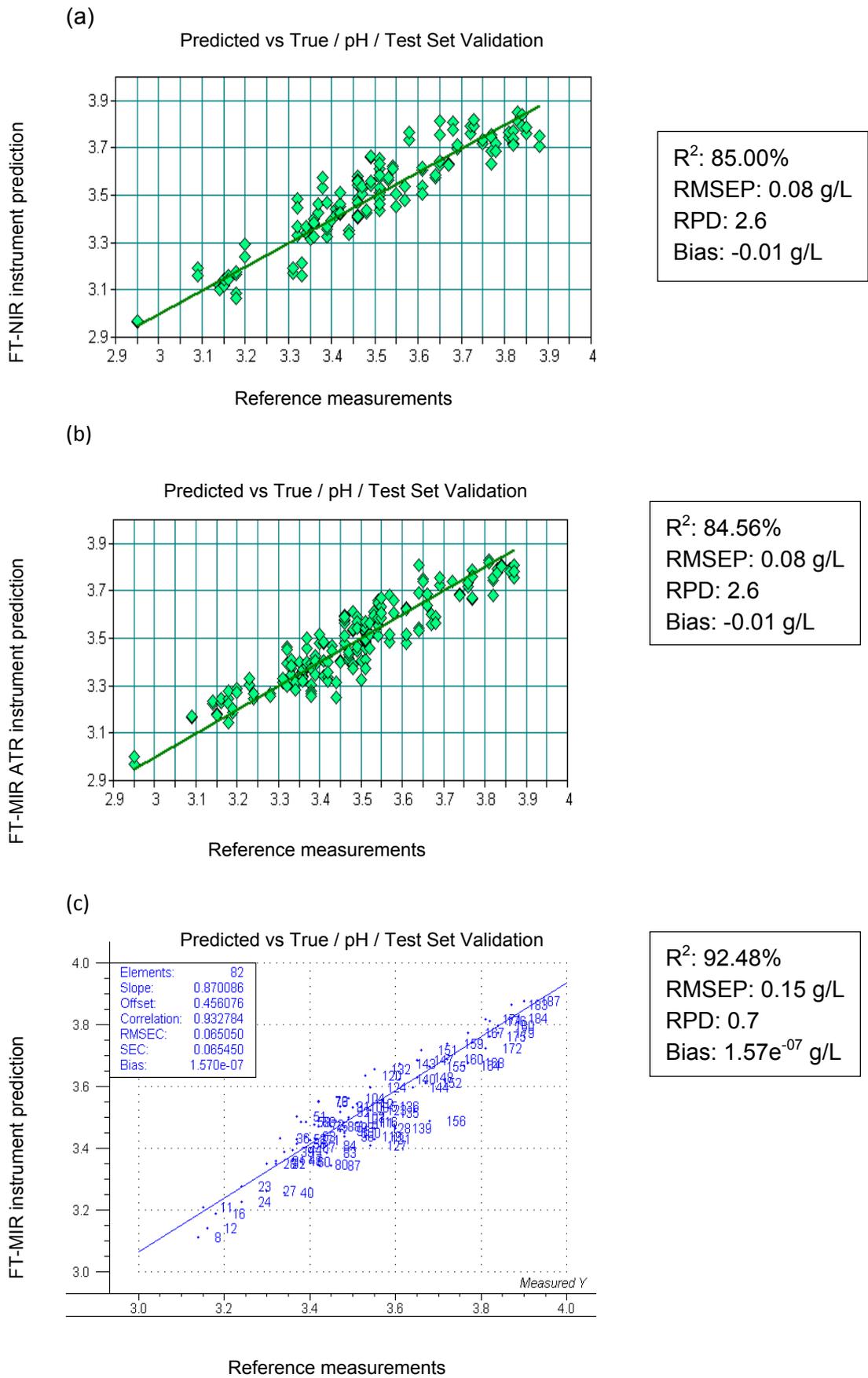


Figure 3.5 Test set validation plots for pH on the (a) FT-NIR-, (b) FT-MIR ATR and (c) FT-MIR instruments with the respective regression statistics obtained in the inserted textbox. Individual samples are represented by the blocks in the FT-NIR- and FT-MIR ATR instruments plots and by numbers in the FT-MIR instrument plot.

3.4.4.3 TA

A total of 9 factors obtained the lowest RMSEP of 0.48 g/L on the FT-NIR instrument. The bias of the new calibration model was small (-0.03 g/L). The bias value was lower than the SEL of ± 0.2 g/L, but the RMSEP value was almost double that. Despite this, this was the only model in this study that is suitable for quantification purposes based on both the R^2 which was equal to 90.77% and a RPD value of 3.3 that was obtained.

For the new TA calibration model on the FT-MIR ATR instrument a total of 4 factors obtained the lowest RMSEP of 0.56 g/L. The bias of the new calibration model was larger (-0.10) than on the FT-NIR instrument. The bias value was once again lower than the SEL, but the RMSEP value was higher. The R^2 was equal to 86.95% and a RPD value of 2.8 was obtained. The value of 2.8 indicates that the model is fit for screening purposes and the R^2 of 86.95% that the model is suitable for quantification purposes.

On the FT-MIR instrument a total of 4 factors obtained the lowest RMSEP of 0.80 g/L. The bias of the new calibration model was 0 g/L. Here the bias value was also again lower than the SEL, but the RMSEP value was much higher. The R^2 was equal to 95.28% and a RPD value of 0.5 was obtained. The value of 0.5 indicates that the model is unsuitable for quantification, although the R^2 of 95.28% indicates that it is suitable for quantification purposes.

The R^2 on all three instruments were lower than the 96% obtained when FT-MIR spectroscopy was used to quantify TA by Swanepoel *et al.* (2007). Here, however, the distribution or variation in the larger values seemed to play a role in this.

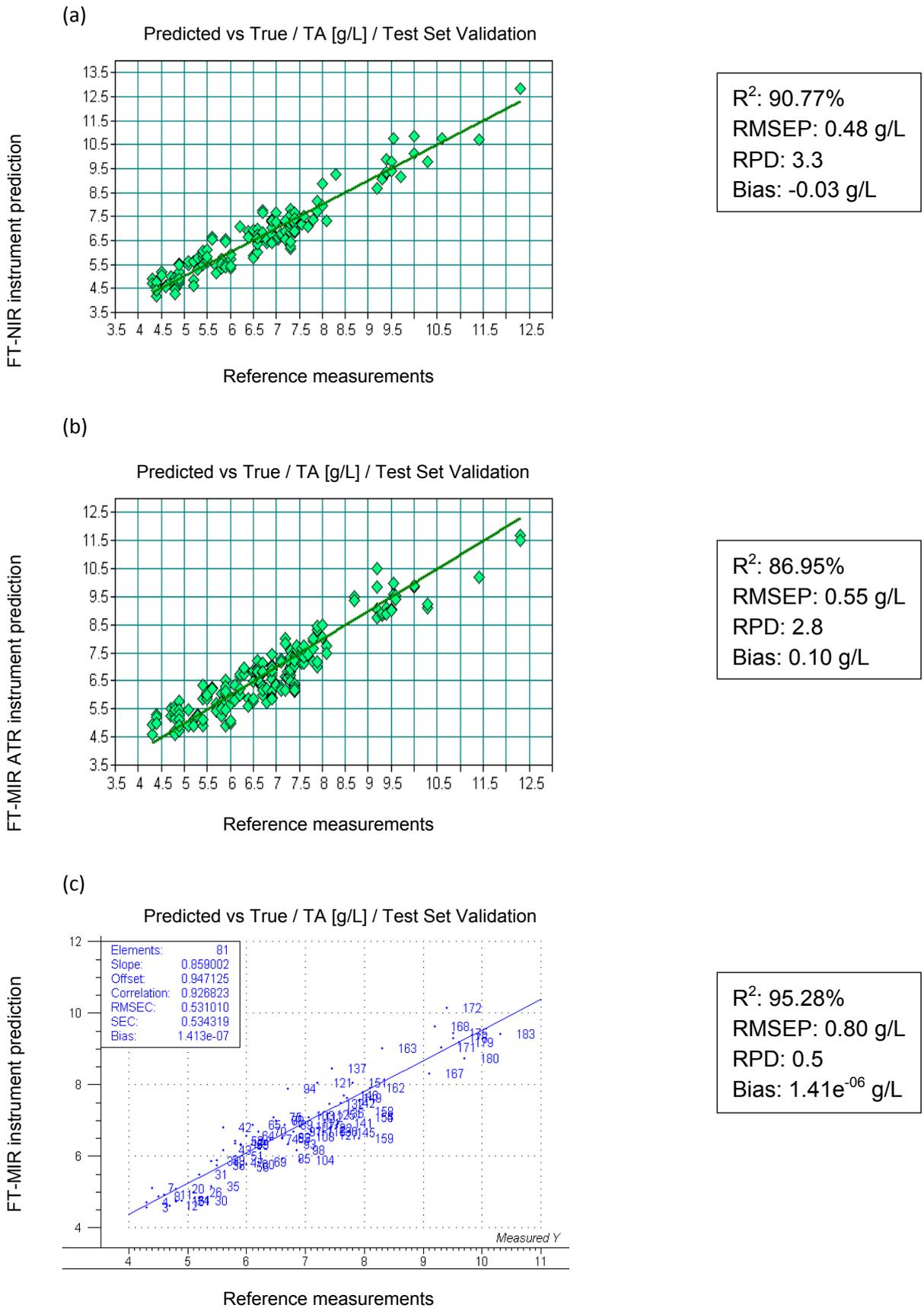


Figure 3.6 Test set validation plots for TA on the (a) FT-NIR -, (b) FT-MIR ATR - and (c) FT-MIR instruments with the respective regression statistics obtained in the inserted textbox. Individual samples are represented by the blocks in the FT-NIR- and FT-MIR ATR instruments plots and by numbers in the FT-MIR instrument plot.

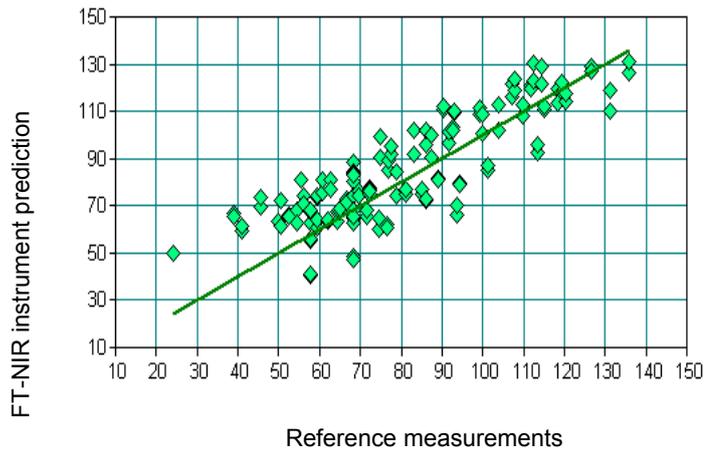
3.4.4.4 GLUCOSE

A total of 3 factors obtained the lowest RMSEP of 13.1 g/L on the FT-NIR instrument. The bias of the new calibration model was very large (3.65 g/L), the R^2 was equal to 65.88% and a RPD value of 1.8 was obtained. The value of 1.8 indicates that the model is not yet suitable for quantification purposes and so does the R^2 of 65.88%.

For the new glucose calibration model on the FT-MIR ATR instrument a total of 2 factors obtained the lowest RMSEP of 11.10 g/L. The bias of the new calibration model was large (1.64 g/L), the R^2 was equal to 70.71% and a RPD value of 1.9 was obtained. The value of 1.9 indicates that the model is not yet suitable for quantification purposes, but the R^2 of 70.71% indicates that it is fit for screening purposes.

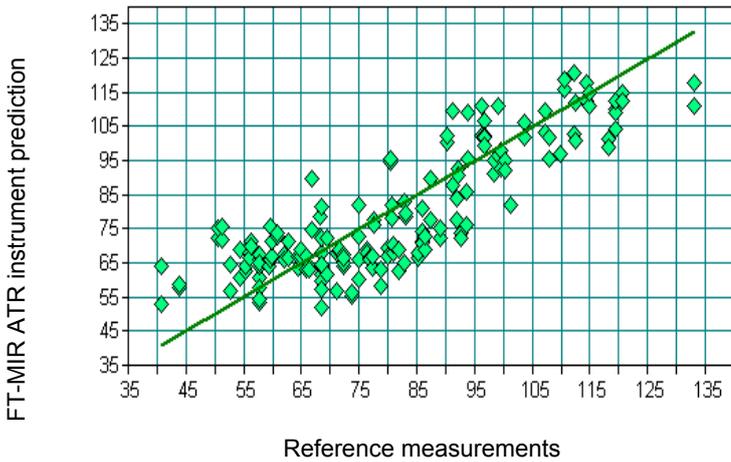
On the FT-MIR instrument a total of 2 factors obtained the lowest RMSEP of 18.72 g/L. The bias of the new calibration model was 0 g/L, the R^2 was equal to 87.58% and a RPD value of 0.6 was obtained. The value of 0.6 indicates that the model is not yet suitable for quantification or screening purposes, although the R^2 of 87.58% indicates that it is fit for quantification purposes.

(a) Predicted vs True / Glucose [g/L] / Test Set Validation



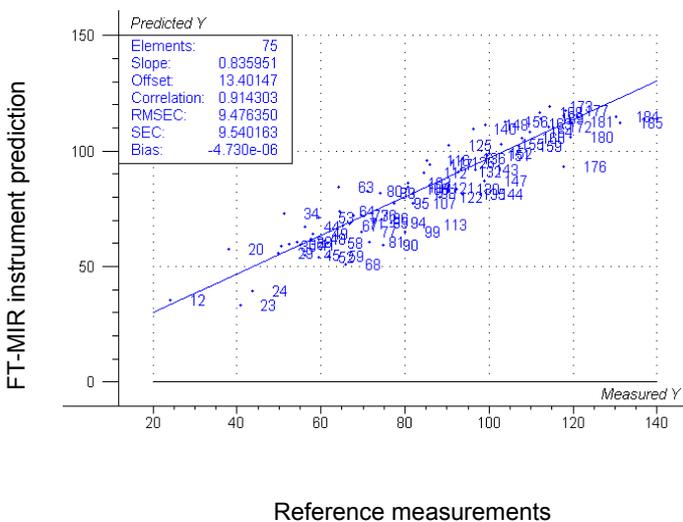
R^2 : 65.88%
 RMSEP: 13.10 g/L
 RPD: 1.8
 Bias: 3.65 g/L

(b) Predicted vs True / Glucose [g/L] / Test Set Validation



R^2 : 70.71%
 RMSEP: 11.10 g/L
 RPD: 1.9
 Bias: 1.64 g/L

(c)



R^2 : 87.58%
 RMSEP: 18.72 g/L
 RPD: 0.6
 Bias: $-4.73e^{-06}$ g/L

Figure 3.7 Test set validation plots for glucose on the (a) FT-NIR-, (b) FT-MIR ATR - and (c) FT-MIR instruments with the respective regression statistics obtained in the inserted textbox. Individual samples are represented by the blocks in the FT-NIR- and FT-MIR ATR instruments plots and by numbers in the FT-MIR instrument plot.

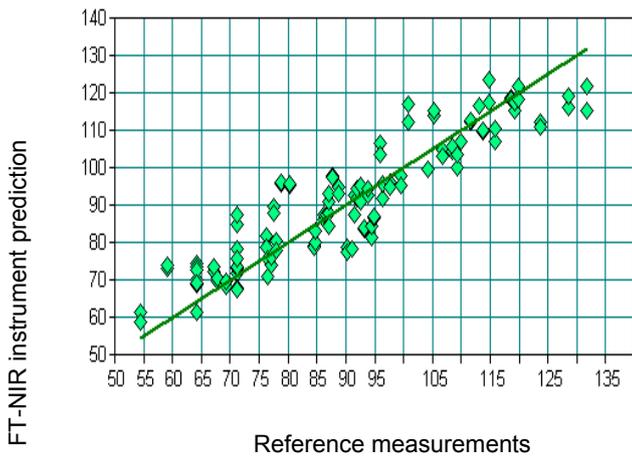
3.4.4.5 FRUCTOSE

A total of 7 factors obtained the lowest RMSEP of 9.28 g/L on the FT-NIR instrument. The bias of the new calibration model was large (1.10 g/L), the R^2 was equal to 74.66% and a RPD value of 2 was obtained. The value of 2 indicates that the model is fit for screening purposes and the R^2 of 74.66% indicates that the model is fit for quantification purposes.

For the new glucose calibration model on the FT-MIR ATR instrument a total of 3 factors obtained the lowest RMSEP of 11.60 g/L. The bias of the new calibration model was very large (13.24 g/L), the R^2 was equal to 58.88% and a RPD value of 1.6 was obtained. The value of 1.6 indicates that the model is not yet suitable for quantification purposes and so does the R^2 of 58.88%.

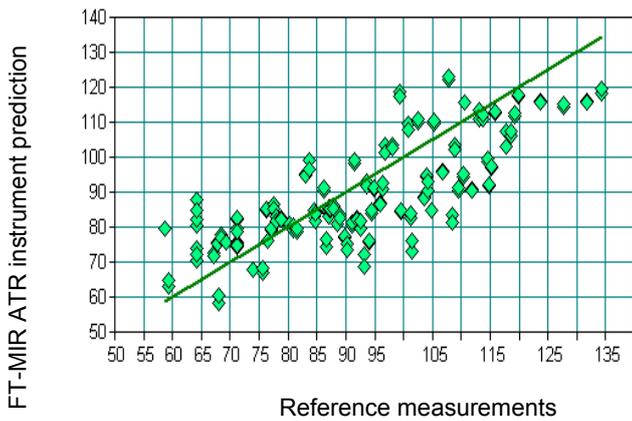
On the FT-MIR instrument a total of 5 factors obtained the lowest RMSEP of 34.59 g/L. The bias of the new calibration model was 0 g/L, the R^2 was equal to 77.78% and a RPD value of 1.6 was obtained. The value of 1.6 indicates that the model is not yet suitable for quantification or screening purposes, although the R^2 of 77.78% indicates that it is fit for quantification purposes.

(a) Predicted vs True / Fructose [g/L] / Test Set Validation



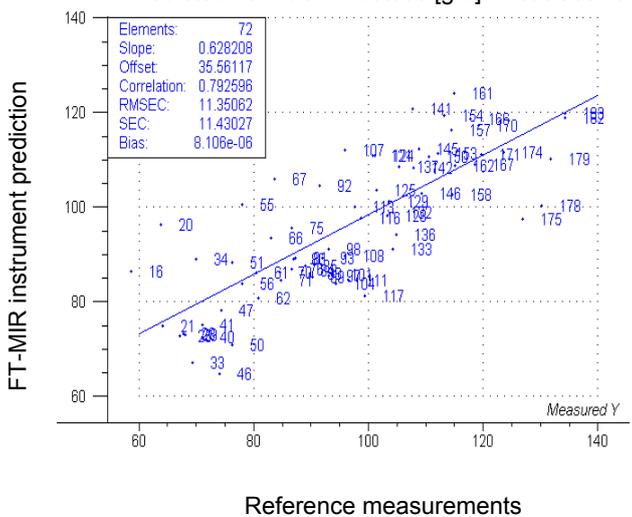
R^2 : 74.66%
 RMSEP: 9.28 g/L
 RPD: 2
 Bias: 1.10 g/L

(b) Predicted vs True / Fructose [g/L] / Test Set Validation



R^2 : 58.88%
 RMSEP: 11.60 g/L
 RPD: 1.6
 Bias: 3.24 g/L

(c) Predicted vs True / Fructose [g/L] / Test Set Validation



R^2 : 77.78%
 RMSEP: 34.59 g/L
 RPD: 0.6
 Bias: $8.11e^{-06}$ g/L

Figure 3.8 Test set validation plots for fructose on the (a) FT-NIR-, (b) FT-MIR ATR - and (c) FT-MIR instruments with the respective regression statistics obtained in the inserted textbox. Individual samples are represented by the blocks in the FT-NIR- and FT-MIR ATR instruments plots and by numbers in the FT-MIR instrument plot.

3.5 CONCLUSION

The construction of calibration models using PLS regression for five different parameters namely TSS, pH, TA, glucose and fructose on three different IR instruments (FT-NIR, FT-MIR ATR and FT-MIR) was quite successful given that it has never been done for table grape juice samples before. Models obtained for TSS, TA and pH proved suitable for quantification purposes on all three instruments based on the R^2 values, but only fit for screening purposes based on the RPD values of the FT-NIR- and FT-MIR ATR instruments. All three parameters were not yet suitable for quantification or screening purposes on the FT-MIR based on the RPD values. Only the model for TA proved to be suitable for quantification purposes on the FT-NIR instrument based on the RPD value of 3.3. The fact, however, that the RMSEP and bias values for all three instruments (except for the RMSEP on the FT-MIR and FT-MIR ATR instrument sometimes) were lower than the SEL, is a good sign that the accuracy of the instruments is better than that of the reference methods.

The model for glucose proved not yet suitable for quantification purposes on the FT-NIR instrument, fit for screening purposes on the FT-MIR ATR instrument and not yet suitable for quantification purposes on the FT-MIR instrument based on the R^2 values. Based on the RPD values, the models for glucose on all three instruments were unsuitable for quantification purposes. For fructose the models obtained on the FT-NIR and the FT-MIR instruments proved suitable for quantification purposes and not suitable for quantification purposes on the FT-MIR ATR instrument based on the R^2 values. Based on the RPD values the model on the FT-NIR instrument was fit for screening purposes and not yet suitable for quantification purposes on both the FT-MIR ATR and FT-MIR instruments.

Both the FT-NIR and FT-MIR ATR instruments seems to be the best instruments to determine the maturity indexes on, since they gave models for TSS, pH, TA, glucose and fructose that were suitable for screening purposes in most instances. This was with the exception of TA where the FT-NIR instrument gave a model that was suitable for quantification purposes. This was with the exception of glucose gave a model that was not yet suitable for quantification purposes on the FT-NIR instrument and fructose that gave a model that also was not suitable yet for quantification purposes on the FT-MIR ATR instrument.

Whilst it is clear that the FT-MIR instrument did not deliver any model that was suitable for quantification purposes or fit for screening yet, future work will have to include analysing this data with the software on the instrument, which has a built-in algorithm that is much stricter with variable selection, than the Unscrambler software that was used to analyse the data of the FT-MIR instrument in this study. Other cultivars from other locations and also at different sugar levels will also have to be included in future analysis/quantification of these parameters so that the obtained models will be suitable for quantification purposes on all three instruments and not only fit for screening purposes. It was done for TSS, pH and TA in wine grape juice on the FT-MIR instrument by Swanepoel *et al.* (2007). Thus it is anticipated that development of models suitable for quantification purposes can also be achieved for the five parameters investigated in this study. This especially now that a platform of technologies has been made available through this study for the determination of the respective parameters in future table grape samples, by just collecting their spectra on one of the instruments. Indeed something that has not been possible or available for the South African table grape industry before.

3.6 LITERATURE CITED

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Chapter 4

Research results

Preliminary evaluation of monitoring and detection of browning of white seedless table grapes with near-infrared spectroscopy

Chapter 4: Research results

Preliminary evaluation of monitoring and detection of browning in white seedless table grapes with near-infrared spectroscopy

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4.1 ABSTRACT

BACKGROUND: The postharvest browning of table grapes is a serious problem for the South African table grape industry. Grapes that suffer from browning disorders normally have a shorter postharvest shelf life and may not be suitable for long-distance markets where the biggest profits are often gained. To date, rapid monitoring techniques were not available to monitor and detect browning on white seedless grapes, only visual inspection and detection are possible when the browning has already manifested on the grapes. It is known that Regal Seedless and Thompson Seedless are prone to various types of browning. A non-destructive way to determine whether browning will occur before the grapes are exported is, therefore, an absolute necessity. In this preliminary work, the utility of near-infrared (NIR) spectroscopy was evaluated to distinguish between non-brown and berries affected by external and/or internal browning as a first step towards detection of browning on table grapes.

RESULTS: NIR spectroscopy combined with Principal Component Analysis (PCA) of the spectra of whole berries removed from bunches was used to develop classification models to distinguish between non-brown and affected berries. NIR spectra were obtained at two stages, namely before cold storage, where no visible signs of browning were present on the berries and again, after cold storage on the same berries with visible browning present on some berries. The different types of browning were grouped together when the PCA analysis was done. Classification models were built with spectra obtained of berries before cold storage (non-brown) and after cold storage (non-brown and brown) put together. Classification accuracies obtained with berry spectra taken after cold storage was high: 90% of the non-brown berries and 83% of the brown berries were correctly classified. The overall classification accuracy was 87%. With models based on spectra obtained before cold storage non-brown berries were classified with an accuracy of 68%, brown berries with 59% and the total accuracy was 64%.

CONCLUSION: NIR spectroscopy successfully captured the information that is related to changes that occur in the grape berries during cold storage. At this moment it is not clear whether these changes are related to browning, but preliminary indications are some information that correlates with browning incidence is present, based on the results obtained with the classification models. The classification models that were constructed of the berries before and after cold storage show potential for the prediction of browning before cold storage. The possibility of predicting whether the grapes will turn brown after they have reached their destination is now available thus the loss of huge amounts of money (because of export grapes turning brown) can now be prevented.

Further analysis of the data, however, through the selection of specific browning types in each cultivar and variable selection techniques will have to be explored.

KEYWORDS: White seedless table grapes, browning, near infrared spectroscopy, principal component analysis, cold storage, classification models

4.2 INTRODUCTION

Ever since it was first reported in 1989 (Wolf, 1996) the browning phenomenon has become more severe worldwide. Numerous studies have been conducted and are still on-going, in South Africa (DFPT Researchers) and in other countries (Australian Table Grape Annual Industry Report, 2007/8) to try and find out what exactly the cause is for it on table grapes. None to date, however, has brought to light the one factor that seems to be related to its development, whether it be internal (restricted to berry flesh) or external (on berry skin). Different phenotypes of browning have been identified based on visual inspection (external browning) and destructive techniques (berry cut open for internal browning detection) (Fourie, 2009). These are currently the only methods which can be used to monitor and detect browning and can only be done after cold storage of grapes.

Over the last couple of years, light-based sensing techniques, especially NIR spectroscopy, have been used extensively to measure external and internal fruit quality attributes and seems to offer great potential (Peirs *et al.*, 2003, Lu, 2007). NIR spectroscopy can be used for a wide variety of fruit and vegetables as reviewed by Nicolai *et al.* (2007). It can be used for qualitative purposes such as measuring the soluble solid content (SSC) of bell pepper (Penchaiya *et al.*, 2009) and acidity and SSC in apples (Lammertyn *et al.*, 1998). It can also be used to measure qualitative attributes of fruit such as firmness, skin and flesh colour, and dry matter content of pickling cucumbers (Kavdir *et al.*, 2007). NIR spectroscopy, where browning is concerned, has been used destructively for the measurement of browning in tissue slices and extracts (Nicolas *et al.*, 1994) and also non-destructively for detecting brown heart of pear (Fu *et al.*, 2007). Following these successful applications of NIR spectroscopy the next logical step was to try and develop similar non-destructive methods to monitor and detect browning of whole table grape berries. Although it is not conventional to remove berries during transport and storage of table grapes, it was done in this study for proof of the concept of scanning whole berries for the detection of browning. Whole bunches would have proved difficult and challenging, therefore, measurements could not be done *in situ* (still on the vine). The generated NIR spectrum on a spectrometer contain a huge amount of information, so multivariate statistical techniques such as PCA had to be used to develop classification models. The objectives of this study were, therefore: (i) to evaluate the potential of NIR spectroscopy in capturing information related to changes occurring in whole table grape berries during cold storage, and (ii) to evaluate if classification models could be constructed based on IR spectra obtained, respectively, before and after cold storage, to predict browning in whole table grape berries.

4.3 MATERIALS AND METHODS

4.3.1 EXPERIMENTAL DESIGN

Two commercially important white seedless table grape cultivars, Thompson Seedless and Regal Seedless, respectively, were harvested over two harvest seasons from two different vineyards in the Hex River Valley, Western Cape, South Africa. In the Regal Seedless vineyard a different crop

load (the total amount of bunches on the vines per hectare) were left in each row that was used as an experimental unit. In row 1 the total crop load was 75 000 bunches, in row 2, 50 000 bunches and in row 3, 35 000 bunches. Each row had six sections and two bunches were harvested from each section every harvest date. The harvest date corresponded to the sugar level on that specific day of harvest. For Thompson Seedless there were only two crop loads left on the vines, 75 000 in row 1 and 50 000 in row 2. Each row also had six sections and two bunches were also harvested from each section every harvest date. In 2008 the cultivars were harvested three times during the season; early at 16°Brix, at optimum ripeness at 18°Brix and late at 20°Brix. In 2009 the two cultivars were each harvested four times during the season, early at 16°Brix, twice at the optimum (18°Brix) (László and Saayman, 1993; Fraser, 2007) and late at 20°Brix. Each section represented a repetition, thus for each harvest date or sugar level 18 samples were obtained for Regal Seedless and 12 samples for Thompson Seedless. This resulted in a total of 210 samples (54 for Regal Seedless in 2008 and 72 in 2009, for Thompson Seedless 36 in 2008 and 48 in 2009).

4.3.2 GRAPE BERRY SAMPLING

In order to ensure that berries were selected to be representative berries were picked at different positions along the longitudinal bunch axis; twelve berries were randomly cut off a few millimetres above the stem of the berry with a clean, sharp scissor from the top, middle and bottom of each bunch. The 24 berries were put into peach trays marked from 1 to 24 with a permanent marker (Figure. 4.1). Berries were also marked at the top from 1-24. The cultivar name and harvest date were indicated at the bottom right hand side of the peach tray.



Figure 4.1 Peach tray with Regal Seedless berries stored at 0°C for 4 weeks, followed by storage at 7.5°C for one week to follow the incidence of browning in individual berries.

4.3.3 COLD STORAGE AND INSPECTION

The peach trays with the 24 berries were stacked into 4.5 kg boxes which were lined with 2 mm perforated low density polyethylene (LDPE) liners. The three trays per box were separated by placing plastic bottle lids between the trays in order to prevent chaffing of the berries. Uvasys® sulphur dioxide (SO₂) generator sheets were placed in the plastic bags on top of the grapes before the boxes were closed. These were then carefully transported to the cold rooms for storage at 0°C for 4 weeks followed by storage at 7.5°C for one week. This was done to simulate storage conditions during export of grapes by ship and on the shelves in supermarkets, although it is not conventional to remove berries from the bunch in the industry. This, however, had to be done to make the scanning of whole berries with the solid probe of the FT-NIR spectrometer possible. After

cold storage berries were examined for browning, scored and coded (Table 4.1) according to the pamphlet of Fourie (2009) for further statistical analysis. Steps as indicated in Figure 4.2 were followed further.

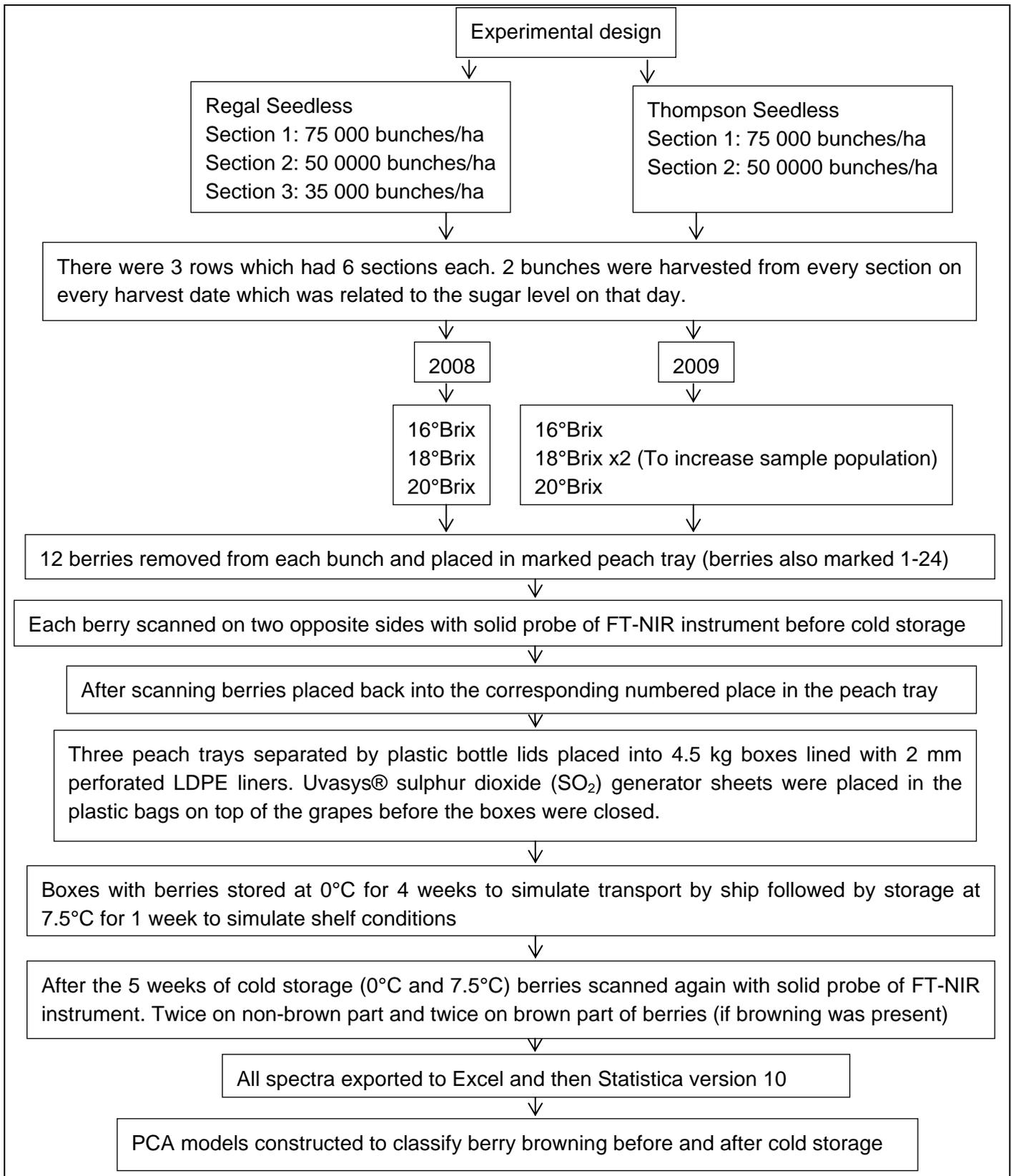


Figure 4.2 Experimental design for preliminary monitoring and detection of browning of white seedless table grapes with NIR spectroscopy.

Table 4.1 Abbreviations for different types of browning that was found on the berries

Browning types	Abbreviation
glassy berry external symptoms	GBES
contact browning	CT
abrasion	ABR
bruising	BR
netlike browning	NLB
friction	FR
mottled browning	MTLD
fungal infection	FI
stylar-end russet spots	SERS
no specific type of browning	B
internal browning	IB
chocolate browning	CB
showed browning after cold storage	Yes
remained clear after cold storage	No

4.3.5 NEAR INFRARED SPECTROSCOPY

It was determined that the best quality repeatable spectra would be obtained with the solid probe of the FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany). The spectral data were collected with a FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) over the range 12500 to 4000 cm^{-1} , using the following conditions: resolution 8 cm^{-1} ; scanner velocity 10 kHz; background with air, 16 scans; sample 16 scans. The berries were scanned on each side along the longitudinal axis within 24 hours of harvest and were kept cool (22 °C) during this period. The peach trays with the 24 berries were then stacked into 4.5 kg boxes (three trays per box) separated by plastic bottle lids, and stored at 0°C for four weeks followed by one week at 7.5°C week.

After cold storage of five weeks, each berry was scanned again, once on each non-brown part/side (Figure 4.3 a). If it had any browning on the skin or showed any symptoms of internal browning, it was scanned twice on the same spot/side (Figure 4.3b). Fourie (2009) released a pamphlet which contained all the different types of browning that can be found on white seedless table grapes, hence in 2009 each berry that was scanned after cold storage was inspected for the presence of any of those types of browning (Table 4.1). Each harvest date represented two datasets for each cultivar; one for berries scanned before cold storage and one for berries scanned after cold storage. Six datasets were, therefore, created for each cultivar in 2008 and eight datasets were created for each cultivar in 2009. There was thus a total of fourteen datasets available for analysis per cultivar.

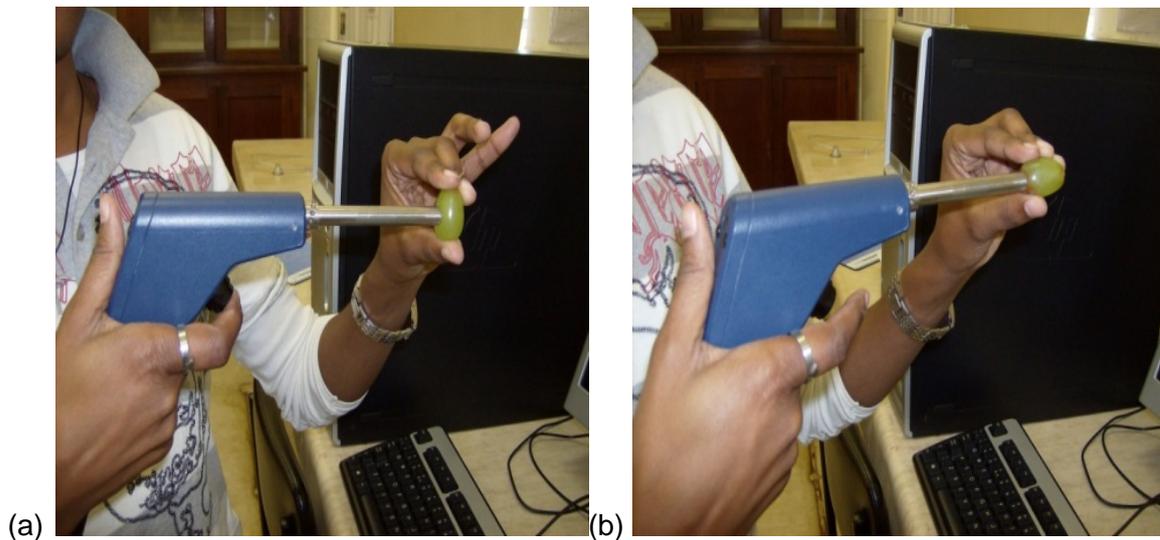


Figure 4.3 Scanning of (a) a non-brown berry before cold storage (once on one of the longitudinal sides and then once on the opposite side of the berry); and (b) scanning of a brown berry (twice on the non-brown part and twice on the brown part) with the solid probe of the FT-NIR spectrometer.

4.3.6 STATISTICAL ANALYSIS

The OPUS spectral files were imported into Unscrambler v9.2 software (CAMO ASA, Oslo, Norway) and then exported to Excel after absorbance spectra of whole berries were obtained with the solid probe of the FT-NIR spectrometer. Different codes were then assigned to the berries in the Excel files (Table 4.1) before analysis in Statistica version 10. The different datasets (before cold storage and after cold storage) were also combined into one for each harvest date. PCA of all the samples together (Regal Seedless and Thompson Seedless for both years) as well as apart (Regal Seedless alone and Thompson Seedless alone) were composed to determine the optimum number of principal components to use in the models. As a control measure three Regal Seedless datasets 27J09R, 26F08R and 17F09R were also analysed separately. All these datasets were divided into training data (70% of the samples which contained non-brown and brown berries) and test data (30% of the samples which also contained non-brown and brown berries) to classify the browning status of the berries based on their spectra before cold storage and after cold storage. Only spectra of berries that actually turned brown during cold storage were added to the spectra before cold storage to see if the browning that occurred could already be predicted before cold storage. When the training data models were tested the same samples as those used to construct the model was used. When the test data models were tested the 30% of samples that was that was not used in the construction of the models was used. Classification models were built based on the whole spectral range ($12800 - 4000 \text{ cm}^{-1}$) and no variable selection was done.

4.4 RESULTS AND DISCUSSION

4.4.1. INCIDENCE OF BROWNING TYPES

Some of the different types of browning that were observed on the berries after five weeks of cold storage are shown in Figures 4.3 to 4.8. In Figure 4.3 (a) it can be clearly seen that not all the berries turned brown or shows any signs of browning after cold storage. In Figure 4.3 (b) a completely clear berry is right next to a completely chocolate brown berry. This type of browning was caused by a fungal infection as can be seen by the mycelia of the fungus growing out of the stem end of the berry in Figure 4.4 (a). This shows that although browning is predominantly of a physiological nature, it can also have a pathological cause (Kruger *et al.*, 1999). In Figure 4.4 (b) only the seed traces of the Regal Seedless berry and some of the tissue surrounding them is still non-brown. This is in stark contrast with the internal browning of the Thompson Seedless berry in Figures 4.5 (b) and 4.6 (b), where only the internal tissue is brown and the rest of the berry tissue is non-brown. This confirms that browning in Thompson Seedless starts parallel to the vascular system in the centre of the berry and never develops on the skin as it does in the white seedless table grape cultivar, Princess (Vial *et al.*, 2005). It was also found that the berry browning symptoms of Princess are frequently expressed on white table grape cultivars like Italia and Regal Seedless (Vial *et al.*, 2005). Figure 4.5 (a) shows what internal browning looks like seen from the outside of a berry and Figure 4.6 (a) what a healthy non-brown Thompson Seedless berry looks like after five weeks in cold storage. Figure 4.7 (a) shows browning caused by abrasion damage. This type of browning could possibly be the cause for the fungal infections seen on the berries in the peach tray in Figure 4.7 (b). From personal observation it is not surprising that Thompson Seedless is much more prone to abrasion damage browning due to its very thin skin, in comparison with the much tougher skin of Regal Seedless. Figure 4.8 shows netlike browning symptoms on Regal Seedless berries as seen from the outside (a) underneath the skin (b) inside the berry (c) and compared to a part of the berry not showing any browning (d).

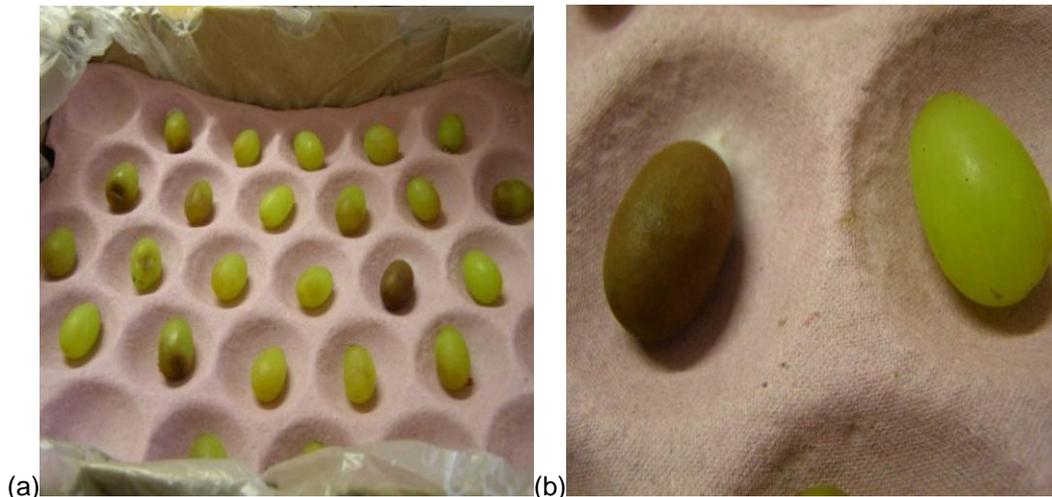


Figure 4.3 (a) The browning stage of Regal Seedless berries in peach tray after 5 weeks of cold storage and (b) a berry showing chocolate browning (caused by a fungal infection) and a berry not showing any browning.

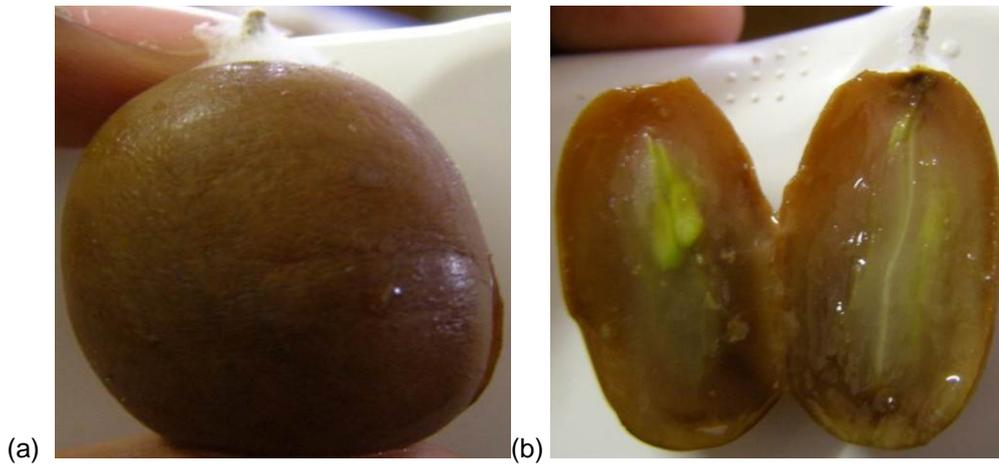


Figure 4.4 A Regal Seedless berry showing chocolate browning on the outside (a) and (b) on the inside caused by a fungal infection.

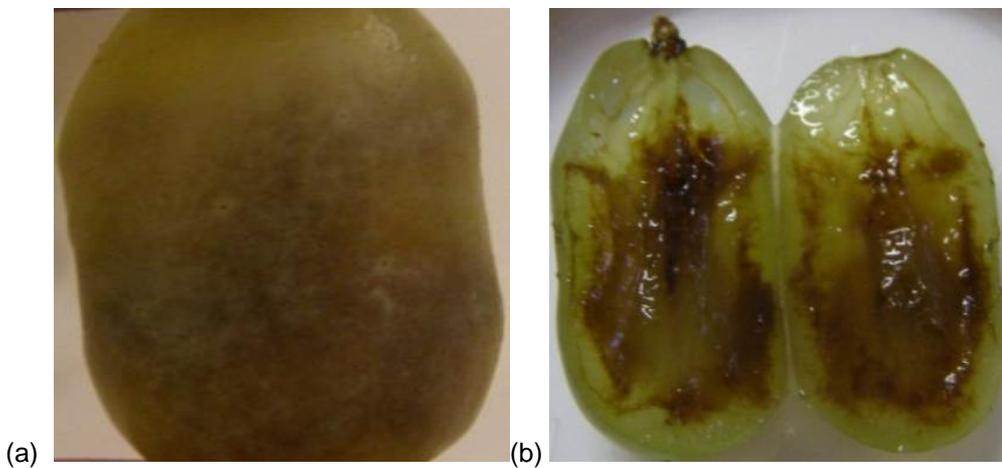


Figure 4.5 Internal browning as seen (a) from the outside and (b) on the inside of a Thompson Seedless berry.

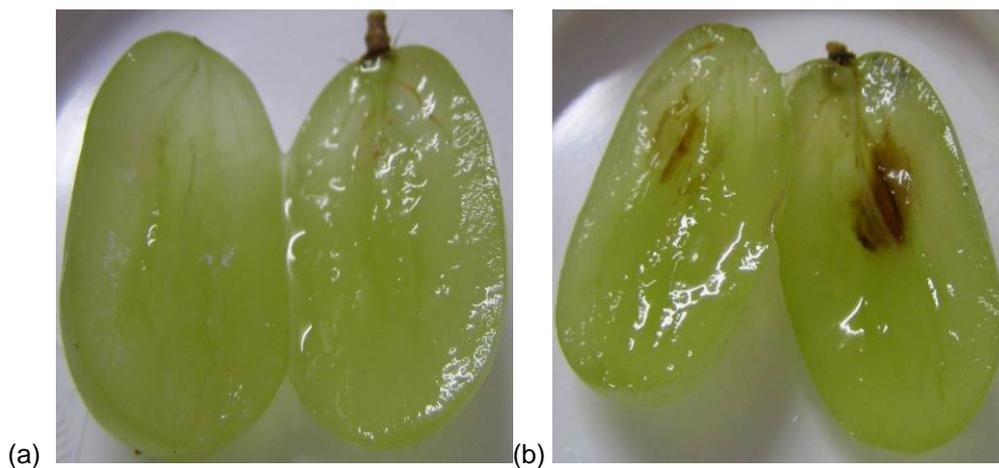


Figure 4.6 A Thompson Seedless berry showing (a) no signs of browning on the inside and (b) onset of internal browning around the vascular tissue.

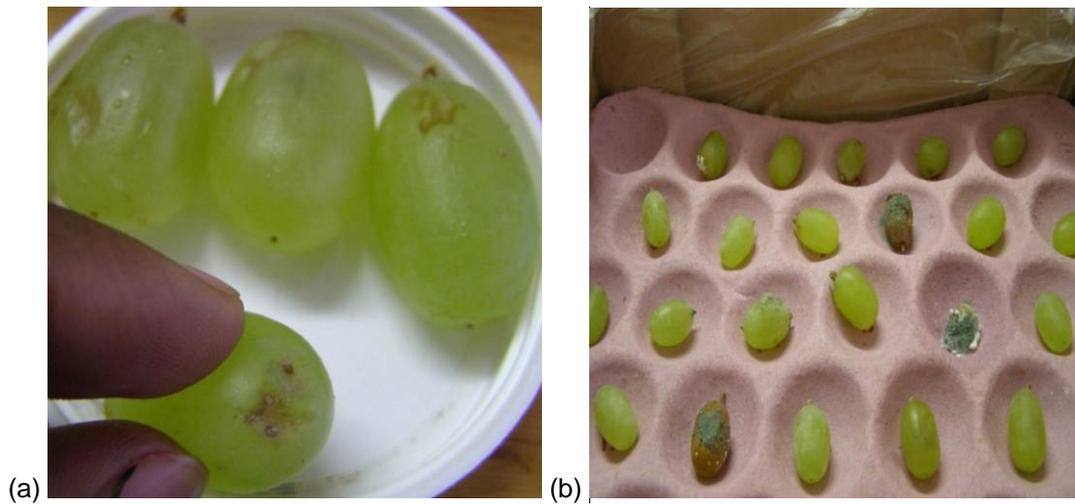


Figure 4.7 (a) Thompson Seedless berries showing abrasions and (b) berries showing fungal infection.

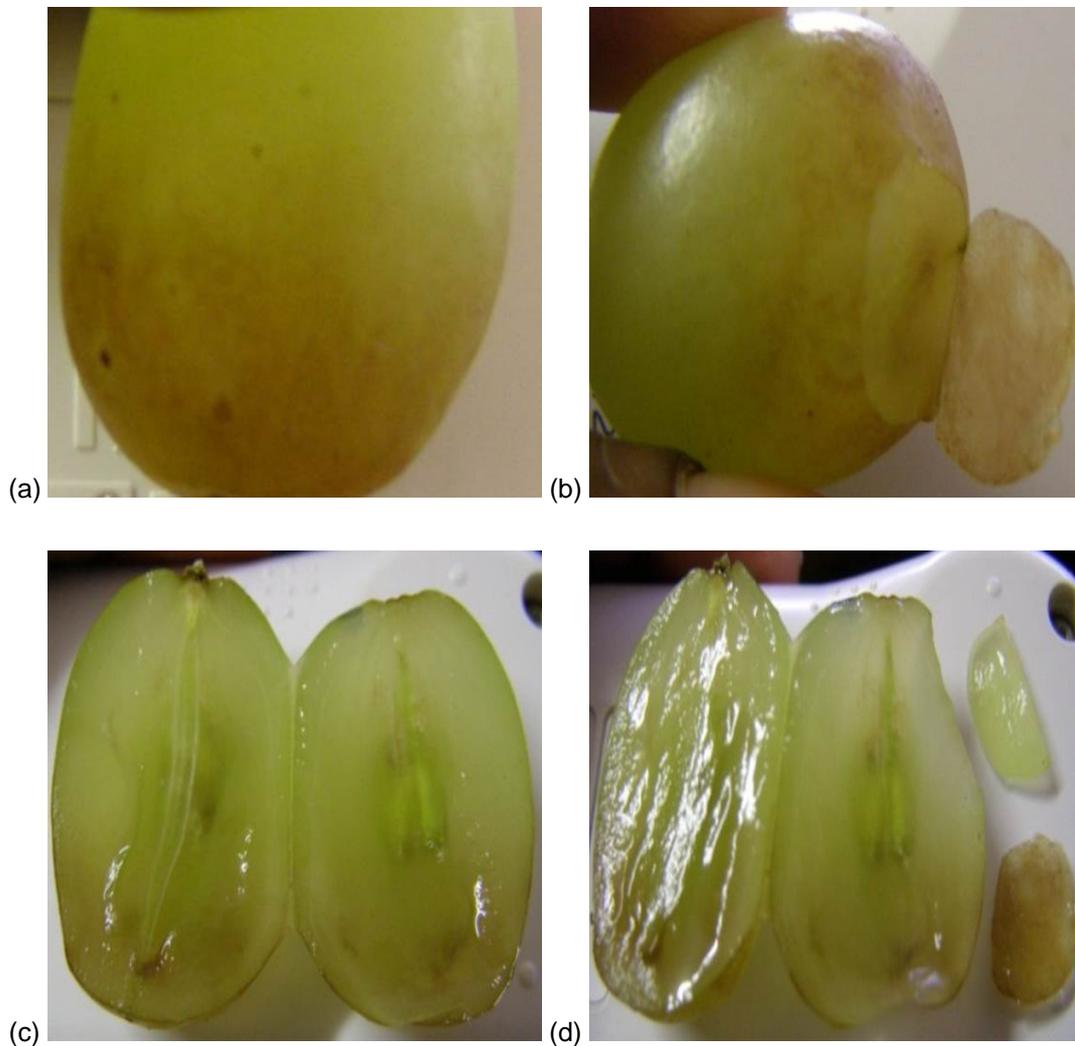


Figure 4.8 Netlike browning symptoms on Regal Seedless berries as seen (a) from the outside (b) underneath the skin (c) inside the berry and (d) compared to a part of the berry not showing any browning.

The number of berries that showed different types of browning after cold storage is shown in Table 4.2 and Table 4.3. The data was not analysed statistically because only an indication was needed

of which type of browning was more prevalent on which cultivar. Internal browning was more prevalent in berries of Thompson Seedless (13%) than berries of Regal Seedless (5%) in 2008. A correlation between increased sugar levels of berries and the percentage of berries that developed internal browning has been found more than 40 years ago (Pool and Weaver, 1970). Therefore, it is still speculated that internal browning potential may increase with advanced harvest maturity, especially on Thompson Seedless (DFPT Researchers, 2009). In this study, however, this seemed to hold true with the Regal Seedless 2008 data and the Thompson Seedless 2009 data (Table 4.2 and 4.3), but not for the Thompson Seedless 2008 data and the Regal Seedless 2009 data (Table 4.2 and 4.3) where incidence of browning decreased with advanced maturity. Regal Seedless was more prone to other no specific types of browning (61%) in 2008. Browning of berries seemed to occur more during the lower sugar level harvest date of Thompson Seedless (24%) but not for Regal Seedless (83%). Regal Seedless had the second most browning during the second harvest date (53%) and Thompson Seedless also during the second harvest date (23%).

Table 4.2 Number of berries that showed signs of browning on respectively Regal Seedless and Thompson Seedless berries harvested in 2008 with the corresponding sugar level they were harvested at.

	REGAL 2008			Total	THOMPSON 2008			Total
	16°Brix	18°Brix	20°Brix		16°Brix	18°Brix	20°Brix	
CB	0	12	8	20	1	8	2	11
IB	0	30	6	36	48	40	24	112
B	119	112	205	436	21	19	16	56
Total Brown	119	154	219	492	70	67	42	179
Total Berries	144	288	288	720	288	288	288	864

In 2009 Regal Seedless again had a higher incidence of browning than Thompson Seedless. Thompson Seedless in 2009 showed no signs of contact, netlike, mottled and stylar-end russet spots browning. However, the cultivar should have a high incidence of these types of browning, since most of the enzyme activity of polyphenol oxidase (the enzyme responsible for the browning reaction) is located in its skin (Radler, 1964). It, however, had a high incidence of chocolate-, abrasion- and internal browning. This was the exact opposite for Regal Seedless which had high incidences of external types of browning like glassy berry external symptoms-, bruising-, friction-, netlike-, fungal infection- and other no specific types of browning. With Regal Seedless, the highest incidence of browning in 2009 was observed during the third harvest (77%). This was followed by the second (74%) and first harvest (63%) and then the last (50%). With Thompson Seedless it was the last harvest date that had the highest incidence of browning (38%). The first harvest date had the second highest (27%), followed by the third (21%) and then the second (14%). This definitely did not follow the expected trend of increasing browning with increasing sugar level. Regal Seedless also seemed to have more external symptoms of browning than Thompson Seedless, which is no surprise, since the skin of Regal Seedless is known to be more sensitive to marks causing it to brown easily (Avenant, 2007). Unlike what Wolf (1996) found for Waltham Cross, a white seeded table grape cultivar, netlike browning did not seem to increase with increasing ripeness for Regal Seedless, it actually decreased. This was in agreement with what Fourie (2010) suggested which is that netlike browning is the dominant type of browning on Regal Seedless and it decreases with advanced harvest maturity (Table 4.3).

Table 4.3 Number of berries that showed signs of browning on Regal Seedless and Thompson Seedless berries harvested in 2009 with the corresponding sugar level they were harvested at.

	REGAL 2009				THOMPSON 2009					
	16°Brix	18°Brix	18°Brix	20°Brix	Total	16°Brix	18°Brix	18°Brix	20°Brix	Total
GBES		36	22	12	70	6		21	3	30
CB	6		3	3	12	2	1		38	41
CT		2			2					0
ABR		9	5	2	16	<u>66</u>	17	7	1	<u>91</u>
BR		15	11	2	28		1			1
NLB		218	200	170	<u>588</u>					0
FR		18	9		27	1	1	5		7
MTLD		1	2		3					0
FI		18	1	1	20		4			4
SERS		1	3	2	6					0
IB	4			2	6	3	7	27	16	53
B	<u>263</u>		75	20	358	1	10	1	51	63
Total Brown	273	318	<u>331</u>	214	<u>1136</u>	79	41	61	<u>109</u>	<u>290</u>
Total Berries	432	432	432	432	1728	288	288	288	288	1152

4.4.2. NIR SPECTRA AND PCA

The spectrum of a whole table grape berry scanned with the solid probe of the FT-NIR instrument is shown in Figure 4.9. Figure 4.10 (a) shows a score plot of spectra taken of whole table grape berries before storage (red b's on the left) and of spectra taken after cold storage (blue a's on the right) and what the distribution looks like on the main axis. This is because a PCA models most of the differences in the data set by displaying the spectra in an environment demarcated by principal components (PC's) and the scores show where those samples are. Principal components (PC's) are a combination of the original variables and contain the information of most importance that can be found in the data and they are also known as latent variables (Esbensen, 2006). The first PC in Figure 4.10 (a) explains 88% of the variation in the data, the second PC 10% and the third one only (1%). This clearly shows that cold storage has an effect on the berry and this information is contained within the spectrum of the berry taken before and after cold storage. This is an important finding that can be used in future research that is focussed on investigations into the effects on cellular level of cold stored table grapes. The loadings plot in Figure 4.10 (b) shows that the full spectrum carries most of the information in this particular dataset.

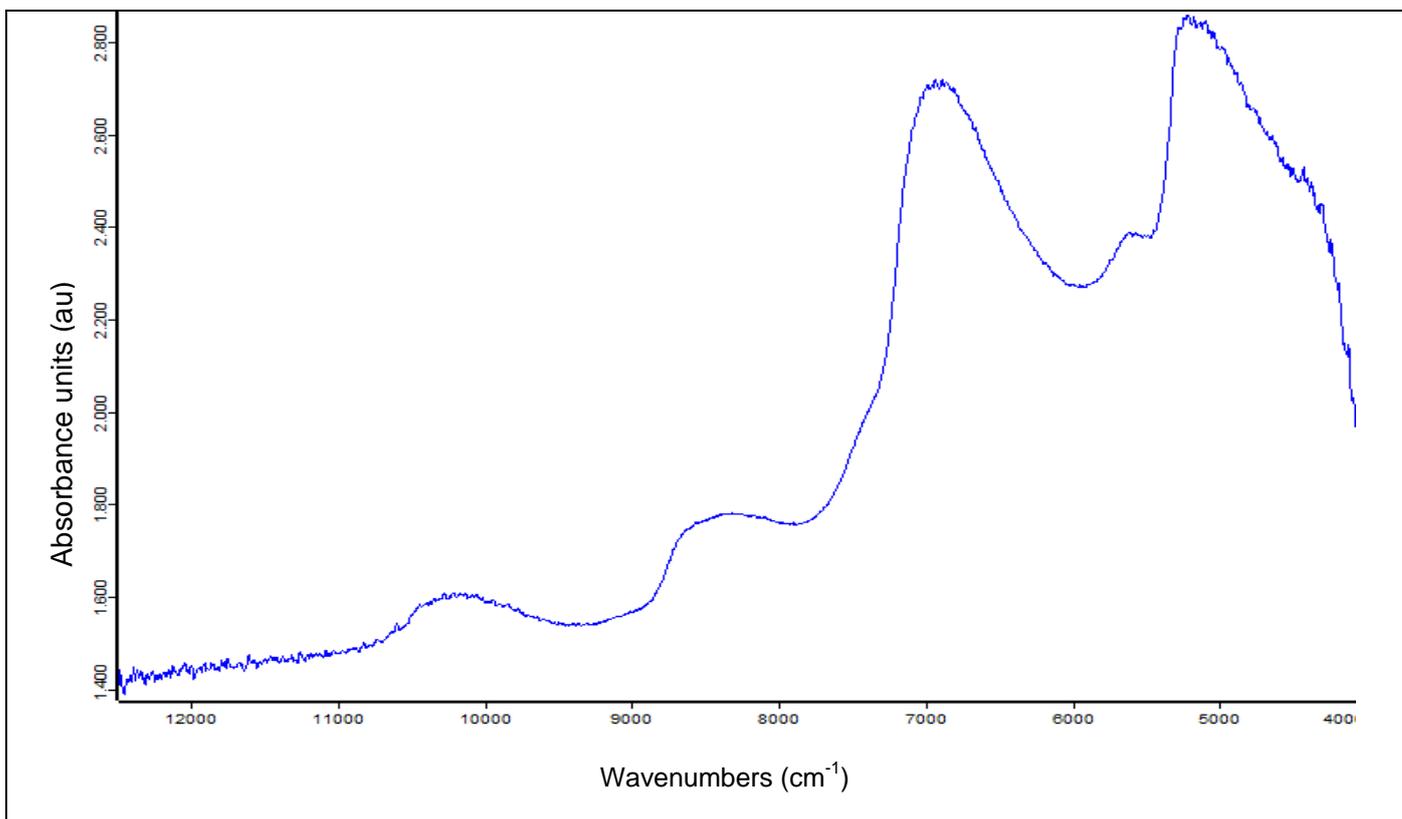


Figure 4.9 Example of FT-NIR spectrum taken of a whole table grape berry.

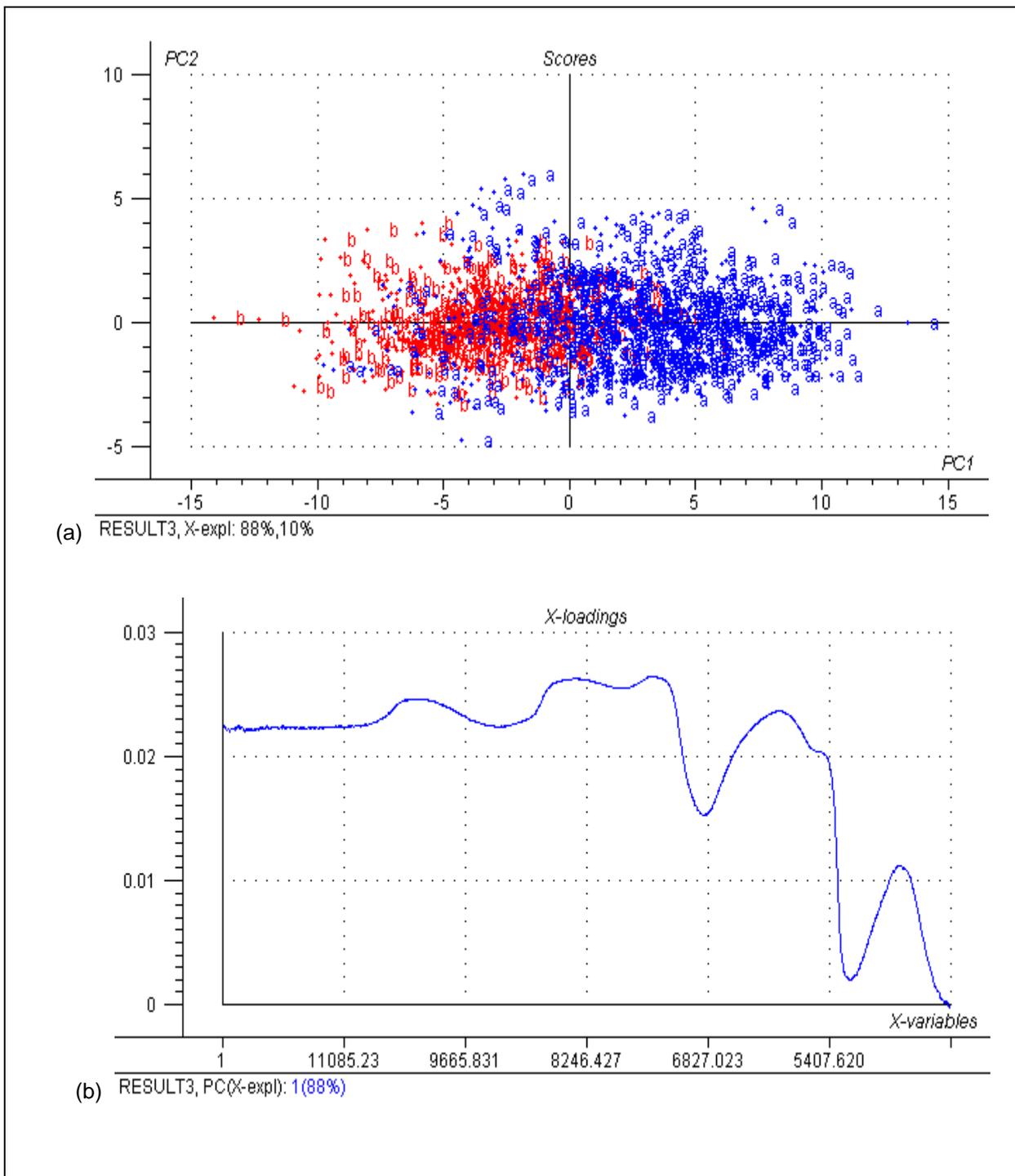


Figure 4.10 (a) PCA score plot showing spectral distribution on the main axis of table grape berries scanned before cold storage (red group on the left = b) and of table grape berries scanned after cold storage (blue group on the right = a), (b) Loadings plot showing that the full spectrum carries most of the information in this particular dataset.

4.4.2 CLASSIFICATION OF BERRIES BASED ON SPECTRA OBTAINED AFTER COLD STORAGE

After examining, scoring and coding the berries after cold storage, the data was used for statistical analysis in Statistica version 10. An example of the PCA's constructed for the different after cold storage data sets are shown in Figure 4.11. The optimum number of principal components to construct the model for all the data sets together and Thompson Seedless alone was eleven where

almost 90% and more than 93% of the variance in the spectra was explained, respectively. For Regal Seedless it was ten and nine for the Regal Seedless 27J09R, 26F08R and 17F09R datasets where a variance of more than 90% and almost 88% was explained, respectively. Scatterplots of Component 2 against Component 1 is shown in Figure 4.12 for Regal Seedless and in Figure 4.13 for Thompson Seedless depicting the outliers which were not removed during model construction.

The results of the training data (Table 4.2) always showed better statistics than those of the test data (Table 4.3). This is because re-substitution was used where the same 70% of the total samples used to construct the models was used to test the models. With the test data the models were tested with the 30% of the samples that was not used in the construction of the models. The Regal Seedless 27J09R, 26F08R and 17F09R datasets gave the best classification for the training data with a total accuracy of 99%, followed by Thompson Seedless (98%), Regal Seedless (97%) and then all the datasets used together (94%).

For the test data (Table 4.3) Thompson Seedless achieved the best total accuracy (92%) followed by all the datasets (87%), Regal Seedless (83%) and then the Regal Seedless 27J09R, 26F08R and 17F09R dataset (79%). The non-brown berries (no), always gave the best classification than the berries that showed signs of browning after cold storage (yes) for the training dataset classification (Table 4.2). This was with the exception of the Regal Seedless 27J09R, 26F08R and 17F09R datasets, where the classification for both (yes and no) was 99%. For the test data classification the Regal Seedless (86%) and Regal Seedless 27J09R, 26F08R and 17F09R datasets (80%) obtained better accuracies for the berries that turned brown than the ones that did not. Overall the best classification for non-brown berries after cold storage was obtained with all the data together; 97% for the training data and 90% for the test set data. This was also true for the classification of berries that turned brown after cold storage: 92% for the training data and 83% for the test data.

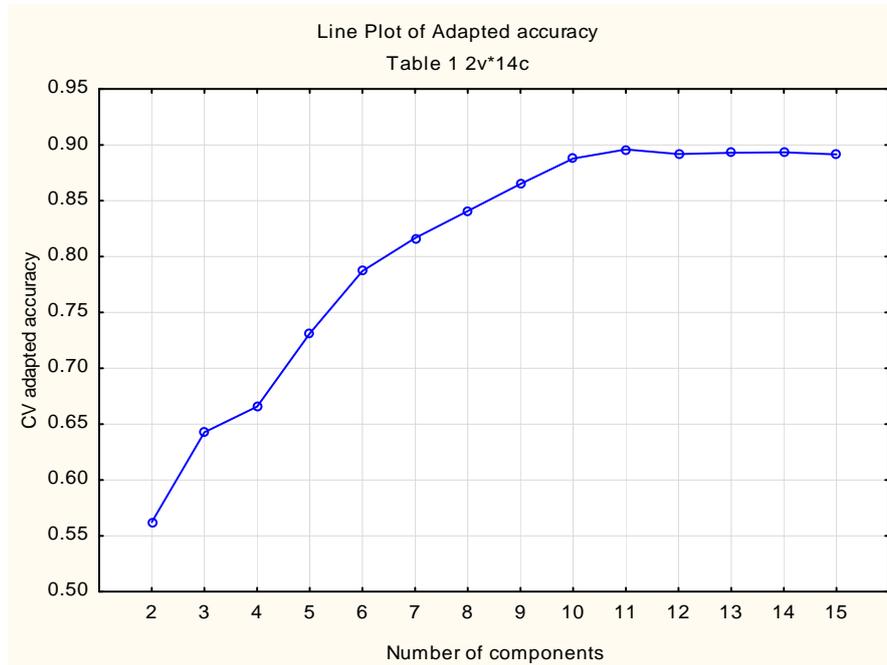


Figure 4.11 PCA line plot of adapted accuracy for all data showing the optimum number of principal components used to construct the model for all the data sets together as 11, where almost 90% of the variance in the spectra is explained.

Table 4.2 Regal Seedless and Thompson Seedless training data for after cold storage classification

All	% correct	no	yes	RS ^a	% correct	no	yes	RS ^a 27J09R 26F08R 17F09R	% correct	no	yes	TS ^b	% correct	no	yes
no	97%	1390	46	no	97%	473	15	no	99%	337	2	no	100%	948	0
yes	92%	104	1122	yes	96%	35	950	yes	99%	4	447	yes	92%	19	222
TA ^c	94%			TA ^c	97%			TA ^c	99%			TA ^c	98%		

^aRS = Regal Seedless, ^bTS = Thompson Seedless, ^cTA = total accuracy

Table 4.3 Regal Seedless and Thompson Seedless test data for after cold storage classification

All	% correct	no	yes	RS ^a	% correct	no	yes	RS ^a 27J09R 26F08R 17F09R	% correct	no	yes	TS ^b	% correct	no	yes
no	90%	554	62	no	78%	189	54	no	78%	129	37	no	98%	364	9
yes	83%	90	435	yes	86%	57	350	yes	80%	38	154	yes	75%	29	89
TA ^c	87%			TA ^c	83%			TA ^c	79%			TA ^c	92%		

^aRS = Regal Seedless, ^bTS = Thompson Seedless, ^cTA = total accuracy

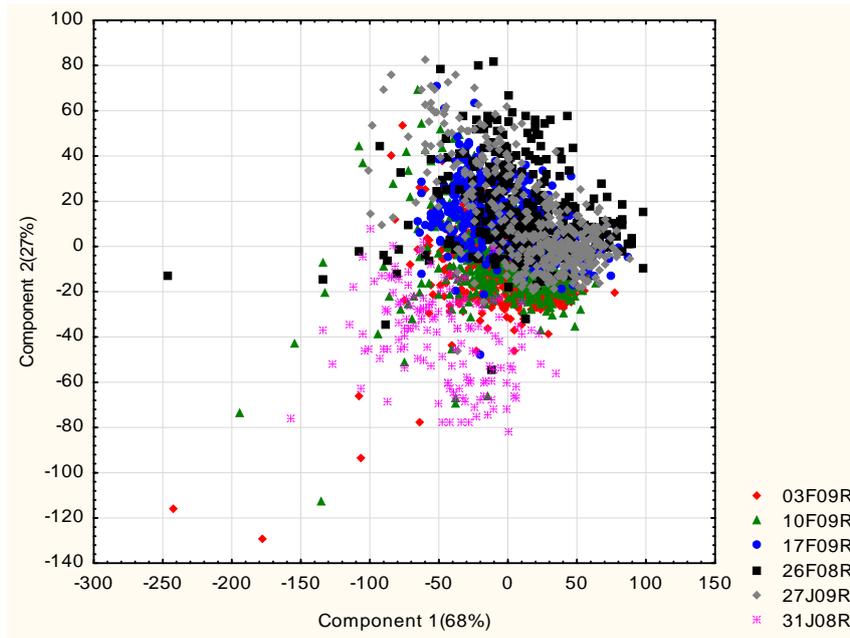


Figure 4.12 Scatterplot for the Regal Seedless samples harvested on different dates which are related to the sugar content/ripeness level on that specific date. Red diamond = Regal Seedless harvested on 03 February 2009 (03F09R = 18°Brix), green triangle = Regal Seedless harvested on 10 February 2009 (10F09R = 18°Brix), blue circle = Regal Seedless harvested on 17 February 2009 (17F09R = 20°Brix), black square = Regal Seedless harvested on 26 February 2008 (26F08R = 20°Brix), grey star = Regal Seedless harvested on 26 January 2009 (27J09R = 16°Brix) and pink snowflake = Regal Seedless harvested on 31 January 2008 (31F08R = 16°Brix).

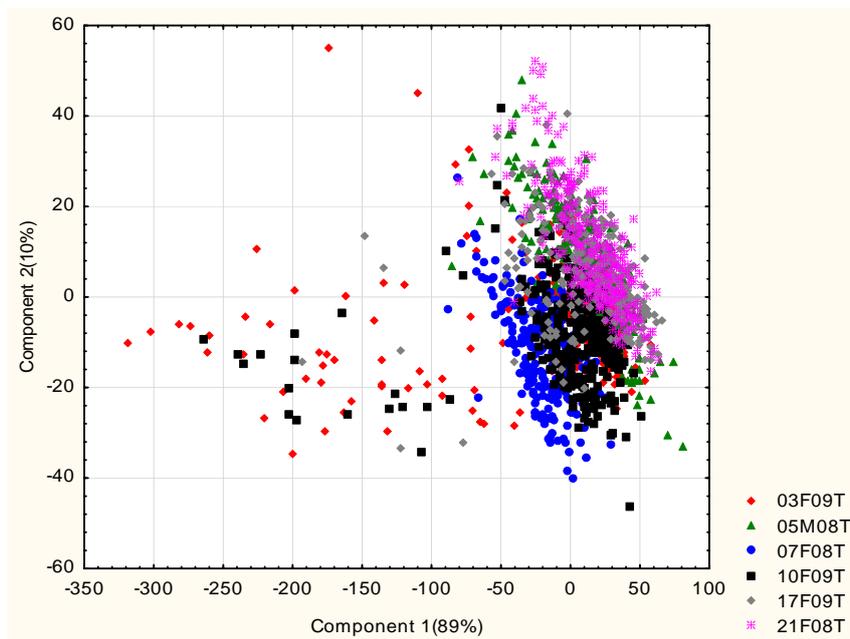


Figure 4.13 2D Scatterplots for Thompson Seedless depicting different dates (sugar level) grapes were harvested on. Each shape is representative of a single berry. Red diamond = Thompson Seedless harvested on 03 February 2009 (03F09T = 16°Brix), green triangle = Thompson Seedless harvested on 05 March 2008 (05M08 = 20°Brix), blue circle = Thompson Seedless harvested on 07 February 2008 (07F08T = 18°Brix), black square = Thompson Seedless harvested on 10 February 2009 (10F09T = 18°Brix), grey star = Thompson Seedless harvested on 17 February 2009 (17F09T = 20°Brix) and pink snowflake = Thompson Seedless harvested on 21 February 2008 (21F08T = 18°Brix).

4.4.3 CLASSIFICATION OF BERRIES BASED ON SPECTRA OBTAINED BEFORE COLD STORAGE

An example of the PCA's constructed for the different before cold storage datasets are shown in Figure 4.14. The optimum number of principal components to construct the model for all the datasets together and Thompson Seedless alone was seven, where more than 70% and almost 80% of the variance in the spectra was explained, respectively. This is less than the ones for the after cold storage classification. It, however, seems as if Thompson Seedless is a driving force in the determination of the optimum number of principal components to be used for the construction of the models for all the data, since the number was also the same (eleven) for the classification based on the after cold storage spectra in the previous section. For Regal Seedless it was six and a variation of almost 70% was explained in the spectra.

The results of the training data (Table 4.4) always looked better than those of the test data (Table 4.5) as was also the case with the after cold storage classifications models. This is because re-substitution was also used here to construct the models. With the test data the models were tested with the 30% of the samples that was not used in the construction of the models. Thompson Seedless had the best classification for the training data with a total accuracy of 91%, followed by Regal Seedless (84%) and then all the datasets used together (79%).

For the test data Thompson Seedless again achieved the best total accuracy (68%) followed by all the datasets (64%) and then Regal Seedless (59%). The non-brown berries (no), always gave the best classification compared to the berries that showed signs of browning after cold storage (yes) for the training dataset classification. This was with the exception of Regal Seedless where the classification for yes was 91%. For the test data classification, Regal Seedless obtained the best accuracy (72%) for the berries that turned brown than the ones that did not.

Overall, the best classification for non-brown berries after cold storage was obtained with all the data together which was 82% for the training data and 68% for the test set data. This was also true for the classification of berries that turned brown after cold storage which was 76% for the training data and 59% for the test data. This was based on the total amount of berries used in the classification. Although it appears that Thompson Seedless on its own, which obtained 98% for non-brown berries after cold storage has better classification accuracies than Regal Seedless on its own which obtained 91% for berries that showed browning after cold storage. It has to be kept in mind that less berries were used in the construction of the Thompson Seedless classification models (Table 4.4). There were also more different types of browning present on the Regal Seedless berries than on the Thompson Seedless ones. This was also true for the test set classification data (Table 4.5).

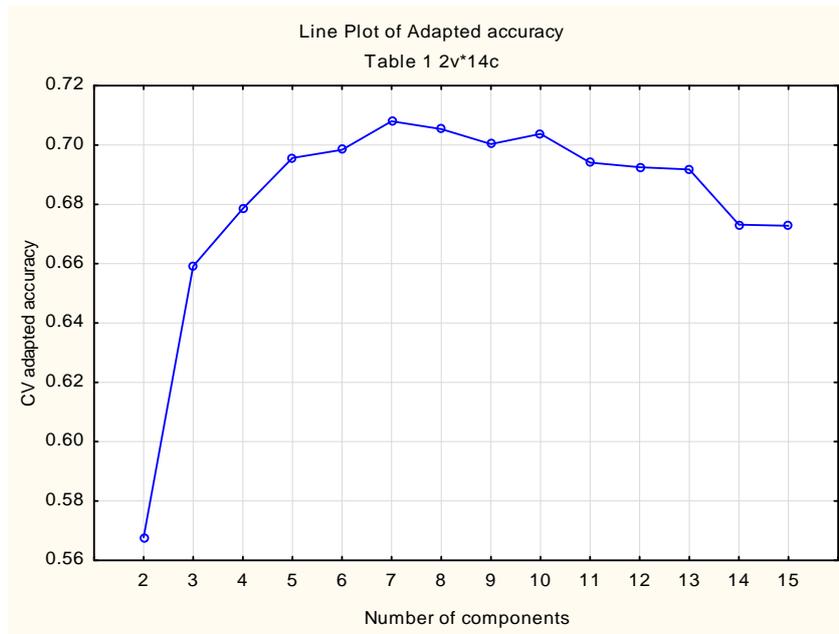


Figure 4.14 2D line plot showing the optimum number of principal components used to construct the model for Regal Seedless and Thompson Seedless as seven where almost 90% and more than 93% of the variance in the spectra was explained respectively.

Table 4.4 Regal Seedless and Thompson Seedless training data for before cold storage classification

All	% correct	no	yes	RS ^a	% correct	no	yes	TS ^b	% correct	no	yes
no	82%	1167	249	no	72%	352	140	no	98%	908	16
yes	76%	281	882	yes	91%	82	847	yes	61%	91	143
TA ^c	79%			TA ^c	84%			TA ^c	91%		

^aRS = Regal Seedless, ^bTS = Thompson Seedless, ^cTA = total accuracy

Table 4.5 Regal Seedless and Thompson Seedless test data for before cold storage classification

All	% correct	no	yes	RS ^a	% correct	no	yes	TS ^b	% correct	no	yes
no	68%	410	197	no	36%	84	147	no	85%	321	55
yes	59%	205	293	yes	72%	106	277	yes	10%	104	11
TA ^c	64%			TA ^c	59%			TA ^c	68%		

^aRS = Regal Seedless, ^bTS = Thompson Seedless, ^cTA = total accuracy

4.5 CONCLUSION

Regal Seedless had a higher incidence of browning than Thompson Seedless under the experimental conditions used in this study, indicating that it is possibly much more susceptible to browning than Thompson Seedless. This might be due to a lot of factors like a difference in the phenolic content of the two cultivars (Boulton *et al.*, 1996), a difference in climatic conditions of the two experimental sites (Singleton and Trousdale, 1992) as well as cultivation practices followed (Avenant, 2007). Regal Seedless was more prone to netlike browning and Thompson Seedless more prone to internal browning. The classification models based on training data overall had better results than those of the test data classification models. This was no surprise due to the reasons explained above.

The classification models based on the spectra obtained of berries after cold storage were also better than those based on the spectra obtained of berries before cold storage. With the models based on the spectra obtained of berries after cold storage non-brown berries can be

classified with an accuracy of 90%, brown berries with 83% with an overall accuracy of 87%. With the models based on the spectra obtained of berries before cold storage, which is actually the stage which is the most important for determining whether white seedless table grapes will turn brown, non-brown berries could be classified with an accuracy of 68%, brown berries with 59% and a total accuracy of 64%. At this stage it is only 10% better than chance (50% correct classification). This is clearly not as good as the classification accuracy that can be obtained with the after cold storage spectra at this moment, but it does at least show that there is definitely some chemistry in the spectra that shows the potential to predict browning already before cold storage.

This has major implications for the table grape industry, since it is the first time that the possibility to predict browning with methods other than visual inspection, especially before cold storage, is shown. It is not that accurate at the moment, but it should, however, be noted that these models were constructed with the full spectrum taken of every berry in the sample sets and no variable selection was done. No outliers were also removed. The use of variable selection is very important because once this technology is transferred to the industry an instrument that will only measure the wavelengths associated with the specific browning phenotypes will be necessary. An instrument containing all the wavenumbers that the berries were scanned in will be impractical and very expensive, especially if the wavenumbers that the instrument chooses is far apart. It is, therefore, highly recommended that the data be analysed further by first applying variable selection and try to get adjacent wavenumbers that are associated with the browning phenomenon.

The models might also have been unstable due to the different phenotypes of browning that were used in the models. The different types of browning will, therefore, also have to be investigated on their own in all the datasets, with netlike browning being the obvious one to look at first in Regal Seedless and internal browning in Thompson Seedless.

When new data will be acquired in the future, a rubber head will also be attached at the end of the solid probe of the FT-NIR instrument to improve the spectrum quality by reducing the pathlength (distance between berry and instrument). This will also be to eliminate any influence that the solid probe might have on the development of browning after cold storage, since berries tend to be softer than when they were first harvested and packed. To further eliminate the influence of contact on the pathlength a separate attachment like an emission head can be also used to scan the berries contactless. Since in this study the pathlength could also have been influenced by the stretching and squeezing of the berries during measurements due to the contact that was made. This will ultimately be the way to go if whole bunches will be scanned for browning on the packing line after harvest. If bunches will be scanned in the vineyard already then the way to go will be a handheld instrument. It will also be attempted to scan berries two weeks after they have been in cold storage and then again after the whole five weeks of cold storage have been completed to determine how the incidence of browning progress throughout cold storage. For now, the table grape industry can rejoice over this breakthrough that has been made.

4.6 LITERATURE CITED

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Chapter 5

General discussion and conclusions

Chapter 5: General Discussion and Conclusions

The export of South African table grapes earns valuable foreign currency for the country. In addition to the risks associated with the current sensitive and volatile economic climate, the implications of the dramatically changing world climate, as seen in global warming, on the future of table grape production, are not to be overlooked. Everything possible should thus be done to ensure consistent and excellent fruit quality for the sustainability of future crops. Aspects of critical importance are the maintenance and improvement of the product quality that consumers have become used to over the years. The variability in the quality between individual berries on a bunch, between bunches on the same vine and different vines in a vineyard, makes this task very challenging for the producer. There is thus really no secure way by which mere looking or testing of one berry on a bunch can lead to assessment of whether the grapes are at the right harvest maturity level or optimum quality. Rather, different parameters, including sugar level (TSS, glucose and fructose) and the acidity (pH and TA), should simultaneously be measured collectively for this objective.

Determination of these parameters, especially TSS, can be easily done in the vineyard or in the laboratory with a handheld refractometer. However, as mentioned earlier, the consumer perceives quality as taste (sweet and sour), so determining the correct acidity or sugar/acid ratio which is the norm in South Africa, is just as important. This, however, is not easy since the titratable acidity has to be determined in the laboratory where specific chemicals and instruments are used. The same problem exists with glucose and fructose for which specialised enzymatic assay kits and instruments are necessary. The individual determination of these two sugars were done since, their concentrations can also vary during the development of the grape berries. With strict laboratory discipline (accurate determinations) and commitment (laborious and time consuming), the optimum eating quality for export grapes can still be achieved using conventional wet chemistry methods. Ensuring, however, that the grapes look as fresh as the day they were harvested and are free of any defects, are more difficult especially with a postharvest quality defect like browning that only becomes visually apparent after storage.

Browning is a complex chemical process which occurs on the cellular level and is expressed either on the inside (internal browning) or surface (external browning) of table grapes. Table grape browning is not visible during harvest, packing, or transport of table grapes, but only once grapes reach their destination.

This study thus had two major aims. One was to find a way to determine if all the harvest maturity and quality parameters, namely TSS, pH, TA, glucose and fructose could be quantified fast, accurately and simultaneously and secondly to find a way by which browning can be determined before it becomes visibly apparent. Infrared (IR) spectroscopy was therefore used for quantitative (sugar or acid content) and qualitative (brown or not) purposes.

Results of the calibration models constructed for TSS, pH and TA on the FT-NIR instrument were very good. All the models constructed for these parameters can be used for quantification purposes. The calibration model for fructose on the FT-NIR instrument is fit for screening purposes, but the model for glucose is not yet suitable for quantification purposes. Results obtained for TSS, pH and TA on the FT-MIR ATR were similar to those obtained on the FT-NIR instrument. All the models can be used for screening purposes. The one for glucose, however, is fit for screening purposes on FT-MIR ATR instrument, whilst the one for fructose is not yet suitable for quantification purposes. Results for calibration models constructed for all the different parameters on FT-MIR instrument was suitable for quantification purposes based on the R^2 value, but was not yet suitable for quantification based on the RPD values. Although the bias values of all the parameters on the FT-MIR instrument were all zero, which is excellent, the RMSEP values, however, were quite high. This means that the uncertainty with which these parameters can be

determined in future table grape juice samples are very high. Nevertheless, a platform of technologies has now been made available through this study for the determination of the respective parameters in future table grape juice samples.

The scanning of whole table grape berries before and after cold storage showed that storage effects can definitely be captured with NIR, although it was not possible to interpret in exact chemical or physical detail. It can, however, be speculated that this could be related to the dehydration that grape berries undergo as soon as they are harvested. To determine whether this is true and quantify storage effects even better, one might go about taking the spectra of grapes that has not undergone any form of cold storage and then construct a principal component analysis (PCA) of all the data.

When the berries were investigated for the incidence of browning after cold storage, some of the same browning phenotypes were present as those that were identified by Fourie (2009). It therefore does not seem as if the removal of berries from bunches had any effect on the type or incidence of browning. Regal Seedless had a higher incidence and also more different types of browning than Thompson Seedless. Regal Seedless was also more prone to netlike browning and Thompson Seedless more prone to internal browning.

Classification models were built of spectra obtained of berries that remained clear as opposed to those that turned brown after cold storage. Classification models based on spectra of berries that were obtained after cold storage had better accuracies than classification models based on spectra obtained of berries before cold storage. The classification models based on spectra obtained of berries after cold storage could be used to classify non-brown berries with an accuracy of 90%, brown berries with 83% and an overall accuracy of 87%. Classification models based on spectra obtained of berries before cold storage could be used to classify non-brown berries with an accuracy of 68%, brown berries with 59% and a total accuracy of 64%. The critical stage where browning should be monitored and detected is before cold storage of grapes. The classification models based on spectra obtained of berries before cold storage were not as good as the classification models based on spectra obtained of berries after cold storage. There is, however, definitely still huge potential to monitor and detect browning with this technology. The models based on spectra obtained of berries before cold storage can be refined by applying variable selection techniques instead of using the whole spectrum as was done in this study. In that way noise, especially water peaks, can be removed and only wavelengths which are strongly associated with the browning phenomenon can be selected. Through this the development of a handheld device can also be made possible. Groundwork laid in this study can also be used to evaluate whole table grape bunches two weeks after they have been in cold storage and then again after the whole five weeks of cold storage. NIR hyper spectral imaging, as was done with whole maize kernels (Williams, 2009), can also be investigated. The main point will, however, be to first just focus on the main browning types occurring on Regal Seedless (netlike browning) and Thompson Seedless (internal browning). When successful or classification models based on spectra obtained of berries or bunches before cold storage with better results have been obtained, it will ultimately lead not only to less financial losses by the SA table grape industry due to export of grapes that turned brown after cold storage, but a maintenance of SA as a powerhouse in earning the most agricultural foreign income for the SA economy and also as the third largest exporter of table grapes in the world!

LITERATURE CITED

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