Non-destructive Prediction and Monitoring of Postharvest Quality of Citrus Fruit

By

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The aim of this study was to develop non-destructive methods to predict external and internal quality of citrus fruit. A critical review of the literature identified presymptomatic biochemical markers associated with non-chilling rind physiological disorders. The prospects for the use of visible to near infrared spectroscopy (Vis/NIRS) as non-destructive technology to sort affected fruit were also reviewed. Initial studies were conducted to determine the optimum condition for NIRS measurements and to evaluate the accuracy of this technique and associated chemometric analysis. It was found that the emission head spectroscopy in diffuse reflectance mode could predict fruit mass, colour index, total soluble solids, and vitamin C with high accuracy. Vis/NIRS was used to predict postharvest rind physico-chemical properties related to rind quality and susceptibility of 'Nules Clementine' to RBD. Partial least squares (PLS) statistics demonstrated that rind colour index, dry matter (DM) content, total carbohydrates, and water loss were predicted accurately. Chemometric analysis showed that optimal PLS model performances for DM, sucrose, glucose, and fructose were obtained using models based on multiple scatter correction (MSC) spectral pre-processing. The critical step in evaluating the feasibility of Vis/NIRS was to test the robustness of the calibration models across orchards from four growing regions in South Africa over two seasons. Studies on the effects of microclimatic conditions predisposing fruit to RBD showed that fruit inside the canopy, especially artificially bagged fruit, had lower DM, higher mass loss, and were more susceptible to RBD. The study suggested that variations in microclimatic conditions between seasons, as well as within the tree canopy, affect the biochemical profile of the rind, which in turn influences fruit response to postharvest stresses associated with senescence and susceptibility to RBD. Principal component analysis (PCA) and PLS discriminant analysis (PLS-DA) models were applied to distinguish between fruit from respectively, inside and outside tree canopy, using Vis/NIRS signal, suggesting the possibility of using this technology to discriminate between fruit based on their susceptibility to RBD. Results from the application of optical coherence tomography (OCT), a novel non-destructive technology for imaging histological changes in biological tissues, showed promise as a potential technique for immediate, real-time acquisition of images of rind anatomical features of citrus fruit. The study also demonstrated the potential of Vis/NIRS as a non-destructive tool for sorting citrus fruit based on external and internal quality.

OPSOMMING

Die studie het ten doel gestaan om nie-destruktiewe meeting metodes te toets en ontwikkel wat die interne en eksterne-kwaliteit van sitrusvrugte kan voorspel. In ’n litratuuroorsig is biochemies verandering in die skil en wat geassosieer word met die ontwikkeling van fisiologies skildefekte geïdentifiseer, asook is die moontlikheid ondersoek om Naby Infrarooi spektroskopie (NIRS) as ‘n nie-destruktiewe tegnologie te gebruik om vrugte te sorteer. Eerstens was die optimale toestande waarby NIRS meetings van sitrusvrugte geneem moet word asook die akkuraatheid van die toerusting en chemometrika data-ontleding beproef. Daar is gevind dat die uitstralings-kop spektrofotometer in diffusie-weerkaatsings modus vrugmassa, skilkleur, totale opgeloste stowwe asook vitamien C akkuraat kan voorspel. Daarna van NIRS gebruik om na-oes fisies-chemiese eienskappe wat verband hou met skilkwaliteit en vatbaarheid vir skilafbraak van ‘Nules Clementine’ mandaryn. Deur gebruik te maak van “Partial least squares” (PLS) statistieke was gedemonstreer dat die skilkleur, droë massa (DM), totale koolhidrate en waterverlies akkuraat voorspel kon word. Chemometriese analises het ook getoon dat optimale PLS modelle vir DM, sukrose, glukose en fruktose verkry kan word deur modelle te skep wat gebaseer is op “Multiple scatter correction” (MSC) spektrale voor-verwerking. ’n Belangrike stap in die ontwikkeling van NIRS gebaseerde indeling is om die robuustheid van die kalibrasiemodelle te toets en was gedoen deur vrugte te meet en sorteer van vier boorde en oor twee seisoene. ’n Verder eksperiment om die impak van mikroklimaat op die skil se vatbaarheid vir fisiologiese defekte te ontwikkel het getoon dat vrugte wat binne in die blaardak ontwikkel (lae vlakke van sonlig) ’n laer DM, hoër gewigsverlies het en was ook meer vatbaar vir skilafbraak. Die resultate dui daarop dat verskille in mikroklimaat oor die seisoen asook in die blaardak die skil se biochemiese profiel beïnvloed, wat lei tot ’n negatiewe reaksie op na-oes stres en verhoogde voorkoms van fisiologiese skilafbraak. Die ontwikkelde “Principal component analysis” (PCA) en PLS-diskriminant analise modelle was daarna suksesvol toegepas om vrugte te skei na NIRS meetings, op die basis van vrugpossies in die blaardak. Nuwe, nie-destruktiewe tegniek, nl. “Optical coherence tomography” (OCT) was suksesvol getoets as manier om ’n fotografiese beeld te skep van histologiese veranderinge in die skil. Die resultate dui op die potensiaal van die onontginde tegnologie om intak biologiese-materiaal te analiseer. Hierdie studie het getoon dat daar wesenlike potensiaal is om NIRS verder te ontwikkel tot ’n tegnologie wat gebruik kan word om vrugte te sorteer gebaseer op eksterne (skil) asook interne (pulp) eienskappe.

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ISETHULO

Lomsebenzi ngiwethula ngentobeko, ngenhlonipho, ngentokozo, nangokutusa kubazali bami abangizalayo. Ngizoqala ngomama, uBhekephi Benzeleni Magwaza, uMashombela intombi yakoMbashane eMaKhabeleni. Isikhukhukazi esihamba netshwele elilodwa ezinye zihamba namatshwele ngamaningi. Ngithi kuwe mama wami ngiyabonga ngokungifukamela kubo bonke ubunzima engidlule kubo kusukela ebuncaneni ngize ngibe kulelizinga. Ngibonga imfundiso yakho eyangifundisa ukuthanda imfundo, impuncuko, intuthuko nenkuthalo okuyizinto eziwubisi lwempumelelo kulomhlaba. Kubaba, uNgindi Magwaza, ngithi ngibonga ukungifundisa ubudoda, isandla sakho owangikhulusa ngaso singenze ngakwazi ukumelana nokubekezelela zonke izimo ezinzima kulomhlaba. Ume njalo nje Gidizela, wena Mbenge encane esibekela izithebe ezikhulu, wena mpisi edla inethile endlini kwabo kwaMaNdlovu. Unsizwa ziyamthibela ngoba uthitshelwe uDodofana kwabakaCele. Bese ngithi kunina nonke maqhawe namaqhawekazi omuzi wakwaMagwaza nawakwaMakhaye, asadla amabele nalawo asephumule kwelamathongo, ngithi lelibhuku alibe yisikhumbuzo sokubaluleka kwegama lenu emhlabeni wonke jikelele. Ngokufunda lelibhuku nezizukulwane zenu ezizayo seziyokhongozela lomlando esiwuqophile zifunde ukubaluleka kwemfundo. Ngiyethemba nizowuthokozela umsebenzi wami.

Ngithanda ukubonga kukhokho kababa uShishi, usilo sodonga umakhulukuthu, ubantu bokutholwa abadlelwa mithi, inkunzi ebhixa ezinye ngodaka kwamhlekwa. Ngibonge nasendodaneni yakhe yokuqala, uSonani, uhhayi lukaShishi usihlahla somdlebe, bethi esomdlebe nje kanti esombhelebhele. Ngiphinde ngidlulise ukubonga okukhulu ngokungisingatha kwenye indodana kaShishi, umkhulu kababa, ogama lakhe, uNgxephu, uSobhaduzela ubhaduza kalunyawo, unyawo azikhethi mabala koMmisi. Unyokana eluhlazana, umakhwela emithini noma engenanyawo. Ngibonge kakhulu kukhokho, uMshikashika indaba isocansini. Umfokamsizakali lowo, ozala omkhulu oHluphizwe, nompisi edla emasimba kayise, ubaba omkhulu wakaPhumhambe, nobaba omkhulu wasendulo, uNobhongcula kusale ikhobokhobo. Bese ngiza kubaba oMkhulu ozala ubaba, uMathambo, uNovanzi omuhle ngemilenze, ugadla bakhamise abezizwe nabasekhaya. Ngibonge nakobaba abangasekho, ubaba omdala, uVayisi, uhlabana ngenja emnyama eBhalekisi, umatete nomatete. Nakubaba ophakathi uMpini, usaka elimdudla endlini kwabo kwaMandlovu. Udikadika umuntu ngejozi eangweni koMabala, ngithi ngyabonga Manqondo.

Lelibhuku nilethulelwa uLembe eleqa amanye amalembe ngokukhalipha, usimayedwa onjengelanga ngoba lona lima lodwana esibhakabhakeni. Usakhalukhalweni phakathi kwemimfula emibili ubhucunga nontshalantshala. Unontandakubukwa onjengesakabuli. Ucelemba obugawugawu ngoba ugawule abezizwe kwelikaQueen eNgilandi wabuye wagawula nabasekhaya le konaspoti. Untombi ziyambuka ziyamonyozela kubabaze omama bakamakhelwane bathi “lomfana kaMagwaza useyenzeni lengane ngoba isencane nje?”

PREFACE

This dissertation is presented as a compilation of manuscripts where each chapter is an independent entity introduced separately. Some repetition between chapters has, therefore, been unavoidable. The chapters in this dissertation are written in accordance with the requirements of Elsevier BV Publishers of Postharvest Biology and Technology.

TABLE OF CONTENTS

LIST OF PUBLISHED PEER-REVIEWED PAPERS

1. **Magwaza, L.S.**, Opara, U.L., Nieuwoudt, H.H., Cronje, P.J.R., Saeys, W., Nicolaï, B.M., 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food and Bioprocess Technology, 52, 425–444. IF: 4.11
2. **Magwaza, L.S.**, Opara, U.L., Terry, L.A., Landahl, S. Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Saeys, W., Nicolaï, B.M., 2012. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest Biology and Technology, 74, 1–10. IF: 2. 45
3. **Magwaza, L.S.**, Ford, H.D., Cronje, P.J.R., Opara, U.L., Landahl, S., Tatam, R.P., Terry, L.A., 2013. Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins. Postharvest Biology and Technology, 84, 16–21. IF: 2. 45
4. **Magwaza, L.S.**, Opara, U.L., Cronje, P.J.R., Terry, L.A., Landahl, S., Nicolaï, B.M., 2013. Non-chilling physiological rind disorders in citrus fruit. Horticultural Reviews, Vol. 41, Chapter 3, 131–176.
5. **Magwaza, L.S.**, Opara, U.L., Terry, L.A., Landahl, S. Cronje, P.J.R., Nieuwoudt, H.H., Hanssens, A., Saeys, W., Nicolaï, B.M., 2013. Evaluation of Fourier transform-NIR spectroscopy for integrated external and internal quality assessment of 'Valencia' oranges. Journal of Food Composition and Analysis, 31, 144–154. IF: 2.08
6. **Magwaza, L.S.**, Opara, U.L., Cronje, P.J.R., Landahl, S., Terry, L.A., 2013. Canopy position effect on rind biochemical profile of 'Nules Clementine' mandarin fruit during postharvest storage. Postharvest Biology and Technology, 86, 300–308. IF: 2.45
7. **Magwaza, L.S.**, Opara, U.L., Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2013. Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. Scientia Horticulturae, http://dx.doi.org/10.1016/j.scienta.2013.09.035. IF: 1.40
8. **Magwaza, L.S.**, Opara, U.L., Cronje, P.J.R., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2013. Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. Scientia Horticulturae, http://dx.doi.org/10.1016/j.scienta.2013.09.050. IF: 1.40

LIST OF CONFERENCE PRESENTATIONS

1. **Magwaza, L.S.**, Opara, U.L., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Terry, L.A., 2013. Vis/NIRS-based PLS-DA and PCA models can be used to segregate citrus fruit with different susceptibility to postharvest rind disorders. Presented at the 8th ***CIGR Section VI International Symposium on "Advanced Food Processing and Quality Management"***, South China University of Technology, Guangzhou, China, 3-7 November, 2013. (Oral)
2. **Magwaza, L.S.**, Opara, U.L., Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Landahl, S., Terry, L.A., 2013. Introducing samples from target orchards improves prediction performance of Vis/NIRS models of citrus fruit quality measured at harvest. To be presented in the ***VI International Conference on Managing Quality in Chains (MQUIC 2013)***, Cranfield University, United Kingdom, 2–5 September 2013. (Oral)
3. **Magwaza, L.S.**, Opara, U.L., Landahl, S., Ford, H.D., Cronje, P.J.R., Terry, L.A., 2013. OCT: A novel, rapid and non-destructive technology to visualise microstructures of mandarin rinds. Presented in the ***Combined Congress 2013***, University of KwaZulu-Natal, Durban, 21-24 January 2013. Pp 80. (Oral)
4. **Magwaza, L.S.**, Opara, U.L., Landahl, S., Cronje, P.J.R., Terry, L.A., 2012. A rapid HPLC method for the determination of phenolic compounds composition of citrus rind and pulp samples. Presented at the ***7th CIGR Section VII International Technical Symposium on "Innovating the Food Value Chain" Postharvest Technology and Agri-Food Processing***, Stellenbosch University, Stellenbosch, South Africa, 25-29 November, 2012. (Poster)
5. **Magwaza, L.S.,** Opara, U.L., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Terry, L.A., 2012. The use of Vis/NIRS and chemometrics to predict fruit defects and postharvest behaviour of 'Nules Clementine' mandarin fruit. Presented at the ***7th CIGR Section VII International Technical Symposium on "Innovating the Food Value Chain" Postharvest Technology and Agri-Food Processing***, Stellenbosch University, Stellenbosch, South Africa, 25-29 November, 2012. (Oral)
6. **Magwaza, L.S.**, Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H.H., 2012. Quantifying the effects of fruit position in the canopy on physical and biochemical properties and predicting susceptibility to rind breakdown disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) using Vis/NIR spectroscopy.

Presented in the ***2nd All Africa Horticultural Congress***, 21-25 January 2012, Skukhuza, Kruger National Park, South Africa. Acta Horticulturae, 1007, 83–91. (Oral)

7. **Magwaza L.S.**, Opara, U.L., Nieuwoudt, H.H., Cronje, P.J.R., 2011. FT-NIR spectroscopy for non-destructive quality assessment of 'Valencia' orange. Presented in the ***Unilever Colworth and Cranfield University: Regional partners working together for global success Workshop***, 23 June 2011, Unilever Colworth Science Park, England. Pp. 46. (Poster)
8. **Magwaza L.S.**, Opara, U.L., Nieuwoudt, H.H., Cronje, P.J.R., 2011. Non-destructive quality assessment of 'Valencia' orange using FT-NIR spectroscopy. Presented in the ***15th International Conference on NIR Spectroscopy***, 13-20 May 2011, Cape Town, South Africa. Pp. 375. (Poster)

SECTION I

GENERAL INTRODUCTION AND LITERATURE REVIEW

A. General Introduction
B. Paper 1: Non-chilling Physiological Rind Disorders in Citrus Fruit
C. Paper 2: NIR Spectroscopy Applications for Internal and External Quality Analysis of Citrus Fruit – a Review

CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIMS

GENERAL INTRODUCTION AND RESEARCH AIMS

1. Introduction

Citrus is the biggest component of South African exported subtropical fruit. Globally, the South African citrus industry is ranked the third largest exporter of fresh citrus fruit after Spain and Turkey, respectively (Citrus Growers' Association of Southern Africa, 2012), worth over 6.5 billion rand (ZAR) ($733 million; April 2013) annually (Bonorchis, 2013). The position of the industry in the international market is strengthened by the production of a wide range of cultivars over an extended period of time - from March through to November. However, citrus fruit are prone to develop various types of physiological rind disorders, manifested by a multitude of symptoms during handling and storage (Kader and Arpaia 2002; Alquezar et al., 2010). The amount of fruit affected by these disorders can reach up to 60% of total production in the worst seasons (Agustí et al., 2001). These physiological disorders affect the rind and in general do not compromise the edible internal portion of the fruit, they do, however, decrease postharvest fruit market value, since external appearance is the primary specification used to evaluate quality of fresh citrus fruit (Alquezar et al., 2010). Limited knowledge of the physiological mechanisms underlying these disorders hinders the development of cost-effective solutions to minimise losses, and assure consistent supply of quality fruit.

A postharvest physiological rind disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit, commonly referred to as rind breakdown (RBD), is among several commercially important defects affecting the citrus industry (Cronje et al., 2011a, b). During early stages, the visual symptoms of RBD on 'Nules Clementine' mandarin are manifested as small, irregular, and slightly sunken patches of about 3 to 6 mm in diameter randomly scattered on the flavedo (the outer-most, pigmented part of citrus rind) of affected fruit (Cronje et al., 2011a). The sunken areas associated with this non-chilling related rind disorder are 0.1 to 0.7 mm in depth, occurring directly above and among the oil glands of the affected flavedo tissue. RBD is anatomically similar to other non-chilling rind physiological disorders, such as rind staining and breakdown of 'Navel' orange [*C. sinensis* L. (Osb.)] and mandarin (Agustí at el., 2001; Lafuente and Zacarìas, 2006), and noxan of 'Shamouti' orange (Ben-Yehoshua et al., 2001). In all these disorders, the

affected areas coalesce, producing larger affected areas. These affected areas turn reddish-brown to dark-brown, and become dry and necrotic in the severe stages of the disorder with extended storage periods (Alférez et al., 2003, 2004; Alférez and Burns, 2004; Cronje, 2005, 2009; Assimakopoulou et al., 2009).

One of the main difficulties related to RBD is its progressive nature. The disorder does not manifest during harvest or fruit grading in the packing house, but only starts developing symptoms during storage, about three to five weeks postharvest (van Rensburg and Bruwer, 2000; van Rensburg et al., 2004; Cronje et al., 2011a, b). The manifestation of RBD symptoms during the later stages of postharvest handling is in essence the main difficulty that faces the industry and importers alike, because if fruit are shipped over long distances, symptoms of the disorder usually coincide with the commercial shipping period and/or point of sale. This is extremely problematic as rind disorders can lead to tremendous financial losses and customer complaints. To date, knowledge of the biochemical changes occurring in the rind of citrus fruit that could be used to predict fruit rind condition, and therefore susceptibility to rind disorders, is limited. It is therefore crucial to identify potential biochemical markers of rind condition that are related to fruit susceptibility to RBD. The assessment of such biochemical markers and correlation with the disorder constitute the principal framework of this research towards understanding the mechanism(s) which influence RBD, which in turn, may lead to a pre-symptomatic detection and/or prediction of the disorder.

Previous studies have reported that different microclimatic conditions during the growing season and within a tree canopy may influence the biochemical profile of the rind and subsequently could play a significant role in fruit susceptibility to RBD (Cronje et al. 2011a, b). These authors further quantified aspects of different light levels within the canopy and their influence on rind concentration of carbohydrate and rind condition. The results showed that fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affected the rind concentration of carbohydrates and mineral elements during fruit development. The concentrations of the main carbohydrates (sucrose, glucose and fructose) in the rind of ‘Nules Clementine’ mandarin were lower on fruit located under low light conditions inside the canopy, compared to outside exposed fruit (Cronje et al., 2011b). It was therefore hypothesised that limited photosynthetic active radiation (PAR) on shaded portions of the canopy reduced the fruit photosynthesis rate and solute potential, which in turn reduced the accumulation of

carbohydrates, rind quality and ultimately increased fruit susceptibility to RBD (Cronje et al., 2011a, b). These studies also suggested that rind carbohydrates concentration could be used as potential biochemical indicator of fruit susceptibility to RBD.

Currently, the concentration of carbohydrates in the rind is measured on "representative" samples of a batch of fruit, using conventional and destructive techniques such as high performance liquid chromatography. Although destructive techniques are widely used, they are expensive, and require time-consuming and specialised sample preparation. The results of these tests only reflect the properties of the specific fruit evaluated. The high variability in quality attributes of fruit batches, coupled with industry demand for innovative tools for the detection, have spurred considerable interest among researchers to search for rapid and non-destructive tools for detection, prediction, segmentation, and monitoring of physiological rind disorders. As a result, the trend in postharvest research and industry has shifted towards developing reliable and cost effective technologies to non-destructively predict fruit physiological disorders (Zheng et al., 2010). Non-destructive, instrument-based methods are preferred because they allow the analysis of individual fruit, reduce waste, permit repeated measures on the same item over time, and increase sample size.

In order for the South African citrus industry to maintain its competitive edge and increase its market share in the lucrative export markets, there is a need to develop such non-destructive methods that can be used to monitor and predict citrus fruit susceptibility to physiological rind disorders such as RBD. A wide range of objective instruments for sensing and measuring the quality attributes of fresh produce has been reported. These include X-ray computed tomography (CT) imaging (Lammertyn et al., 2003a, b; Herremans et., 2013), magnetic resonance imaging (MRI) (Defraeye et al., 2013), visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS)-based imaging systems such as optical coherence microscopy (OCM) (Reeves et al., 2002; Kutis et al., 2005) and optical coherence tomography (OCT) (Sapozhnikova et al., 2003; Kutis et al., 2005; Meglinski et al., 2010). Among non-destructive quality assessment techniques, Vis/NIRS is arguably the most advanced with regard to instrumentation, applications, accessories, and chemometric software packages. As such, Vis/NIRS has become one of the most used candidates for non-destructive evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Miyamoto and Kitano, 1995; Ou et al., 1997; Peirs et al., 2003; Guthrie et al., 2005; Nicolaï et al., 2007; Bobelyn et al., 2010; Wedding et al., 2013). Most Vis/NIRS studies report

the application of the technique to assess fresh fruit according to their internal quality attributes (Nicolaï et al., 2007). In contrast, very limited research has been conducted to evaluate Vis/NIRS to predict and monitor the physiological disorders and rind physiological disorders of citrus fruit in particular. Vis/NIRS offers a potential for the non-invasive assessment of rind chemical composition of citrus fruit.

Currently, the microstructural changes associated with these disorders on citrus fruit are evaluated using conventional methods such as light microscopy and transmission electron microscopy (Chikaizumi, 2000; Vitor et al., 2000; Cajuste et al., 2011). These microscopic techniques require laborious and specialised destructive sample preparation. Monitoring the internal structural changes of intact fruit using such techniques is therefore difficult and sometimes impossible. NIRS-based imaging systems known as OCT is one such non-invasive and real-time analytical technique currently available to researchers and is suitable for examining internal structure of plant tissues.

2. Research aims and objectives

The overall aim of this PhD study was to develop Vis/NIRS-based non-destructive methods to predict external and internal quality of citrus fruit. To achieve this aim, the study included the following specific objectives:

1. Investigate the effects of preharvest factors (spatial location of fruit within the canopy and bagging treatments) to identify potential biochemical markers that can be analysed non-destructively to predict RBD.
2. Determine the optimum conditions of NIR measurements and evaluate the accuracy of this technique and associated optimised chemometric analysis to detect physico-chemical properties.
3. Explore the feasibility of diffuse reflectance Vis/NIR spectra collected at harvest to predict susceptibility of intact fruit to RBD, which showed symptoms about 3-8 weeks postharvest, by detecting rind physico-chemical properties of individual intact fruit from different canopy positions.
4. Investigate the feasibility of OCT for imaging histological changes associated with the development of RBD.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415–422.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Burns, J.K., Zacarìas, L., 2004. Postharvest peel pitting in citrus is induced by changes in relative humidity. Proc. Fla. State Hort. Soc. 117, 355–358.

Alférez, F.M., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Assimakopoulou, A., Tsougrianis, C., Elena, K., Fasseas, C., Karabourniotis, G., 2009. Pre-harvest rind-spotting in 'Clementine' mandarin. J. Plant Nutr. 32, 1486–1497.

Ben-Yehoshua, S., Peretz, J., Moran, R., Lavie, B., Kim, J.J., 2001. Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*Citrus sinensis*, Osbeck). Postharvest Biol. Technol. 22, 19–27.

Bobelyn, E., Serban, A., Nicu, M., Lammertyn, J., Nicolaï, B.M., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55,133–143.

Bonorchis, R., 2013. South Africa citrus fruit disease ban may cause dispute with EU. http://www.bloomberg.com/news/2013-04-11/south-africa-citrus-fruit-disease-ban-may-cause-dispute-with-eu.html. Last accessed 29 May 2013.

Cajuste, J.F., García-Breijo, F.J., Armiñana, R., Lafuente, M.T., 2011. Ultrastructural and histochemical analysis reveals ethylene-induced responses underlying reduced peel collapse in detached citrus fruit. Microsc. Res. Tech. 74, 970–979.

Chikaizumi, S., 2000. Mechanism of rind-oil spot development in 'Encore' (*Citrus nobilis* Lour. X *C. Deliciosa* Ten.) fruit. J. Japan. Soc. Hort. Sci. 69, 149–155.

Citrus Growers' Association of Southern Africa, 2012. Key industry statistics for citrus Growers 2012. http://www.cga.co.za/site/files/5438/CGA%20Stats%20Book%202012%20e(1).pdf. Last accessed 07 May 2013.

Cronje, P., 2005. Peteca spot of lemons. S. African Fruit J. Feb/March, 26–28.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Defraeye, T., Lehmann, V., Gross, D., Holatc, C., Herremans, E., Verboven, P., Verlinden, B.E., Nicolai, B.M., 2013. Application of MRI for tissue characterisation of 'Braeburn' apple. Postharvest Biol. Technol. 75, 96–105.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. Aust. J. Agric. Res. 56, 417–426.

Herremans, E., Verboven, P., Bongaers, E., Estrade, P., Verlinden, B.E., Weverse, M., Hertog, M.L.A.T.M., Nicolaï, B.M., 2013. Characterisation of 'Braeburn' browning disorder by means of X-ray micro-CT. Postharvest Biol. Technol. 75, 114–124.

Kader, A.A., Arpaia, M.L., 2002. Postharvest handling systems: Subtropical fruits. p. 375–384. In: A.A. Kader (ed.), Postharvest Technology of Horticultural Crops. Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA.

Kutis, I.S., Sapozhnikova, V.V., Kuranov, R.V., Kamenskii, V.A., 2005. Study of the morphological and functional state of higher plant tissues by optical coherence microscopy and optical coherence tomography. Rus. J. Plant Physiol. 52, 559–564.

Lafuente, M.T., Zacarìas, L., 2006. Postharvest physiological disorders in citrus fruit. Stewart Postharvest Rev. 2, 1–9.

Lammertyn, J., Dressalaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003a. MRI and X-ray CT study of spatial distribution of core breakdown in 'Conference' pears. Magn. Reson. Imag. 21, 805–815.

Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003b. Analysis of the time course of core breakdown in 'Conference' pears by means of MRI and X-ray CT. Postharvest Biol. Technol. 29, 19–28.

Meglinski, I.V., Buranachai, C., Terry, L.A., 2010. Plant photonics: Application of optical coherence tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3, 227–237.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I. K., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Ou, A.S., Lin, S., Lin, T., Wu, S., Tiarn, M., 1997. Studies on the determination of quality-related constituents in 'Ponkan' Mandarin by near infrared spectroscopy**.** J. Chinese Agric. Chem. Soc. 35**,** 462–474.

Peirs, A., Tirry, J., Verlinden, B., Darius, P., Nicolaï, B.M., 2003. Effect of biological variability on the robustness of NIR models for soluble solids content of apples. Postharvest Biol. Technol. 28, 269–280.

Reeves, A., Parsons, R.L., Hettinger, J.W., Medford, J.I., 2002. *In vivo* three-dimensional imaging of plants with optical coherence microscopy. J. Microsc. 208, 177–189.

Sapozhnikova, V.V., Kamenskii, V.A., Kuranov, R.V., 2003. Visualization of plant tissues by optical coherence tomography. Rus. J. Plant Physiol. 50, 282–286.

van Rensburg, P.J.J., Bruwer, M., 2000. Factors influencing rind breakdown of 'Clementine' mandarin fruit. Proc. Int. Soc. Citricul. 3, 1173.

van Rensburg, P.J.J., Cronje, P.J.R., Joubert, J., Gambetta, G., Bruwer, M., 2004. Factors influencing rind breakdown in citrus fruit. Proc. Int. Soc. Citricul. 2, 1051–1061.

Vitor, R.F., Lidon, M., Barreiro, M.D., Maia, M.I., Medeira, M.C., Guerreiro, A., 2000. 'Peel pitting' of 'Encore' mandarin fruits: etiology, control and implications in fruit quality. Fruits 56, 27–35.

Wedding, B.B., Wright, C., Grau, S., White, R.D., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biol. Technol. 75, 9–16.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort. 125, 401–405.

CHAPTER 2

LITERATURE REVIEW

PAPER 1: NON-CHILLING PHYSIOLOGICAL RIND DISORDERS IN CITRUS FRUIT*

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NON-CHILLING PHYSIOLOGICAL RIND DISORDERS IN CITRUS FRUIT

Abstract

Appearance is the primary parameter used to evaluate quality of citrus fruit for the fresh market; so the condition of fruit rind is an important quality attribute. Preventing the development of non-chilling physiological rind disorders such as rind breakdown, rind pitting, rind staining, puffiness, and peteca spots is one of the key challenges in postharvest handling of citrus fruit. Intensive research has been conducted towards better understanding of the factors contributing to the incidence of these disorders. This review examines the preharvest and postharvest factors contributing to the incidence and severity of non-chilling physiological rind disorders in citrus fruit, and presents a physical characterisation of these disorders. It also describes the molecular, biochemical and physiological basis of rind disorders and highlights the techniques for inducing rind disorders for research investigations. To date, the mechanism of citrus rind pitting disorders and the relationship with other physiological rind disorders is still not well understood. However, it has been established that fruit susceptibility to these disorders is influenced by the microclimate inside the fruit canopy. The prospects of using biochemical markers to predict the development of rind disorders at harvest and the use of non-destructive technologies to sort affected fruit are discussed.

Keywords: Rind breakdown · Rind pitting · Rind staining · Peteca · Physiological disorders · Postharvest physiology · Rind quality.

1. Introduction

Citrus fruit are the highest value fruit crop in international trade. Current annual worldwide citrus production is estimated at over 122 million tons, worth over 20 billion US dollars (Spreen, 2009; FAO, 2010). However, citrus fruit are prone to various types of physiological rind disorders, which manifest as a multitude of symptoms during handling and storage (Kader and Arpaia, 2002; Alquezar et al., 2010). The incidence of these disorders can affect up to 60% of total fruit production (Agustí et al., 2001). Physiological rind disorders include chilling injury, peteca spot, rind breakdown, non-chilling rind/peel pitting and rind staining, puffiness, creasing, oleocellosis, stem-end rind breakdown (SERB), and stylar-end breakdown (Giffilan et al., 1981; Gilfillan, 1990; Cronje, 2007).

Although these physiological disorders affect the rind and do not compromise the edible internal portion of the fruit, they do however decrease postharvest fruit market value since external appearance is the primary specification used to evaluate quality of fresh citrus fruit and a major reason for consumer complaint (Alquezar et al., 2010). Limited knowledge of the physiological mechanism underlying these disorders affects supply, profits and losses. The challenge is significant regarding citrus physiological rind disorders such as rind breakdown of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) (Cronje et al., 2011a, b) rind breakdown of 'Navel' orange [*C. sinensis* L. (Osb.)] (Agustí et al., 2001) and non-chilling postharvest rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Alférez and Burns, 2004; Alférez et al., 2005), which characteristically do not manifest during harvest grading, but develop about 1 to 5 weeks after harvest (Cronje et al., 2011a). If fruit are shipped over long distances, symptom development usually coincides with the commercial shipping period and is visible at the point of sale. This is therefore extremely problematic as rind disorders can lead to tremendous financial losses at this stage of the logistical supply chain.

This review examines citrus fruit rind quality with particular emphasis on the current knowledge of morphological and physiological aspects of non-chilling physiological rind disorders of citrus fruit. The authors will first emphasise the magnitude of the problem, followed by characterising each of these disorders by encompassing their visual symptomology, microstructural periphery of fruit affected by these disorders. This section will include a summarised tabular quick reference version for different physiological rind disorders. The next

extended section includes causal factors, both preharvest and postharvest, physiology and molecular biology of these disorders. Finally, the authors will discuss possible preventative or control measures and list future research avenues including the potential of non-destructive technologies to predict and determine fruit susceptibility to physiological rind disorders.

2. Citrus rind disorders

2.1. Terminology

Numerous rind disorders that are discussed in the literature can be classified as non-chilling physiological rind disorders of citrus fruit. The terminology used to refer to these physiological rind disorders depends mostly on symptomology, species, cultivar, region in which the research was conducted, etc. It is not well understood how the different rind disorders described in the literature in different citrus cultivars are related to each other.

In the literature, the collective term referring to the rind non-chilling physiological disorder is "non-chilling rind (or peel) pitting", which is sometimes used interchangeably with "peel pitting", although some authors distinguish between "rind pitting" and "rind breakdown" or necrosis (Petracek et al., 1998b; Ben-Yehoshua et al., 2001). For the reason that the different terminology relating to specific symptoms observed on a specific cultivar has been established in literature, in this review we will try as much as possible to stick to this common terminology and its definitions. "Rind" or "peel" both describe the same structure (flavedo and albedo). The use of either one follows the general use in the country a specific disorder is seen in, for example, peel pitting in Spain (Cajuste and Lafuente, 2007) and USA (Alférez et al., 2005), whereas rind breakdown is used in South Africa (van Rensburg et al., 2004) and Australia (Treeby et al., 1995). In general, the common name of a disorder is always associated with a cultivar, and this combination, as for example noxan of 'Shamouti' (Peretz et al., 2001), rind breakdown of 'Nules Clementine' mandarin (Fig. 1A) (Cronje et al., 2011a), or rind staining of 'Navel' (Fig. 2A, 2B and 3A) (Alférez et al., 2003), separates the disorder from similar ones and helps to identify associated causal factors.

2.2. Symptomology

Externally, symptoms of non-chilling physiological rind disorder may resemble the symptoms of postharvest chilling injury (Fig. 4A and 4B) (Freeman, 1976; Arpaia et al., 1991). Chilling injury is defined as "the permanent or irreversible physiological damage to plant cells, tissues, or organs, which results from exposure of plants to temperatures below some critical threshold, but non-freezing temperature, that causes injury" (Lyons and Breidenbach, 1987). Non-chilling rind disorders are distinguished from chilling injury in that the symptoms develop at non-chilling cold storage temperatures (Petracek et al., 1995; Cronje, 2007). Non-chilling rind disorders are also distinguished from chilling injury because most of these non-chilling physiological disorders affect both the albedo (the inner, white part of the peel) and the flavedo (the outer-most, pigmented part of citrus peel) tissues (Petracek et al., 1995; Alférez and Burns, 2004; Cronje, 2007) and that fruit may be predisposed to the disorder while still attached to the tree (Sala et al., 1992).

An overview of symptomology, time to occurrence and causal factors of different non-chilling physiological rind disorders reported on oranges, grapefruit, and mandarin fruit are respectively summarised in Tables 1, 2, and 3, with selected recent review publications in Table 4. The time of symptom development is thought to offer some suggestion as to the cause of a rind disorder. Therefore, it is highly possible that "peel pitting", "rind pitting", "rind staining" on 'Navel' oranges, "rind breakdown", "non-chilling peel pitting", "rind staining" of 'Navelate', 'Pinalate' and 'Navelina' oranges, "peel pitting" of 'Fallglo' and 'Fortune' mandarins, which all can develop within one week after harvest, refer to the same disorder. However, "Noxan" of 'Shamouti' orange, differs from rind pitting because of its superficial nature. Although the symptoms and the terminology are the same, "peel pitting" of 'Marsh' grapefruit differs from the rest of the listed disorders, in that it develops within two days after harvest. In addition, there have been several reports of fruit developing non-chilling "rind pitting", "peel pitting", "rind staining" and "Noxan" while still on the tree at non-chilling temperatures (Almela et al., 1992; Duarte and Guardiola, 1995; Vitor et al., 2000; Tamim et al., 2001; Agustí et al., 2001, 2003; Assimakopoulou et al., 2009). Rind breakdown of 'Nules Clementine' mandarin and SERB are differentiated appreciably from all these disorders by the fact that no pre-harvest symptoms have been previously reported (Cronje, 2009).

In general, rind postharvest physiological disorders, with the exception of SERB, are initially manifested on the equatorial plane as small, irregular, slightly sunken and colourless patches of about 3 to 6 mm in diameter, scattered about the flavedo of the fruit. Typical visual symptoms of rind breakdown of 'Nules Clementine' mandarin (Fig. 1A) (Cronje et al., 2011a), non-chilling postharvest pitting of 'Nova' mandarin (Fig. 1B) (Duarte and Guardiola, 1995), rind breakdown (Fig. 5A) and preharvest rind pitting of 'Navelate' sweet orange (Fig. 5B) (Agustí et al., 2001), rind staining of 'Navelate'(Fig. 2A), 'Navelina' (Fig. 2B) and 'Navel' oranges (Alférez et al., 2003) are portrayed. Chilling injury in 'Navel' orange and 'Satsuma' mandarin are respectively displayed in Fig. 4A and Fig. 4B.

The sunken areas associated with non-chilling rind disorders are 0.1 to 0.7 mm in depth (Petracek et al., 1995), occurring directly above and among the oil glands of the affected flavedo. The affected areas coalesce producing larger affected areas, turning reddish-brown to dark-brown, becoming dry and necrotic in the severe stages of the disorder with extended storage (Alférez et al., 2003; Alférez and Burns, 2004; Cronje, 2005, 2009; Assimakopoulou et al., 2009). Browning of affected rind surface of 'Navelate' oranges (Fig. 5B) appears to be the result of oxidative processes (Agustí et al., 2001). In contrast, SERB (Fig. 6) involves the collapse and darkening of the epidermal tissues around the stem end of the fruit and does not target oil glands as postharvest rind pitting (Grierson, 1986; Porat et al., 2004).

3. Rind anatomy and histological characteristics

A healthy citrus flavedo is characterised by an epidermis with an apolyhedral arrangement of cells, covered by a cuticle (Fig. 7A) (Albrigo, 1972a, b; Medeira et al., 1999). The flavedo consists of an external layer of epidermal cells and inner tightly packed parenchyma/collenchyma cells with no intercellular air spaces. Under cryo-scanning electron microscopy, flavedo cells gradually increase in size towards the inner parts of the rind (Alquezar et al., 2010). The albedo of the rind is a complex mesh of meristematic cells in which each single cell has direct plasmodesmata connections with eight other adjacent cells (Storey and Treeby, 1994; Alquezar et al., 2010). This structure results in large intercellular air spaces between the cells and provides the typical spongy morphology of albedo tissue. Intercellular spaces are bigger at the inner layers of the albedo near the pulp (Agustí et al., 2001).

In ‘Fortune’ mandarin, the damage caused by non-chilling rind pitting disorders is manifested by flattened and collapsed parenchyma cells immediately above the oil glands (Fig. 7B) (Vercher et al., 1994). Similarly, sub-epidermal cell orientation in affected areas of ‘Encore’ mandarin resembles the damage described in ‘Fortune’ mandarin as well as the postharvest chilling injury symptoms of lemons (Obenland et al., 1997; Medeira et al., 1999). Damaged cell layers were found to be orientated parallel to the rind surface, extending all along the flavedo (Vercher et al., 1994; Medeira et al., 1999). The deterioration processes were found to begin in epidermal cells, later extending to hypodermal cells. The first signs of cellular damage are associated with internal membrane disorganisation of the plastids, followed by vesiculation and degradation of the cytoplasm (Medeira et al., 1999).

Rind breakdown of ‘Navelate’ sweet orange fruit begins at the transitional zone of the flavedo-albedo and subsequently advances across the flavedo reaching the epidermis (Agustí et al., 2001). It was further substantiated by these authors that cells from fruit affected by the disorder had reduced amount of cytoplasm located in a central position and have twisted and squashed walls, forming areas of collapsed cells amongst healthy cells of the flavedo and albedo. Collectively, these observations suggest that citrus non-chilling rind pitting begins in the cell membrane structure and progresses to epidermal and subepidermal tissues, followed by flattening of several layers of surrounding cells, causing the collapse of these cells and oil glands.

Petracek et al. (1998) were of the opinion that oil glands are the primary sites of damage and that the rupture of oil bodies could, in turn, release oil into the surrounding cells, causing rind pitting injury to citrus fruit. However, the oil glands of ‘Fortune’ (Almela et al., 1992) and ‘Clementine’ (Assimakopoulou et al., 2009) mandarins affected by rind spotting remained intact and unaffected (Medeira et al., 1999). Similarly, no relationship has been shown between the oil gland and peel pitting disorder in ‘Encore mandarins (Medeira et al., 1999; Vitor et al., 1999, 2000). These observations suggest that rind spotting in ‘Fortune’ and ‘Clementine’ mandarins, as well as peel pitting injury in ‘Encore’ mandarin, might not originate from oil gland disruption. In contrast, the collapse of oil glands was the initial symptom of postharvest peel pitting in grapefruit (Alférez and Burns, 2004). In the advanced stages of the disorder, the collapsed oil glands became deformed, with layers of flavedo cells below oil glands and adjacent deeper areas appeared twisted and wrinkled (Fig. 8), concomitant with browning of the flavedo. Epidermal

and sub-epidermal cells appeared strongly stained and crushed, indicating a collapse in these tissues, and that some layers of enveloping cells of the oil glands had become flattened.

Vitor et al. (1999, 2000) investigated the interactions between hydroxyl radical production and the composition of the epicarp cells associated with pitted tissues, and observed that the phosphatidylinositol content of dark stained cells was lower in unpitted than in pitted tissues. The significant change of the membrane composition in pitted tissues is closely associated with acyl lipid peroxidation, mediated by hydroxyl radical production (Vitor et al., 2000). It was concluded that degradation of fatty acids in pitted tissues was incomplete. In addition, the disorder has been shown to be related to tonoplast disruption and unrepaired damage to cell membranes in the flavedo (Sawamura et al., 1984; Vitor et al., 2000). Tonoplast disruption in injured cells accelerated propagation of disorder to adjacent cells through oxidative product release preventing the normal function of organelles and membranes (Vitor et al., 2000).

4. Causes of physiological rind disorders of citrus fruit

Physiological rind disorders of citrus fruit are believed to be affected by various preharvest, harvest and postharvest conditions to which the fruit are exposed. Most fruit physiological disorders are theoretically associated with preharvest, ecophysiological and postharvest factors (Witney et al., 1990; Peiris et al., 1998a). Similar to most physiological disorders, some of the causes of non-chilling rind pitting disorders are genetical and cannot be changed (Davies and Albrigo, 1994; Agustí et al., 2003), while others are external and can be altered (Agustí et al., 2004; Alférez and Burns, 2004). Intensive research has been conducted in the past towards determining factors triggering rind pitting. For example, there was a correlation of preharvest and postharvest factors to non-chilling rind pitting physiological disorders (Agustí et al., 2001; Alférez et al., 2003). However, the mechanism(s) governing the occurrence of these non-chilling disorders and the manner it is related to other physiological rind disorders is still not well understood (Agustí et al., 2004). Since little is known about the exact causative factors, the occurrence of various non-chilling pitting disorders in citrus fruit appears to be erratic and unpredictable, showing high variability from year to year, among orchards, cultivar, harvest dates, canopy microclimate, postharvest handling, length of storage, and even among fruit of a given tree (Alquezar et al., 2010; Cronje et al., 2011a).

4.1. Pre-harvest factors

Most of the earlier experiments on rind disorders focused on the effect of postharvest factors, such as ethylene treatments and wax applications (Alférez and Zacarìas, 2001; Cronje, 2005). Recently, preharvest aspects influencing rind condition have received attention due to the fact that a number of fruit disorders do not always require specific conditions to be expressed but are closely related with later stages of fruit ripening (Ferguson et al., 1999). The great number of references that relate fruit physiological disorders with climatic conditions, indicate that variations in environmental conditions during the growing season and within orchard variation, including canopy microclimate may influence the susceptibility of fruit to physiological disorders (Ferguson and Watkins, 1999; Assimakopoulou et al., 2009).

4.1.1. Scion cultivar and rootstock

The sensitivity of a fruit to develop a physiological rind disorder varies among different cultivars. Although these disorders occur on most citrus fruit, certain cultivars are more susceptible to these disorders than others. For instance, 'Navelina', 'Washington' navel, 'Shamouti' orange and 'Lane late' are among orange cultivars that have been listed to be highly sensitive to non-chilling rind pitting disorders (Table 1) (Agustí et al., 2003, 2004; Alférez et al., 2003; Estables-Ortiz et al., 2009). Fruit of 'Navelate' oranges are sensitive to peel pitting on the tree as well as under postharvest storage. 'Navelina' oranges on the other hand only develop the disorder during postharvest storage (Lafuente and Sala, 2002). 'Marsh' grapefruit (Table 2) and 'Fallglo' tangerine are also among citrus fruit liable to suffer from non-chilling rind pitting (Petracek et al., 1995, 1998), while 'Shamouti' orange and 'Nules Clementine' mandarin are respectively sensitive to superficial flavedo necrosis "noxan" and rind breakdown (Ben-Yehoshua et al., 2001; Cronje et al., 2011a). It is of interest to note that some mutants of cultivars, which are prone to one rind physiological disorder, are tolerant to another. For instance, 'Nules Clementine' mandarin is susceptible to progressive postharvest rind breakdown, whereas the 'Oroval Clementine' mandarin produced in the same production unit is not susceptible to rind breakdown (Table 3) (van Rensburg and Bruwer, 2000; van Rensburg et al.,

2004; Khumalo, 2006). Furthermore, it has been shown that 'Pinalate' orange fruit are tolerant to chilling injury, but very prone to develop non-chilling peel pitting, in contrast to 'Navelate' orange fruit, which are prone to chilling injury. This suggests that the sensitivity of fruit to a particular type of physiological rind disorder is genetically inherited and 'Navelate' orange fruit might have an additional defence mechanism, which assists in reducing non-chilling peel pitting. Furthermore, this mutant may be used as a model to understand the mechanisms underlying chilling-induced superficial scald and non-chilling peel pitting disorders.

In addition to the scion effect, rootstock has been reported to play a role in fruit sensitivity to disorders (Zacarìas et al., 2000). Rootstocks may influence water balance, water circulation, mineral nutrition, plant growth regulators, growth rate of the fruit and the whole plant (Alférez et al., 2010) thus affecting water and osmotic potentials in the fruit rind (Albrigo, 1977). Rootstocks may also modulate the susceptibility of fruit to develop non-chilling peel pitting on the tree and during postharvest storage (Treeby et al., 1995; Alférez et al., 2010). In cultivars such as 'Navelate' orange, rootstock has been shown to influence both incidence and severity of fruit peel pitting. Fruit harvested from trees grafted on 'Carrizo Citrange' are more susceptible to postharvest rind pitting (Zacarìas et al., 2000). This phenomenon is not unique to orange fruit, as 'Marsh' grapefruit grafted on 'Carrizo citrange' rootstock had higher susceptibility to develop rind disorders compared to those grafted on 'Cleopatra' mandarin or sour orange (Agustí et al., 2003; 2004; Ritenour et al., 2004). The flavedo and albedo of fruit harvested from trees grafted on 'Carrizo citrange' rootstock have been shown to undergo more pronounced decreases in water potential under water stress conditions and had better ability to recover when transferred to a high relative humidity (RH) environment (Zacarìas et al., 2000), suggesting that more rapid water movement through peel tissues may be linked to peel pitting (Alférez et al., 2010).

4.1.2. Canopy position

A number of research groups have established that microclimate can influence the sensitivity of fruit to physiological rind disorders (El-Otmani et al., 1989; Arpaia et al., 1991; Wild, 1991; Almela et al., 1992; Duarte and Guardiola, 1995). They demonstrated that the susceptibility of fruit to different rind physiological disorders varies according to seasonal climatic and orchard environmental conditions. In a study conducted in Spain (Northern Hemisphere), Almela et al.

(1992) showed that these fluctuations could also be observed among fruits from the same tree; the incidence of rind spots in 'Fortune' mandarins was higher in fruit exposed to the sun than non-exposed fruits, and higher on sun-exposed than the non-exposed side of individual fruits. These investigators further showed that fruit oriented to the North-West (NW) position in the canopy were most affected by the disorder. In a later study conducted in the same country, Agustí et al. (2001) corroborated that sun-exposed 'Navel' fruit on the NW side of the tree were more prone to developing rind breakdown disorder and this was reported to be consistent over five seasons. Similar observations were previously made in 'Fortune' mandarin fruit, where it was maintained that rind pitting disorder was higher in the exposed fruit from the NW quadrant of the tree, and was higher on fruit exposed to the sun than non-exposed (covered by foliage) fruit (Duarte and Guardiola, 1995). Similarly, Vitor et al. (2000) showed that shaded 'Encore' mandarin fruit had significantly lower peel pitting compared to sun-exposed fruit. Chikaizumi (2000) further substantiated that the sun-exposed side had higher incidence of pitting than the shaded side of the same individual fruit. The mechanism defining the trend of lower rind physiological disorder on fruit borne on different canopy positions is related to temperature, water potential and irradiation as discussed in the next two subsections (4.1.2.1) and (4.1.2.2).

In a study conducted in Australia (Southern Hemisphere), Wild (1991) reported a similar observation with peteca of lemon (Fig. 3B) (*C. limon* Burn), a disorder similar to rind pitting, where fruit hanging on the eastern side of trees were found to be more susceptible than those on the west side. Moreover, the disorder has been reported to be more prevalent on the exposed side of individual fruit, irrespective of location on the tree. A study conducted in Portugal (Northern Hemisphere), showed mandarin fruit from the South and South-western areas of the canopy to be severely affected by non-chilling rind pitting (Meidera et al., 1999). The authors speculated that solar radiation may be involved in the structural alteration of the cuticle, and a high rind temperature over a long period may induce localised dehydration in the epidermal and sub-epidermal cells leading to plasmolysis and membrane collapse.

The positional effect on development of rind disorder is not unique to non-chilling rind pitting. It has also been demonstrated to affect chilling injury (McDonald et al., 2000). These investigators conducted a study to determine the effect of canopy position on the sensitivity of grapefruit to gamma irradiation and if a short-term heat treatment before irradiation would reduce the severity and incidence of rind pitting and chilling injury. They reported more rind

pitting in exterior canopy fruit than in interior canopy fruit. These results agree with earlier observations reported by McDonald et al. (1993), whereby grapefruit from the sun-exposed exterior canopy were more susceptible to chilling injury than those from the shaded interior canopy. These studies further showed that the development of chilling injury symptoms on exterior canopy grapefruit was related to epicuticular wax morphology and composition. The exposed side of a fruit reached higher temperatures than the shaded side (Sylvertsen and Albrigo, 1980) and has been reported to age faster (El-Otmani et al., 1989). Therefore, the higher susceptibility of the exposed side to non-chilling injury may be the consequence of accelerated senescence.

4.1.2.1. Direct solar radiation and temperature effect

The great number of references reporting non-chilling physiological rind disorder indicates that visual symptoms of non-chilling physiological rind disorders mostly appear in the portions of the rind most exposed to solar radiation (Wild, 1991; Meideira et al., 1999; Chikaizumi, 2000). From the listed observations, it would seem logical to assume that preharvest conditions such as high temperature and radiation, may induce localised flavedo dehydration, plasmolisis and cell collapse, resulting in rind pitting of citrus fruit (Meideira et al., 1999). Direct radiation could result in temperatures exceeding 38 °C on exposed fruit (Chikaizumi, 2000). High light intensity induces oxidative stress in the epicarp which is characterised by an increased peroxidation and degradation of cell membranes (Vitor et al., 2000). An accumulation of H_2O_2 radicals was demonstrated and the peroxidase system that shields the epicarp against photo-oxidative stress had an inhibited catalase activity and increased superoxide dismutase functioning (Poiroux-Gordon et al., 2013). McDonald et al. (2000) demonstrated that radiation induced a transient increase in phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) activity and was correlated with fruit peel pitting. This could be related to stress related synthesis of phenolic compounds (McDonald et al., 2000).

Maia et al. (2004) showed that rindstain of ‘Encore’ mandarin started after summer high temperatures and when the relative humidity increased. With regard to effect of high temperature on rind pitting, results by McDonald et al. (2000) showed that pitting was reduced by a postharvest heat treatment. This is difficult to explain as fruit exposed to higher preharvest

temperatures had a higher incidence of the disorder while the postharvest heat treatment at the same temperature had a lower incidence of rind pitting. Considering this, one would expect fruit treated with postharvest heat treatment to a have higher incidence of the disorder. Although there are limited reports of physico-chemical differences between exterior and interior canopy of citrus fruit (McDonald et al., 1993, 2000), it is apparent that canopy position exerts a very strong influence on the biochemical makeup of the flavedo and the whole fruit. These observations suggest that variations in microclimatic conditions during the growing season and within an orchard influence the biochemical profile of the rind and subsequently play a role in the development of non-chilling disorders than postharvest factors.

4.1.2.2. Effect of canopy position on carbohydrates content in the flavedo

Fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affects sugar concentration in the flavedo uring fruit development (Cronje et al., 2011a). The flavedo from fruit borne on the outside of the canopy had significantly higher sucrose, glucose and fructose content concentration than the fruit borne inside the canopy. Lower osmotic potential due to the higher sucrose, an osmoregulatory compound in plant cells, was also observed, demonstrating a possible link between fruit position, rind sugar concentration and ultimately the development of the disorder (Cronje et al., 2011a). The incidence of rind breakdown in 'Nules Clementine' mandarin was higher on inside fruit compared with the outside fruit and this was consistent from season to season and could be attributed to their exclusion from adequate sunlight during development (Cronje, 2009). In addition, fruit borne inside the canopy had lower chlorophyll and carotenoid contents, and therefore poorer rind colour (Khumalo, 2006), and lower carbohydrates, which were correlated to their susceptibility to the disorder (Cronje, 2009). Although fruit canopy position has been consistently reported to significantly affect rind pigment and antioxidant capacity at harvest, earlier results by Khumalo (2006) showed that the development of rind breakdown after storage was not affected by fruit canopy position. To the knowledge of the authors, the work by Cronje et al. (2011a) is the only work conducted thus far, which has attempted to relate the effect of fruit position on rind biochemical composition. It was hypothesised that rind photosynthesis contributes significantly to rind condition. A reduction in photosynthetically active radiation on shaded portions of the

canopy reduced fruit photosynthesis rate and osmotic potential, which are thought to contribute to rind breakdown. Although very limited research has been conducted to study the effect of canopy position on the rind biochemical profile, the effect on fruit internal quality is well documented in the literature (Genizi and Cohen, 1988; Morales et al., 2000; Barry, 2000; Vitor et al., 2000; Barry et al., 2003, 2004).

4.1.3. Fruit maturity

Fruit maturity is believed to play a critical role in the development of citrus postharvest rind disorders (Wild, 1991). Fruit susceptibility to rind pitting is mostly at the beginning of colour break and develops with pigmentation until harvest (Assimakopoulou et al., 2009). Green fruit are not susceptible to rind pitting disorder (Duarte and Guardiola, 1995). Almela et al. (1992) studied the occurrence of rind breakdown in 'Fortune' mandarins and noted that fruit were highly sensitive to this disorder during maturation. On hanging fruit, the occurrence of rind pitting in citrus fruit is reported to extend from the onset of colour break and continue for several weeks thereafter, depending on climatic conditions (Agustí et al., 2001). During postharvest storage, the incidence of the disorder takes place irrespective of harvest time but increases quantitatively with fruit maturity (Alférez and Zacarìas, 2001). Furthermore, a positive correlation between maturity and peteca spot on lemons has been reported, with fruit harvested with yellow rinds developing more rind pitting than green fruit (Duarte and Guardiola, 1995; Undurraga et al., 2006).

Application of GA_3 at colour-break delayed chlorophyll loss and the accumulation of carotenoid pigments in rind tissue, and retard the development of rind pitting (Coggins, 1981, 1989; Agustí et al., 1981; Duarte and Guardiola, 1995). The primary quality benefit from using GA_3 is a reduction in preharvest and postharvest rind disorders due to delayed rind senescence (Duarte and Guardiola, 1995). On the other hand, results by Gambitta et al. (2011) suggested that gibberellins (GA1 and GA4) are involved in regulating sugar (sucrose) translocation and ABA accumulation in the flavedo of citrus fruit in relation with colour development, but not in regulating ethylene production. A positive correlation between sugar concentration in the flavedo and peel colour has been previously reported (Huff 1983; Holland et al. 1999; Cronje et al. 2011a). From these observations it could be hypothesised that ABA levels in the flavedo may be used as an indicator of the fruit susceptibility to postharvest rind physiological disorders. In

considering these observations, it could be possible that the disorder is dependent upon fruit maturity and/or an increase in flavedo pigments such as carotenoids. The above observations consistently support hypotheses that sun-exposed warmer fruit mature faster than shaded fruit (Barry et al., 2003) and pitting susceptibility develops with maturity and rind pigmentation (Cronje, 2009).

4.1.4. Fruit mineral nutrition

Mineral elements fill a vital role in plant growth and development. They are involved in synthesis of metabolites, heredity, energy processes and are essential components of plant constituents, taking part in enzyme activation, osmotic regulation and membrane permeability (Clarkson and Hanson, 1980; Mengel and Kirby, 1982; Devlin and Witham, 1983; Tisdale et al., 1985). Similar to other plants, citrus trees require at least thirteen mineral elements, the so-called 'essential elements', for normal growth and reproduction (Joiner et al., 1983; Marschner, 1995). Resnizky and Sive (1993) verified that various physiological disorders arising from the extension of the storage life of a fruit are related to the mineral content present before harvest. For the purposes of this review, only those nutrients known to play a key role in fruit quality such as nitrogen, calcium, potassium, phosphorus, magnesium, and boron will be discussed.

The best documented relationship between the fruit mineral content and fruit disorders concerns Ca. Inter-elemental relationships also exist between minerals such as Mg, K, B, Ca, and N. Ca is known to have a considerable influence on fruit physiology, and the effect that this element has on important aspects of fruit quality has been of particular interest for many years. Due to its role in cell wall structure and membrane function, calcium has been implicated in physiological disorders of many fruits (Poovaiah et al., 1988). Low Ca levels have been directly coupled with physiological disorders such as bitter pit, internal breakdown and lenticel discolouration in apples (Snay and Bramlage, 1973; Wills, et al. 2004), blossom-end rot in pepper (Ehert and Ho, 1986; Morley et al., 1993; Li et al., 2004) and tomatoes (Bangerth, 1979), cuticle cracking in cherries (Seske, 1995) and sweet pepper (Opara et al., 1997; Aloni et al., 1998), as well as vascular and pulp browning in avocado (Bower and Cutting, 1988; Thorp et al., 1997).

In lemons, Ca and B were identified by Khalidy et al. (1969) to play a role in the development of the peteca disorder. Cronje et al. (2011b) studied the possible relationship between the incidence of citrus rind disorders and the mineral composition of fruit. Fruit mineral composition was one of the important factors playing a role in postharvest rind pitting. The mineral composition of citrus fruit was found to vary in relation to position in the tree canopy. Inside fruit were found to have a significantly higher concentration of potassium than outside fruit. Outside fruit were found to contain higher levels of calcium and magnesium. It was interesting to note that visual symptoms of rind breakdown disorder appeared on the fruit from the shaded position of the canopy (Cronje et al., 2011b). Similar observations were reported in an industry experimental report by Kruger et al. (2005). Compared to disorders such as rind staining in 'Fortune' mandarins and rind breakdown in 'Navel' oranges where peel disorders were associated with sun/heat exposure, RBD in 'Nules Clementine' occurred on inside fruit because RBD is associated with low mineral and carbohydrate allocation and therefore premature senescence. Cronje et al. (2011b) concluded that the reduction of transpiration potential by lower temperatures and higher humidity inside the canopy could be responsible for the reduced accumulation of Ca and Mg. The high K concentration of inside fruit flavedo is suggested to be a stress response, due to the low light levels, to maintain osmotic potential in the shaded rind tissue. This imbalance could possibly lead to a reduction in rind condition, which manifests as rind breakdown development.

It is well known that Ca plays an important role in the healthy development of citrus fruit because of its structural role in the cell wall. Storey et al. (2002) revealed that the concentration of Ca in the flavedo and albedo was negatively correlated with the incidence of creasing. Similarly, Storey and Treeby (2002) as well as Treeby and Storey (2002) reported the effectiveness of Ca sprays as a control measure for rind breakdown of 'Navel' orange fruit. These observations were also reported by Li et al. (2009) in 'Navel' oranges, where fruit sprayed with 0.5% $Ca(NO_3)_2$, preharvest, developed significantly lower (50% less) rind pitting compared to untreated fruit. Treating fruit with 1% $CaCl_2$ effectively decreased the development of the disorder. The ability of Ca treatment in reducing the disorder was related to its ability to reduce the electrical conductivity of the cells and reducing the activities of enzymes such as polyphenol oxidase (PPO) and peroxidase (POD; EC 1.11.1.7) (Cajuste and Lafuente 2007). On the other hand, no significant differences in the rind concentration of mineral elements between rind

pitting affected and non-affected 'Clementine' mandarin fruit (Assimakopoulou et al., 2009). Considering that results by the latter authors and the fact that the cracks appeared on the cuticle, strata and oil glands remained intact (Assimakopoulou et al., 2009), the physiological disorder should be attributed to sudden changes of microclimatic conditions and not to nutritional imbalance.

4.1.5. Rainfall and fruit water potential

The amount and distribution of annual rainfall have been reported to play a role in the development of physiological rind disorders. Undurraga et al. (2006) reported that delaying harvest by 3 days after rainfall reduced the incidence of peteca spot in lemons. However, preharvest water stress induced by blocking irrigation and rainfall for 49 days before harvest increased peel breakdown (Ritenour et al., 2008). The effect of water potential on postharvest non-chilling rind pitting disorders has been confirmed in citrus fruit by showing that alteration of rind water potential, particularly water stress, of citrus may be a triggering factor related to rind pitting. Water potential and its components (osmotic and turgor potentials) of citrus fruit provides information on the water status of fruit tissues during postharvest storage and in response to variation in storage conditions (Alférez et al., 2010). In 'Navelate' and 'Navelina' oranges in Spain, sudden changes from low to high relative humidity during postharvest storage induced peel pitting disorder by lowering flavedo water potential (Alférez et al., 2003). Peel water potential of 'Marsh' grapefruit stored at 45% RH for 4 weeks was lower and lead to cell collapse and peel damage but transferring dehydrated fruit to 95% RH after 12 days increased water potential of the flavedo and albedo (Alférez et al., 2010; Alquezar et al., 2010). These observations link the alteration in water, osmotic and turgor potential with the induction of peel pitting in citrus fruit. Therefore the inability to control RH can lead to postharvest peel pitting in 'Marsh' grapefruit and possibly other citrus fruit.

5. Postharvest factors

5.1. Water loss

Citrus fruit are characterised by high postharvest mass loss, and this is essentially due to water loss by transpiration, as it accounts for 90% of total mass loss (Ben-Yehoshua et al., 2001). A percentage water loss rate of 1% per day, during the first 6 days after harvest, and 0.6% thereafter, was reported on 'Marsh' grapefruit stored at 21 °C and 30% RH (Alférez and Burns, 2004). Similarly, Alférez et al. (2010) reported water loss between 9 and 15% on fruit stored for six weeks at 45% RH. Water loss from the fruit is a passive process, resulting from a vapour pressure gradient prevailing between the fruit peel, which is close to saturation with water, and the less saturated outside atmosphere (Kader et al., 1989; Ben-Yehoshua et al., 1994, Macnish et al., 1997). Postharvest water loss has negative implications for the fruit quality, since water is a finite resource (Turner, 1997). Initial symptoms of excessive water loss in a fruit is shrivelling, which is immediately visible on the peel, and affected fruit loses its shine, softens and senesces. For instance, in lemon, a 5% mass loss results in severe shrinkage rate, followed by development of SERB (McCornack, 1975). Water loss is also important in the postharvest softening of fruit such as lemon (Ben-Yehoshua et al., 1983) and oranges (Ben-Yehoshua, 1969). Minimising water loss helps to maintain turgidity and prevents collapse of hypodermal cells and formation of noxan disorder in 'Shamouti' oranges (Peretz et al., 2001). In 'Valencia' oranges, Albrigo (1972a, b) reported that the total concentration of wax on the surface of fruit is inversely correlated to postharvest mass loss.

Several studies have been conducted to identify potential postharvest causes of the rind pitting disorders (e.g. Arpaia, 1994; Petracek et al., 1995, 1998). Change in water status of flavedo and albedo is a primary postharvest factor in the susceptibility of citrus to rind pitting at non-chilling temperatures (Alférez et al., 2010). Evidence in the literature indicates that fruit exposed to low RH after storage at high RH are prone to develop rind pitting (Agustí et al., 2001; Alférez et al., 2003, 2005). Cohen et al. (1994) suggested that low humidity storage may be responsible for citrus rind pitting.

Alquezar et al. (2010) examined morphological and structural changes, and their relationship to water potential, in flavedo and albedo of 'Navelate' fruit exposed to postharvest storage conditions inducing rind breakdown. After one month, fruit stored at high (95%) RH had no signs of cell disruption or collapse of epidermal or flavedo cells. However, albedo cells of fruit stored for the same period at lower (45%) RH were dehydrated and compact, forming clusters of

amorphous and flattened cells in which the typical spongy structure of the albedo was lost, showing typical symptoms of peel pitting.

It was further noted that transfer of fruit to high RH after a week of storage at lower RH produced a marked increase in the incidence of rind pitting in 'Navelate' oranges (Alquezar et al. 2010). These observations were in accord with those previously reported by Alférez et al. (2003) and Alférez and Burns (2004) and further confirm that changes in postharvest RH and consequent rind water potential are important factors inducing rind pitting in citrus fruit. Higher incidence of rind pitting in fruit stored at high RH compared to those stored at lower RH has been previously reported by Lafuente and Sala (2002). Sequentially, this phenomenon was also reported by Alférez et al. (2003) in 'Navelina' and 'Navelate' oranges, Alférez and Burns (2004) in 'Marsh' grapefruit, Alférez et al. (2005) in 'Fallglo' tangerines, Henriod (2006) in 'Navel' oranges and Alférez et al. (2010) in 'Marsh' grapefruit.

All the above evidence suggests that alterations of postharvest RH and, in particular, the shift of water stressed fruit to high RH, improves water potential within rind tissues by reducing the water vapour pressure deficit (VPD), which is somehow detrimental to cell integrity (Syvertsen and Albrigo, 1980). Treating fruit in the reverse order, from high to low RH, or at low or high RH alone, did not result in the same incidence of the disorder (Burns et al., 2007). Alférez et al. (2005) conducted a study to determine the minimum time of dehydration prior to storage at high RH, sufficient to induce rind pitting in 'Marsh' grapefruit and 'Fallglo' tangerines. Alférez et al. (2004) demonstrated that three hours of low RH storage was sufficient to induce rind pitting after transfer to high RH. This implies that fruit harvested during or after being exposed to weather conditions promoting rind dehydration, are very susceptible to develop the disorder when they undergo the industry's degreening practice that requires fruit to be held at 90-95% RH. However, it is worth noting that while on the tree, the fruit has a soil water supply and may not undergo sufficient peel dehydration to lead to this problem. Earlier work in Florida on SERB clearly showed that hot, drying conditions in the field could lead to rind breakdown in 'Navel' oranges (McCornack, 1970). Long adverse water and temperature conditions under Florida conditions also lead to a peel breakdown in 'Valencia' oranges while still hanging on tree (Albrigo, 1972b).

Alquezar et al. (2010) investigated water potential of the albedo and flavedo before and after storage and reported a 30% decrease in water potential of flavedo while that of albedo remained almost unaltered. The vascular system lies beneath the albedo, with no connection to the pulp or

flavedo, thus postharvest water loss is mainly from the rind (Bain, 1958; Ben-Yehoshua, 1987). Therefore, Alquezar et al. (2010) suggested that the flavedo is more sensitive to water stress than albedo. This suggestion concurs with the statement by Ben-Yehoshua (1969) that most water lost to the atmosphere in the initial period of storage originates from the flavedo. However, the results by Agustí et al. (2001, 2004) suggested that excessive water loss from hypodermal, flavedo and albedo cells is responsible for the disorder. Water loss is probably from both the flavedo and albedo, but greatest stress may be on the flavedo since most of the anatomical research shows that tissue damage is near surface (Agustí et al., 2001; Alquezar et al., 2010).

A study on 'Marsh' grapefruit showed a significant positive correlation between rind pitting index and percent cumulative mass loss prior to postharvest storage at high relative humidity, although a very low correlation coefficient (0.54) was obtained (Alférez and Burns, 2004). Estables-Ortiz et al. (2009) also argued that although the severity of the disorder may be enhanced by dehydration-rehydration, the disorder also occurs naturally in non-stressed fruit held continuously at 22 °C and high RH (90-95%). This observation and low correlation coefficient between the rind pitting index (RPI) and mass loss obtained by Alférez and Burns (2004) suggested that development of the disorder might be attributed to other factors in addition to change in RH during postharvest storage. It might also be argued that these fruit might have been held for an extended period under low RH conditions before they were moved to high RH. However, VPD is more important to water loss than RH (El-Otmani et al., 2011).

5.2. Postharvest wax application

Citrus fruit, with the exception of those marketed as organic fruit, are usually waxed to improve appearance, and reduce water loss and the wax serves as a medium to apply 2,4-dichlorophenoxyacetic acid (2,4-D) and fungicides such as thiabendazole (TBZ), and sodium o-phenyl phenate (SOPP), thus, extending shelf life (Alférez et al., 2010). Although beneficial, coating fruit with commercial waxes coupled with warm temperature (20°C) storage has been reported to promote peteca spots on lemons (Wild, 1991). Commercial packing line treatments of fruit may alter susceptibility to rind disorder by altering the natural waxes on the fruit surface. A study by Albrigo (1972a) showed that natural wax is brushed down, reorganised, but not removed by normal commercial washing and brushing. This removal and re-organisation of

natural waxes on the fruit surface is thought to alter water and osmotic potential, consequently reducing turgor pressure potential of 'Marsh' grapefruit (Alférez et al., 2010). For fruit stored at 45% RH for 30 days, the percentage water loss was higher (15%) in packing line-processed fruit than in those hand-washed and waxed (shellac-based wax) which had 9% water loss. In a previous study, the effect of water potential on rind pitting was greater on fruit exposed to prolonged periods of dehydration at lower relative humidity (Alférez and Burns, 2004). These authors concluded that waxing fruit enhanced severity of the disorder, only if there was a previous dehydration period.

In 'Marsh' grapefruit, 'Fallglo' tangerine and 'Temple' oranges waxing has been reported to stimulate postharvest rind pitting and the hypothesis was that the disorder results from decreasing O_2 and increasing CO_2 internal levels (Petracek et al., 1995, 1997, 1998b). Later results showed that shellac-based waxes resulted in higher rind pitting disorder, while fruit coated with carnauba- or polyethylene-based wax developed low incidence and non-waxed fruit did not develop the disorder (Petracek et al., 1998a). Internal O_2 levels were lower (1.8–3.5%) on fruit coated with shellac-based waxes, higher (10%) for carnauba- and polyethylene-based waxes and highest (19%) for non-waxed fruit. Conversely, internal CO_2 levels were high for fruit coated with shellac-based waxes (7.8-8.3%), lower for fruit coated with carnauba wax (4.9%) and polyethylene-based wax (5.8%), and lowest for non-waxed fruit (1.5%). Another finding supporting this idea, showed that waxing the fruit on its own is not detrimental for the development of peel pitting, but alteration in RH of waxed fruit may be deleterious (Alférez and Zacarìas, 2001). In a later study, 'Marsh' grapefruit under controlled atmosphere storage (5% CO_2 and 14% O_2) did not develop rind pitting if there was no previous dehydration (Alférez and Burns, 2004). It is noteworthy that Petracek's group often observed pitting developing at O_2 levels below 9%, perhaps, 14% O_2 used by Alférez and Burns (2004) was not low enough to cause pitting. In 'Blanco' mandarins, Bajwa and Anjum (2006) reported that polyethylene-based and shellac-based coatings resulted in poor water distribution in the rind and exacerbated rind staining symptoms compared to non-waxed fruit. As a result, components contained within commercial wax formulations were implicated in causing the disorder, but no work has been conducted to study morphology of commercially applied waxes. However, a comparison of natural wax morphology between healthy and damaged areas of affected 'Navelate' orange fruit,

conducted by Agustí et al. (2001), revealed no significant differences in wax morphology, cuticular thickness or permeability.

These observations supported the idea that postharvest rind pitting was a result of a previous dehydration event and commercial wax exacerbates the disorder because wax affects fruit water potential. The above arguments collectively suggest that rind pitting is a collapse of sub-epidermal cells that does not affect the morphology and water permeability of the cuticle. Exposure of fruit to low RH conditions followed by high RH storage, and an unfavourable internal CO_2/O_2 ratio, increases the risk of rind pitting. It could therefore be concluded that commercial waxes that alter water potential and levels of internal gases of the fruit, can enhance non-chilling rind pitting, if fruit underwent a previous water stress event.

5.3. Ethylene

Ethylene has been reported to play a protective role against stress conditions causing postharvest loses in citrus fruit (Porat, et al., 1999). Conditioning 'Navelate' fruit with ethylene reduced the incidence of rind pitting (Cajuste and Lafuente, 2007). Ethylene increased the epicuticular wax content of 'Navelate' oranges and showed that ethylene induces changes in surface wax morphology, thus might be related the formation of new waxes (El-Otmani and Coggins, 1985; Cajuste et al., 2010).

6. Molecular and physiological basis of physiological rind disorders

To obtain a general picture of the nature of the main postharvest disorders in citrus, it is necessary to delineate the precise function of the identified physiological and molecular disorder-associated response (Lafuente and Zacarìas, 2006). In recent years, researchers have turned their focus to study the molecular mechanisms underlying rind physiological disorders of citrus fruit. The classical example of a study conducted to establish the correlation between rind pitting and molecular characteristics of the rind was published by Sanchez-Ballesta et al. (2001). These researchers investigated the expression of cDNAs by screening the cDNA library from non-chilling pitting sensitive citrus cultivars ('Navelate' and 'Fortune') and non-chilling pitting tolerant, 'Pinalate' fruit. They further isolated a full-length cDNA, named 3c1, which was

differentially expressed by different postharvest stress conditions. A small region consisting of 35 residues in the 3c1 protein had significant similarity to several proteins known or hypothesised to use an acyl-CoA substrate.

The level of the CrglcQ gene, encoding an acidic class III β-1,3 glucanase isolated from citrus flavedo, increases transiently in fruit exposed to postharvest conditions favouring both chilling injury and non-chilling rind pitting disorders Sanchez-Ballesta et al. (2006, 2008). This increase paralleled those of ethylene production and the appearance of visible symptoms of damage. Thus, although they are fruit-specific, changes in 3c1 and CrglcQ gene expression may serve as biochemical markers for the development of postharvest chilling injury and non-chilling rind pitting disorders. In spite of the disorders being induced by different environmental conditions, different patterns of gene expression in different disorders indicate that patterns may still be used as good molecular markers for the susceptibility of citrus fruit to develop pitting in the flavedo under different postharvest conditions (Sanchez-Ballesta et al. 2008).

In 'Navel' oranges, Gao et al. (2006) and Li et al. (2009) identified differentially expressed genes and showed that genes preferentially expressed in stressed (wounded) fruit had significant similarity to know genes. For instance, the sequence analysis revealed a full length cDNA of one of the isolated clones had significant similarities to a Ca^{2+} binding protein, named CsCAB. Other isolated sequences were similar to cysteine protease, named *CsCP* and to an *NAC* domain protein known as CsNAC. Gao et al. (2009), further investigated the expression of expasin-like gene, named CsEXP. Quantitative analysis performed to determine the expression pattern of these cDNAs during the wounding revealed these genes to be involved in the process of rind pitting development.

The expression of *CsNAC* and *CsCP* genes to be induced under several postharvest abiotic stresses and was enhanced during the development of peel pitting (Fan et al. 2007, 2009). The *CsCP* expression was induced by hypoxia (3% O_2), but repressed by anoxia (0% O_2), wounding, ethylene and high temperature (40 °C). Since ethylene treatment could reduce the rind pitting of orange fruit (Lafuente and Sala, 2002; Cajuste and Lafuente, 2007), the down-regulation of *CsCP* by ethylene might contribute to the suppression of physiological response to stress (Fan et al., 2009). It is important to note that, in the studies by Fan et al. (2007, 2009) and Gao et al. (2006, 2009), the physiological and molecular response was studied by wounding fruit using a knife. Therefore, the conclusion to be drawn from these observations is that these genes are

involved in stress response related to physical damage and possibly to the disorders. The interaction between different rind pitting-related genes would be an interesting aspect in elucidating the molecular mechanism of citrus rind pitting. Furthermore, the cause-result relationship between *CsCP* and rind pitting still needs to be further verified.

The involvement of antioxidant systems in the beneficial effect of ethylene conditioning in reducing the disorder was previously investigated by Cajuste and Lafuente (2007), who demonstrated that increased PAL and reduced POD activities played a significant role in the development of non-chilling peel pitting. Change in phenylpropanoid metabolism is an important response to stress. PAL has been shown to be the rate controlling enzyme in phenylpropanoid synthesis and physiological wounding of citrus rind such as mandarin and grapefruit (McDonald et al., 2000). Activity of PAL in the albedo and flavedo was high, while that of POD was reduced on fruit affected by the disorder (McDonald et al., 2000). In addition, higher levels of lignin and phenols were reported on fruit conditioned with ethylene, and these fruit had lower occurrence of the disorder. These findings concur with results reported by Lafuente et al. (2003) who observed an increase in PAL activity and ethylene production associated with the development of peel pitting. In a similar study, Sala et al. (2005) reported that at non-chilling temperature (12° C) 'Navelate' orange, a cultivar tolerant to non-chilling but susceptible to chilling peel pitting, had lower PAL activity than 'Pinalate', a chilling-tolerant variety. When stored at chilling temperature (2 °C), 'Pinalate' fruit had higher catalase (CAT) than 'Navelate' cultivar. This suggests that the magnitude of PAL and CAT activity and ethylene differ with cultivar as well as the type of disorder. These observations give an indication that the activity of PAL in the flavedo and albedo and a rise in lignin and phenols in the flavedo may be used as a biochemical indicator that more produced phenylpropanoid products led to reduce non-chilling peel pitting (Sala et al., 2005). Furthermore, the decline of POD activity in both albedo and flavedo tissues and also the inability of the albedo to increase lignin and phenolic contents may be indicative of low ability of the fruit to overcome this physiological disorder. CAT activity on the other hand may play a role protecting both 'Navelate' and 'Pinalate' fruit from conditions promoting chilling and non-chilling peel pitting physiological disorders (Cajuste and Lafuente, 2007).

Conditioning 'Navelate' oranges with 10 $\mu L.L^{-1}$ ethylene significantly reduced the incidence of non-chilling peel pitting while inhibiting ethylene action by 1-methylcyclopropene (1-MCP) increased the incidence of non-chilling rind pitting (Estables-Ortiz et al., 2009). Gene ontology

analysis conducted from this study revealed that ethylene induced the metabolism of amino acid derivatives, including phenylpropanoids. These observations are in agreement with Cajuste and Lafuente (2007) who suggested that phenolic compounds may be involved in ethylene-induced protection against peel pitting.

Rind pitting disorder has also been reported to be related to tonoplast disruption, concomitant accumulation of phenolic substances and volatiles under the cuticle, such as acetaldehyde, ethanol and ethylene (Sawamura et al., 1984; Cohen et al., 1990; Zaragoza et al., 1996). Limonene and valencene may be related to postharvest non-chilling peel pitting (Dou et al., 1999; Medeira et al., 1999; Dou, 2003). It was proposed that the internal volatile composition of fruit could be used as an indicator of fruit susceptibility to postharvest pitting. By comparing the volatile composition of pitted and non-pitted 'Fallglo' tangerines and white 'Marsh' grapefruit, Dou et al. (2003) showed that pitted fruit had a higher volatile concentration than non-pitted fruit. However, of more than 20 volatile compounds identified, limonene was the only one found in significantly higher concentrations in pitted 'Fallglo' tangerines and 'Marsh' grapefruit as compared to non-pitted fruit. Medeira et al. (1999) further proposed the possibility that limonene, in the parenchyma of the flavedo of 'Encore' mandarin is released from its compartments, and becomes toxic to the cells. Although significant differences in volatile components of the rind occurred during storage, rind pitting was probably caused by means other than changing rind volatile composition (Sun and Petracek, 1999).

7. Techniques for inducing rind disorders

Studies in different citrus growing regions indicated that rind water status is a key factor prevailing in the susceptibility of citrus fruit to postharvest non-chilling peel pitting (Alférez et al., 2005, 2010). In citrus growing areas; such as Florida during 'Marsh' grapefruit harvesting season and Spain during 'Navelina and 'Navelate' harvesting season, harvested fruit may experience fluctuations in RH from 95% to 25% within a 24-hour period (Alférez et al., 2004, 2005). These conditions have been reported to induce postharvest non-chilling disorders in these fruit. Therefore, to reproduce symptoms of postharvest non-chilling pitting disorders such as noxan of 'Shamouti' oranges (Ben-Yehoshua et al., 2001), postharvest peel pitting in 'Navel' oranges (Alférez and Zacarìas, 2001), 'Marsh' grapefruit (Alférez and Burns, 2004; Alférez et

al., 2010), and rind staining in 'Navel' oranges (Fig. 3A) (Alférez et al., 2003), these fruit should first be stored for a week at low and transferred to higher RH.

The incidence of rind breakdown (RBD) of 'Nules Clementine' mandarin has consistently been shown to be lower in fruit exposed to the sun than non-exposed fruits, and higher on exposed than the non-exposed side of individual fruits (van Rensburg et al., 2004; Khumalo, 2006; Cronje, 2009). Light conditions were also reported to influence fruit colour (van Rensburg et al., 2004; Cronje, 2009) and maturity (Barry et al., 2000). Well coloured 'Nules Clementine' mandarin fruit did not suffer from peel pitting or rind breakdown disorder during storage (Cronje et al., 2011a). These observations prompted investigations to gain better insight into the effect of high light/low light on the occurrence of the disorder as part of testing models which result in an increase in RBD sensitivity.

In order to prove the hypothesis the fruit flavedo should receive direct sun exposure to ensure good rind conditions, Cronje (2009) and Cronje et al. (2011a) used paper bags to cover individual fruit and to manipulate the duration of direct sun exposure. By covering the fruit positioned on the outside of the canopy during stages II and III (immediately after physiological fruit drop or 4 months before harvest) of fruit development, it was possible to induce RBD in 'Nules Clementine' mandarins. This is the first successful technique to induce a physiological rind disorder during preharvest and has opened new avenues of research towards elucidating the mechanisms involved in the disorder and eventually improving rind condition.

8. Prospects for Future Research

Significant progress in understanding non-chilling peel pitting and the factors associated with the disorder has been made. For instance, Cronje et al. (2011a) showed that the reduction of fruit light interception during stage II of fruit development, and probably earlier, is detrimental to rind condition and can lead to a higher incidence of the disorder. This is a major advancement in unravelling the factors predisposing 'Nules Clementine' mandarin fruit rind to rind breakdown disorder.

Although intensive research has been conducted, physiological rind disorders still occur frequently and unpredictably. The difficulty is that these disorders, characteristically, do not manifest during harvest or fruit grading, but develops about one to five weeks after harvest

(Agustí et al., 2003, 2004; Cronje, 2007). This delayed phenomenon is in essence the difficulty that faces the industry and importers alike. The challenge is therefore to develop technology to determine rind quality on the packing line, in order to classify individual fruit for either local or export market, as loses in the postharvest chain can result in a significant reduction of income. There is therefore, a need to develop an objective, fast and non-destructive assessment that can be used to accurately determine or predict susceptibility of presymptomatic citrus fruit to rind disorders. Among non-destructive quality assessment techniques such as visible to near-infrared spectroscopy (Vis/NIRS) (Zheng et al., 2010), hyperspectral or multispectral imaging (Blasco et al., 2009), magnetic resonance imaging (MRI), X-ray computed tomography (CT) (Lammertyn et al., 2003), optical coherence tomography (OCT) (Meglinski et al., 2010), visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS) is arguably the most advanced with regard to instrumentation, applications, accessories, and chemometric software packages (Nicolaï et al., 2007). A review in Paper 2 discusses the recent developments and application of Vis/NIR spectroscopy to non-destructively evaluate internal and external quality of citrus fruit.

It is worth noting that very limited research has been conducted to develop a technology to assess, predict and monitor the physiological disorders and rind physiological disorders of citrus fruit (Geeola et al., 1994). This is because until recently, most non-destructive quality measurements technologies were developed to assess fruit according to their internal quality attributes (Butz et al., 2005) rather than for external quality. However, the success of Vis/NIRS to detect surface bruising in apple (Geeola et al., 1994), surface defects in peach (Miller and Delwiche, 1991), storage disorder in kiwifruit (Clark et al., 2004) and internal drying in tangerine citrus (Peiris et al., 1998c) shows the potential of this technology in determining non-chilling physiological rind disorders. One of the recent successes of Vis/NIR application was demonstrated by Zheng et al. (2010) who used Vis/NIRS to predict oleocellosis sensitivity of citrus fruit.

Imaging techniques have been developed and successfully applied as inspection tools for quality assessment of a variety of fruits (Xing et al., 2003, 2005; Mehl et al., 2004). The development of the multispectral and hyper-spectral imaging technologies provides the possibility to capture images not only in the visible range but also extend to capture multiple images in the Vis/NIR regions (Mehl et al., 2004; Qin et al., 2008). These analytical technologies, which combine Vis/NIRS with digital imaging, now allow both spatial and spectral

information to be obtained simultaneously. Further information on the principles of these technologies can be found in a review by Gowen et al. (2007).

Internal and external quality defects of onions can be effectively observed and screened by the OCT system, and this non-invasive method is suitable for examining intact plants (Meglinski et al., 2010). OCT produces two-dimensional images of plant tissues at a penetration depth of 1–2 mm from the surface (Sapozhnikova et al., 2003). Detailed aspects of OCT theory and applications have been discussed in comprehensive reviews by Fercher et al. (2003) and Tomlins and Wang (2005). Vis/NIRS and OCT offer potential for the non-invasive assessment of the chemical composition such as carbohydrate content in the flavedo and changes in intact fruit that might predispose fruit to the development of rind disorders.

Another area that still needs further research is the relationship between biochemical profile of the rind, antioxidant system and development of citrus non-chilling physiological disorders. Understanding this cause-response relationship will help identify potential presymptomatic biochemical markers for these disorders. Cronje et al. (2011a) showed a negative relationship between rind carotenoids concentration measured postharvest and RBD. It is possible that rind pigments measured only at harvest cannot be used as indicators for fruit susceptibility to RBD (Cronje et al., 2011a). Studies have also suggested the participation of the antioxidant enzymatic system in the chilling and non-chilling conditions causing rind pitting (Sala and Lafuente, 1999; Lafuente et al., 2003; Sala et al., 2005; Cajuste and Lafuente, 2007). These investigations have shown that phenolic metabolism may be required for building protecting barriers that would help 'Navelate' fruit to reduce non-chilling peel pitting. However, further investigations are needed to understand the participation of the antioxidant system in protecting citrus fruit against other rind non-chilling physiological disorders, such as RBD on 'Nules Clementine' and rind staining on 'Navel' oranges.

9. Conclusion

Intensive research has been conducted on determining preharvest and postharvest factors trigger non-chilling rind pitting disorders. Similar to most fruit physiological disorders, non-chilling rind physiological disorders are theoretically associated with preharvest, ecophysiological and postharvest factors. Various studies by different groups of investigators

have found a correlation between preharvest factors such as cultivar, rootstock, microclimate, and nutrient imbalances and postharvest rind pitting. In addition, stressful postharvest conditions such as low and sudden changes in RH exacerbate the disorder by reducing rind water potential. However, the mechanism governing the occurrence of rind pitting disorder and the manner it is related to other physiological rind disorders is still not well understood. Nonetheless, it has been established that different canopy microclimates influence sensitivity of fruit to these disorders. Different research groups maintained that rind pitting disorders affects mainly sun-exposed fruit as opposed to shaded (covered by foliage) fruit. It is hypothesised that solar radiation may be involved in the structural alteration of the cuticle, and a high rind temperature over a long period may induce localised dehydration in the epidermal and sub-epidermal cells, leading to plasmolysis and membrane collapse. In contrast, 'Nules Clementine' mandarins deviated from this trend whereby inside fruit were more susceptible to RBD. Exposure of 'Nules Clementine' to high (outside) or low (inside) light levels in the canopy, affected the rind carbohydrate and carotenoids concentration during fruit development in that outside fruit had higher levels compared to fruit located inside the canopy. Future research on general rind biochemical changes and antioxidants in particular, incorporating multiple sampling times and postharvest pigment analysis is suggested.

The incidence of these disorders can be controlled by maintaining mineral nutrient balance, sufficient light penetration within the canopy by pruning, high water potential, and reducing the rate of fruit senescence. Preharvest and postharvest application GA_3 (10 $mg.L^{-1}$ just before or at fruit colour break) has been demonstrated as a practical method to reduce incidence of physiological non-chilling rind disorders associated with senescence such as rind staining of 'Navel' orange and rind pitting in 'Fortune' mandarin. Incidence and severity of non-chilling pitting disorder can be markedly reduced by several postharvest treatments such maintaining constant humidity and raising the humidity around the fruit to above 95% RH. Basically, postharvest treatments that reduce mass loss and maintain the turgidity and firmness of the fruit also reduce the incidence of these disorders. Literature evidence also showed that transient exposure of fruit to low RH (<45%), followed by transfer to high RH (90%) and 2 hours at low RH was sufficient to induce peel pitting after transfer to RH. It is therefore suggested that the economic losses associated with postharvest peel pitting can be reduced by harvesting susceptible cultivars at high RH and minimising exposure to low RH postharvest. However, it

should be borne in mind that excessive turgidity and wetness can also predispose citrus fruit to a different important physiological disorder, oleocellosis. Considering that oleocellosis results from mechanical impact damage that ruptures oil glands, careful handling during harvest and packhouse operations should be maintained.

References

Agustí, M., Almela, V., Guardiola, J.L., 1981. The regulation of fruit cropping in mandarins through the use of growth regulators. Proc. Int. Soc. Citricul. 1, 216–220.

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415–422.

Agustí, M., Martinez-Fuentes, A., Majeso, C., 2002. Citrus fruit quality. Physiological basis and techniques of improvement. Agrociencia Vol. VI, (2), 1–16.

Agustí, M., Almela, V., Juan, M., Mesejo, C., Martinez-Fuentes, A., 2003. Rootstock influence on the incidence of rind breakdown in 'Navelate' sweet orange. J. Hort. Sci. Biotechnol. 78, 554–558.

Agustí, M., Almela, V., Juan, M., Martinez-Fuentes, A., Masejo, C., 2004. Quality of citrus for fresh consumption as affected by water relationships. Acta Hort. 632, 141–148.

Albrigo, L.G., Alfred, L., Brown, G.E., Fellers, P.J., 1970. Peel and internal quality of oranges as influenced by grove applications of pinolene and benlate. Proc. Fla. State. Hort. Soc. 5, 263–267.

Albrigo, L.G., 1972a. Distribution of stomata and epicular wax on oranges as related to stem-end rind breakdown and water loss. J. Am. Soc. Hort. Sci. 97, 220–223.

Albrigo, L.G., 1972b. Ultrastructure of cuticular surfaces and stomata of developing leaves and fruit of the Valencia oranges. J. Am. Soc. Hort. Sci. 97, 761–765.

Albrigo, L.G., 1977. Rootstocks affect Valencia orange fruit quality and water balance. Proc. Int. Soc. Citricul. 1, 62–65.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Zacarìas, L., 2001. Postharvest pitting in navel oranges at non-chilling temperature: Influence of relative humidity. Acta Hort. 553, 307–308.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarìas, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges. I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222–231.

Alférez, F., Alquezar, B., Burns, J.K., Zacarìas, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49.

Alférez, F.M., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Burns, J.K., Zacarìas, L., 2004. Postharvest peel pitting in citrus is induced by changes in relative humidity. Proc. Fla. State Hort. Soc. 117, 355–358.

Allan, A.C., Fluhr, R., 2007. Ozone and reactive oxygen species. p. 1299–1304. In: K. Roberts, (ed.), Handbook of Plant Science. Vol 2. John Wiley & Sons Ltd.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65.

Aloni, B., Karni, L., Rylski, I., Cohen, Y., Fuchs, M., Moreshet, S., Yao, C., 1998. Cuticular cracking in pepper fruit: I. Effect of night temperature and humidity. J. Hort. Sci. Biotechnol. 73, 743–749.

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Arpaia, M.L., 1994. Preharvest factors influencing postharvest quality of tropical and subtropical fruit. HortScience 29, 982–985.

Arpaia, M.L., Kahn, T.L., El-Otmani, M., Coggins, C.W., DeMason, D.H., O'Connell, N.V., Pehrson Jr., J.E., 1991. Preharvest rind stain of 'Valencia' orange: a histochemical and development characterization. Sci. Hort. 46, 261–274.

Assimakopoulou, A., Tsougrianis, C., Elena, K., Fasseas, C., Karabourniotis, G., 2009. Pre-harvest rind-spotting in 'Clementine' mandarin. J. Plant Nutr. 32, 1486–1497.

Bain, J.M., 1958. Morphologial, anatomical, and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis* (L.) Osbeck. Austral. J. Bot. 6, 1–24.

Bajwa, B.E., Anjum, F.M., 2006. Improving storage performance of *Citrus reticulata* Blanco mandarins by controlling some physiological disorders. Int. J. Food Sci. Technol. 42, 495–501.

Bangerth, F., 1976. A role for auxin and auxin transport inhibitors on the Ca content of artificially induced parthenocarpic fruits. Physiologia Plant. 37, 191–194.

Barry, G.H., 2000. Sources of variation in juice quality of 'Valencia' sweet orange (*Citrus sinensis* L. Osb). PhD thesis, University of Florida, Florida.

Barry, G.H., Castle, W.S., Davies, F.S., 2000. Juice quality of 'Valencia' sweet orange among citrus–producing regions in Florida and between canopy positions. Proc. Int. Soc. Citricul. 1, 308–314.

Barry, G.H., Castle, W.S., Davies, F.S., Litten, R.C., 2003. Variability in juice quality of 'Valencia' sweet orange and sample size estimation for juice quality experiments. J. Am. Soc. Hort. Sci. 128, 803–808.

Barry, G.H., Castle, W.S., Davies, F.S., 2004. Soluble solids accumulation in 'Valencia' sweet orange as related to rootstock selection and fruit size. J. Am. Soc. Hort. Sci. 129, 594–598.

Ben-Yehoshua, S., 1969. Gas exchange, transpiration, and the commercial deterioration in storage of orange fruit. J. Am. Soc. Hort. Sci. 94, 524–528.

Ben-Yehoshua, S., 1987. Transpiration, water stress and gas exchange. p. 113–170. In: J. Weichmann (ed.), Postharvest Biology of Vegetables. Marcel Dekker, Inc., New York.

Ben-Yehoshua, S., Goldschmidt, E.E., Bar-Joseph, M., 1994. Citrus Fruits. p. 357–377. In: C. Arentzen (ed.), Encyclopedia of Agricultural Science, Vol. 1, Academic Press, Inc.

Ben-Yehoshua, S., Shapiro, B., Chen, Z.E., Lurie, S., 1983. Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress. Plant Physiol. 73, 87–93.

Ben-Yehoshua, S., Goldschmidt, E.E., Bar-Joseph, M., 1994. Citrus Fruits. p. 357–377. In: C. Arentzen (ed.), Encyclopedia of Agricultural Science, Vol. 1, Academic Press, Inc.

Ben-Yehoshua, S., Peretz, J., Moran, R., Lavie, B., Kim, J.J., 2001. Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*Citrus sinensis*, Osbeck). Postharvest Biol. Technol. 22, 19–27.

Blasco, J., Aleixos, N., Gómez-Sanchís, J., Moltó, E., 2009. Recognition and classification of external skin damage in citrus fruits using multispectral data and morphological features. Biosyst. Eng.10, 137–145.

Bower, J.P., Cutting, G.J., 1988. Avocado fruit development and ripening physiology. Hort. Rev. 10, 229-271.

Burns, J.K., Alférez, F.M., Lluch, Y.P., 2007. A method to reduce citrus fruit peel pitting and delay senescence. Patent application, World intellectual property organization, WO2009/002824 (PCTR/US2008/067626).

Butz, P., Hofmann, C., Tauscher, B., 2005. Recent developments in non-invasive techniques for fresh fruit and vegetable internal quality analysis. J. Food Sci. 70, 131–141.

Cajuste, J.F., Lafuente, T.M., 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. Postharvest Biol. Technol. 45, 193–203.

Cajuste, J.F., González-Candelas, L., Veyrat, A., García-Breijo, F.J., Reig-Armiñana, J., Lafuente, M.T., 2010. Epicuticular wax content and morphology as related to ethylene and storage performance of 'Navelate' orange fruit. Postharvest Biol. Technol. 55, 29–35.

Chikaizumi, S., 2000. Mechanism of rind-oil spot development in 'Encore' (*Citrus nobilis* Lour. X *C. Deliciosa* Ten.) fruit. J. Japan. Soc. Hort. Sci. 69, 149–155.

Clark, C.J., McGlone, V.A., DeSilva, H.N., Manning, M.A., Burdon, J., Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit (*Actanidia cinensis*) based on visible-NIR spectral characteristics at harvest. Postharvest Biol. Technol. 32, 147–158.

Clarkson, D.T., Hanson, J.B., 1980. The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31, 239–298.

Coggins Jr, C.W., 1981. The influence of exogeneous growth regulators on rind quality and internal quality of citrus fruits. Proc. Int. Soc. Citricul. 1, 214–216.

Cohen, E., 1988. The chemical composition and sensory flavour quality of 'Mineola' tangerines. I. Effects of fruit size and within tree position. J. Hort. Sci. 63, 175–178.

Cohen, E., Shapiro, B., Shalom, Y., Klein, J.D., 1994. Water loss: A non-destructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit. J Am. Soc. Hort. Sci. 119, 983–986.

Cohen, E., Shalom, Y., Rosenberger, I., 1990. Post-harvest behavior of 'Ortanique' ('Toapz') tangor citrus fruit during long-term storage at various temperatures. Sci. Hort. 44, 235–240.

Cronje, P., 2005. Peteca spot of lemons. S. African Fruit J. Feb/March, 26–28.

Cronje, P.J.R., 2007. Postharvest rind disorders of citrus fruit. Citrus Research International, Nelspruit, South Africa.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementines' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Davies, F.S., Albrigo, L.G., 1994. Fruit quality, harvesting and postharvest technology. In: Davies, F.S., and L.G. Albrigo (eds.), Citrus. Chapter 7, pp. 202-225. Cab International, Wallingford, UK.

Devlin, R.M., Witham, F.H., 1983. Functions of essential mineral elements and symptoms of mineral deficiency. Plant physiol. 99:139–153.

Dou, H., 2003. Volatile differences of pitted and non-pitted 'Fallglo' tangerine and white 'Marsh' grapefruit. HortScience, 38:1408–1409.

Dou, H., Ismail, M.A., Petracek, P.D., 1999. Reduction of postharvest pitting of citrus by changing wax components and their concentrations. Proc. Fla. State Hort. Soc. 112, 159–163.

Duarte, A.M.M., Guardiola, J.L., 1995. Factors affecting rind pitting in the mandarin hybrids 'Fortune' and 'Nova'. The influence of exogenous growth regulators. Acta Hort. 379, 59–67.

Ehert, D. L., Ho., L.C., 1986. The effect of salinity on the dry matter partitioning and fruit growth on tomatoes grown in nutrient film culture. J. Hort. Sci. 61, 361–367.

El-Hilali, F., Ait-Oubahou, A., Remah, A., Akhayat, O., 2004. Effects of preharvest spray of Ca and K on quality, peel pitting and peroxidases activity of 'Fortune' mandarin fruit in low temperature storage. Acta Hort. 632, 309–315.

El-Otmani, M., Coggins, C.W., 1985. Fruit age and growth regulator effects on the quantity and structure of the epicuticular wax of Washington navel orange fruit. J. Am. Soc. Hort. Sci. 110, 371–378.

El-Otmani, M., Arpaia, M.L., Coggins Jr., C.W., Pehrson, J.E., O'Connell, N.V., 1989. Developmental changes in 'Valencia' orange fruit epicuticular wax in relation to fruit position on the tree. Sci. Hort. 41, 69–81.

El-Otmani, M., Ait-Oubahouand, A., Zacarías, L., 2011. Citrus *spp.*: orange, mandarin, tangerine, clementine, grapefruit, pomelo, lemon and lime. pp. 437-514. In: E.M. Yahia (ed.), Postharvest biology and technology of tropical and subtropical fruits, Vol. 2.

Estables-Ortiz, B., Lafuente, M.T., Forment, J., Gadea, J., 2009. Transcriptomic analysis of ethylene-induced tolerance to non-chilling peel pitting in citrus fruit. Acta Hort. 839, 555–560.

Fan, J., Gao, X., Yang, Y.W., Deng, W., Li, Z.G., 2007. Molecular cloning and characterization of a NAC-like gene in 'Navel' orange fruit response to postharvest stresses. Plant Mol. Biol. Rep. 25, 145–153.

Fan, J., Yang, W.Y., Gao, X., Deng, W., Falara, V., Kanellis, A.K., Li, Z.G., 2009. Expression of a senescence-associated cysteine protease gene related to peel pitting of 'Navel' orange (*Citrus sinensis* L. Osbeck). Plant Cell Tissue Organ Cult. 98, 281–289.

FAO, 2010. Food and Agriculture Organization of the United Nations, Rome, Italy, www.faostat.org.

Ferguson, I.B., Watkins, C.B., 1989. Bitter pit in apple fruit. Hort. Rev. 11, 289–355.

Ferguson, I.B., Volz, R., Woolf, A., 1999. Preharvest factors affecting physiological disorders of fruit. Postharvest Biol. Technol. 15, 255–262.

Fercher, A.F., Drexler, W., Hitzenberger, C.K., Lasser, T., 2003. Optical coherence tomography-principles and applications. Rep. Prog. Phys. 66, 239–303.

Freeman, D., 1976. Rind blemishes of citrus. I. Initiation and development. Sci. Hort. 4, 317–327.

Gambetta, G., Martínez-Fuentes, A., Bentancur, O., Mesejo, C., Reig, C., Gravina, A., Agustí, M., 2011. Hormonal and nutritional changes in the flavedo regulating rind color development in sweet orange [*Citrus sinensis* (L.) Osb.]. J. Plant Growth Regul. 31, 273–282.

Gao, X., Li, Z.G., Fan, J., Yang, Y.W., 2006. Screening and expression of differentially expressed genes for peel pitting of citrus fruit. Acta Hort. 712, 473–479.

Gao, X., Li, Z.G., Fan, J., Yang, Y.W., Falara, V., Kanelis, A.K., 2009. Molecular cloning and expression of an expasin-like gene in 'Navel' fruit during postharvest stresses. Plant Growth Regul. 59, 13–19.

Geeola, F., Geeola, F., Peiper, U.M., 1994. A spectrophotometric method for detecting surface bruises on 'Golden Delicious' apples. J. Agr. Eng. Res. 58, 47–51.

Genizi, A., Cohen, E., 1988. The chemical composition and sensory flavour quality of 'Mineola' tangerines. II. Relationship between composition and sensory properties. J. Hort. Sci. 63, 179–182.

Gilfillan, I.M., 1990. Physiological disorders. In: Production guidelines for export citrus, Vol. II. South African co-operative citrus exchange limited, Outspan.

Gilfillan, I.M., Stevenson, J.A., Wahl, J.P., 1981. Control of creasing in navels with gibberellins. Proc. Int. Soc. Citricul. 1, 224–226.

Gowen, A.A., O'Donnell, C.P., Cullen, P.J., Downey, G., Frias, J.M., 2007. Hyperspectral imaging – an emerging process analytical tool for food quality and safety control. Trends Food Sci. Technol. 18, 590–598.

Grierson, W., 1986. Physiological disorders, p. 361–378. In: Wardowski, S. Nagy and W. Grierson (Eds.). Fresh citrus fruits. AVI Publ., New York.

Henriod, R.E., 2006. Postharvest characteristics of 'Navel' oranges following high humidity and low temperature storage and transport. Postharvest Biol. Technol. 42, 57–64.

Holland, N., Sala, J.M., Menezes, H.C., Lafuente, M.T., 1999. Carbohydrate content and metabolism as related to maturity and chilling sensitivity, of cv. Fortune mandarins. J.Agric. Food Chem. 47, 2513–2518.

Huff, A., 1983. Nutritional control of regreening and degreening in Citrus peel segments. Plant Physiol. 73, 243–249.

Joiner, J.N., Poole, R.T., Conover, C.A., 1983. Nutrition and fertilization of ornamental greenhouse crops. Hort. Rev. 5, 317–403.

Kader, A.A., Arpaia, M.L., 2002. Postharvest handling systems: Subtropical fruits. p. 375–384. In: A.A. Kader (ed.), Postharvest Technology of Horticultural Crops. Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA.

Kader, A.A., Zagory, D., Kerbel, E.L., 1989. Modified atmosphere packaging of fruits and vegetables. Crit. Rev. Food Sci. Nutr. 28, 1–30.

Khalidy, R., Jamali, A., Bolkan, H., 1969. Causes of the peteca disease of lemons occurring in Lebanon. Proc. First Int. Citrus Symposium 2, 1253–1261.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Kruger, F.J., Penter, M.G., Masevhe, M.R., Combrink, N.K., 2005. The use of fruit mineral content as a tool to investigate the epidemiology of citrus rind disorders. S. African Fruit J. April/May, 54-59.

Lafuente, M.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25, 49–57.

Lafuente, M.T., Zacarìas, L., 2006. Postharvest physiological disorders in citrus fruit. Stewart Postharvest Rev. 2, 1–9.

Lafuente, M.T., Zacarìas, L., Martìnez-Téllez, M.A., Sanchez-Ballesta, M.T., Granell, A., 2003. Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus. Postharvest Biol. Technol. 29, 308–317.

Lafuente, M.T., Zacarìas, L., Martìnez-Téllez, M.A., Sanchez-Ballesta, M.T., Dupille, E., 2001. Phenylalanine ammonia-lyase as related to ethylene in the development of chilling symptoms during cold storage of citrus fruit. J. Agr. Food Chem. 49, 6020–6025.

Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003. Analysis of the time course of core breakdown in 'Conference' pears by means of MRI and X-ray CT. Postharvest Biol. Technol. 29, 19–28.

Larson, R.A., 1988. The antioxidants of higher plants. Phytochem. 27, 969–978.

Li, G., Zhang, W., Benoit, F., Ceustermans, N., 2004. Effects of environment factors on the growth and incidence of blossom-end rot in soilless *Capsicum fructescens* var. *grossum*. Acta Hort. 633, 381–389.

Li, Z., Fan, J., Gao, X., Yang, Y., Deng, W., Yuan, L., 2009. The mechanism and the control of peel pitting of citrus fruits. Acta Hort. 837, 287–292.

Lyons, J.M., Breidenbach, R.W., 1987. Chilling injury. p. 305–326. In: J. Weichmann (ed.), Postharvest Physiology of Vegetables. Marcel Dekker Inc., New York.

Macnish, A.J., Joyce, D.C., Hetherington, S.E., 1997. Packaging to reduce water loss can delay ripening of mango (*Mangifera indica* L. cv. 'Kensington Pride') fruit. Aust. J. Exp. Agr. 37, 463–467.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolaï, B., 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food Bioprocess Tech. 5, 425–444.

Maia, M.I., Medeira, M.C., Pinto, M.J., Duarte, A.M., 2004. Pre-harvest rindstain of 'Encore' mandarin: initial histological signs of epicarp disturbance and extent of the disorder. Sci. Hort. 99, 143–152.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd edition. Academic Press, London. p. 67–73.

Martinez-Tellez, M.A., Lafuente, M.T., 1997. Effect of high temperature conditioning on ethylene, phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of chilled fortune mandarin fruit. J. Plant Physiol. 150, 674– 678.

McCornack, A.A., 1970. Peel injury of Florida navel oranges. Fla. State Hort. Soc. 83, 267–270.

McCornack, A.A., 1975. Postharvest weight loss of Florida citrus fruits. Fla. State Hort. Soc. 88, 333–335.

McDonald, R.E., Nordby, H.E., McCollum, T.G., 1993. Epicuticular wax morphology and composition are related to grapefruit chilling injury. HortScience 28, 311–312.

McDonald, R.E., Miller, W.R., McCollum, T.G., 2000. Canopy position and heat treatments influence gamma-irradiation-induced changes in phenylpropanoid metabolism in grapefruit. J. Am. Soc. Hort. Sci. 125, 364–369.

Medeira, M.C, Maia, M.I., Vitor, R.F., 1999. The first stages of preharvest peel pitting development in 'Encore' mandarin: a histological and ultrastructural study. Ann. Bot. 83, 667–673.

Meglinski, I.V., Buranachai, C., Terry, L.A., 2010. Plant photonics: Application of optical tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Mehl, P.M., Chen, Y-R., Kim, M.S., Chan, D.E., 2004. Development of hyperspectral imaging technique for the detection of apple surface defects and contaminations. J. Food Eng. 61, 67–81.

Mengel, K., Kirby, E.A., 1982. Principles of Plant Nutrition, 3rd edition. International Potash Institute, Worblaufen-Bern, Switzerland. pp. 37.

Miller, B.K., Delwiche, M.J., 1991. Spectral analysis of peach surface defects. Trans. Am. Soc. Agr. Eng. 34, 2509–2515.

Morales, P, Davies, F.S., Littell, R., 2000. Pruning and skirting affect canopy microclimate, yields, and fruit quality of Orlando tangelo. HortScience 35, 30–35.

Morley, P. S., Hardgrave, M., Pilbeam, D.J., 1993. Susceptibility of sweet peppers (*Capsicum annuum* L) cultivars in the calcium deficiency disorder blossom end rot. p. 561–567. In: Fragroso, M.A.C., and M.L. van Beusichem, (eds.), Optimisation of Plant Nutrition. Kluwer Academic Publishers, The Netherlands.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Obenland, D.M., Margosan, D.A., Houck, L.G., Aung, L.H., 1997. Essential oils and chilling injury in lemon. HortScience, 32, 108–111.

Opara, L. U., Studman, C.J., Blanks, N.H., 1997. Fruit skin splitting and cracking. Hort. Rev. 19, 217–262.

Petracek, P.D., Wardowski, W.F., Brown, E.G., 1995. Pitting of grapefruit that resembles chilling injury. HortScience 30, 1422–1426.

Petracek, P.D., Dou, H., Malik, I., 1997. A postharvest pitting of 'Temple' oranges stimulated by high temperature storage and wax application. Proc. Fla. State Hort. Soc. 110, 211–214.

Petracek, P.D., Dou, H., Pao, S., 1998a. The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biol. Technol. 14, 99–106.

Petracek, P.D., Montalvo, L., Dou, H., Davis, C., 1998b. Postharvest pitting of 'Fallgold' tangerine. J. Am. Soc. Hort. Sci. 123, 130–135.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., 1998a. Nondestructive detection of selection drying, an internal disorder in tangerine. HortScience 33, 310–312.

Peiris, K.H.S., Dull, G.C., Leffler, R.G., Kays, S.J., 1998b. Near-infrared (NIR) spectrometric technique for non-destructive determination of soluble solids content in processing tomatoes. Am. Soc. Hort. Sci. 123, 1089–1093.

Peretz, J., Moran, R., Lavie, B., Ben-Yehoshua, S., 2001. Postharvest treatments of high relative humidity (RH) and low temperature reduced the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*C. sinensis*, Osbeck). Acta Hort. 553, 301–302.

Poiroux-Gordon, F., Santini, J., Fanciullino, A.L., Lopez-Lauri, F., Giannettini, J., Sallanon, H., Berti, L., Urban, L., 2013. Metabolism in orange fruits is driven by photooxidative stress in the leaves. Physiol. Plan. (in press). Doi: 10.1111/ppl.12023.

Poovaiah, B.W., Glenn, G.M., Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hort. Rev. 10, 107–152.

Porat, R., Weiss, B., Cohen, L., Daus, A., Aharoni, N., 2004. Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biol. Technol. 33, 35–43.

Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effect of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. Postharvest Biol. Technol. 15, 155–163.

Qin, J., Burks, T.F., Kim, M.S., Chao, K., Ritenour, M.A., 2008. Citrus canker detection using hyperspectral reflectance imaging and PCA-based image classification method. Sens. Instrumen. Food Qual. 2, 168–177.

Resnizky, D., Sive, A., 1993. Mineral composition of different fruits-a dominant factor in determining keeping quality in CA storage. Acta Hort. 343, 168–170.

Ritenour, M.A., Dou, H., 2003. Stem-end rind breakdown of citrus fruit. HS936. University of Florida Institute of Food and Agricultural Science Extension. http://www.docstoc.com/docs/37680812/Stem-End-Rind-Breakdown-of-Citrus-Fruit-1. Last accessed June 2012.

Ritenour, M.A., Dou, H., Bowman, K.D., Bowman, B.J., Stover, E., Castle, W.S., 2004. Effect of rootstock and stem-end rind breakdown and decay of fresh citrus. HortTechnol. 14, 315–319.

Ritenour, M.A., Boman, B.J., Burns, J.K., Hu, C., Contina, J.B., 2008. Effect of pre- and postharvest factors on fresh grapefruit peel breakdown. Proc. Fla. State. Hort. Soc. 121, 322–325.

Rodov, V., Ben-Yehoshua, S., Aharoni, N., 2010. Modified humidity packaging of fresh produce. Hort. Reviews, 37, 281–330.

Sala, J.M., Lafuente, M.T., 1999. Catalase in the heat-induced chilling tolerance of cold-stored hybrid 'Fortune' mandarin fruit. J. Agric. Food. Chem. 47, 2410–2414.

Sala, J.M., Lafuente, M.T., Cunat, P., 1992. Content and chemical composition of epicular wax of 'Navelina' oranges and 'Satsuma' mandarin as related to rindstaining of fruit. J. Sci. Food Agr. 59, 489–495.

Sala, J.M., Sanchez-Ballesta, M.T., Alférez, F., Mulas, M., Zacarìas, L., Lafuente, M.T., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges. II. Antioxidants enzymatic system and phenylalanine ammonia-lyase in non-chilling and chilling peel disorders of citrus fruit. Postharvest Biol. Technol. 37, 232–240.

Sanchez-Ballesta, M.T., Lafuente, M.T., Granell, A., Zacarìas, L., 2001. Isolation and expression of a citrus cDNA related to peel damage caused by postharvest stress conditions. Acta Hort. 553, 293–296.

Sanchez-Ballesta, M.T., Lluch, Y., Gosalbes, M.J., Zacarìas, L., Granell, A., Lafuente, M.T., 2003. A survey of genes differentially expressed during long-term heat-induced chilling tolerance in citrus fruit. Planta 218, 65–70.

Sanchez-Ballesta, M.T., Gosalbes, M.J., Rodrigo, M.J., Granell, A., Zacarìas, L., Lafuente, L.T., 2006. Characterization of a β-1,3-glucanase from citrus fruit as related to chilling-induced injury and ethylene production. Postharvest Biol. Technol. 40, 133–140.

Sanchez-Ballesta, M.T., Zacarìas, L., Granell, A., Lafuente, M.T., 2008. β-1,3-Glucanase gene expression as a molecular marker for postharvest physiological disorders in citrus fruit and its hormonal regulation. Postharvest Biol. Technol. 48, 146–149.

Sapozhnikova, V.V., Kamenskii, V.A., Kuranov, R.V., 2003. Visualization of plant tissues by optical coherence tomography. Russian J. Plant Physiol. 50, 282–286.

Sawamura, M., Manabe, T., Oonishi, S., Yasuoka, K., Kusunone, H., 1984. Effects of rind oils and their components on the induction of rind spots in citrus species. J. Hort. Sci. 59, 575–579.

Seske, L., 1995. Fruit cracking in sweet cherries (*Prunus avium* L.). Some physiological aspects–a mini review. Sci. Hort. 63, 135–141.

Snay, E.R., Bramlage ,W.J., 1973. Effect of calcium on apple mitocondria. HortScience 8, 273.

Spreen, T.H., 2009. Projections of world production and consumption of citrus to 2010. China/FAO citrus symposium. Food and Agricultural Organisation of the United Nations. http://www.fao.org/docrep/003/x6732e/x6732e02.htm.

Storey, R., Treeby, M.T., 2002. Cyro-SEM study of the early symptoms of peteca in 'Lisbon' lemons. J. Hort. Sci. Biotechnol. 77, 551–556.

Storey, R., Treeby, M.T., 1994. The morphology of epicuticular wax and albedo cells of orange fruit in relation to albedo breakdown. J. Hort. Sci. 69, 329–338.

Storey, R., Treeby, M.T., Milne, D.J., 2002. Crease: Another Ca deficiency-related fruit disorder? J. Hort. Sci. Biotechnol. 77, 565–571.

Sun, D., Petracek, P.D., 1999. Grapefruit gland oil composition is affected by wax application, storage temperature, and storage time. J. Agr. Food Chem. 47, 2067–2069.

Syvertsen, J.P., Albrigo, L.G., 1980. Some effects of grapefruit tree canopy position on microclimate, water relations, fruit yield, and juice quality. J. Am. Soc. Hort. Sci. 105, 454-459.

Tamim, M., Goldschmidt, E.E., Goren, R., Shachnai, A., 2001. Potassium reduces the incidence of superficial rind pitting (Nuxan) in 'Shamouti' orange. Acta Hort. 553, 303–304.

Thorp, T.G., Hutching, D., Lowe, T., Marsh, K.B., 1997. Survey of fruit mineral concentrations and postharvest quality of New Zealand grown 'Hass' avocado (*Persea americana* Mill.). New Zealand J. Crop Hort. Sci. 25, 257–260.

Tomlins, P.H., Wang, R.K.W., 2005. Theory, developments and applications of optical coherence tomography. J. Phys. D: Appl. Phys. 38, 2519–2535.

Tisdale, S. L., Nelson, W.L., Beaton, J.D., 1985. Soil fertility and fertilizers. MacMillan Publishing Co, New York. pp. 78–82.

Treeby, M.T., Storey, R., Bevington, K.B., 1995. Rootstock, seasonal, and fruit size influences on the incidence and severity of albedo breakdown in Bellamy navel oranges. Austral. J. Expt. Agric. 35, 103–108.

Treeby, M.T., Storey, R., 2002. Calcium-spray treatments for ameliorating albedo breakdown in navel oranges. Austral. J. Expt. Agric. 42, 495–502.

Turner, D.W., 1997. Bananas and Plantains. p. 47-84. In: Mitra (ed.), Postharvest Physiology and Storage of Tropical and Subtropical Fruits. Cab International.

Undurraga, M.P., Olaeta, C., Retamales, A.J., Brito, P.A., 2006. Effect of dipping in calcium solutions on peteca (rind pitting) in lemons and limes, harvested after a rainfall and cold stored. Agricultura Tecnica. Instituto de Investigaciones Agropecuarias, Chillan, 66, 3–12. [Spanish]. (Cab Abstract).

van Rensburg, P.J.J., Bruwer, M., 2000. Factors influencing rind breakdown of 'Clementine' mandarin fruit. Proc. Int. Soc. Citricul. 3, 1173.

van Rensburg, P.J.J., Cronje, P.J.R., Joubert, J., Gambetta, G., Bruwer, M., 2004. Factors influencing rind breakdown in citrus fruit. Proc. Int. Soc. Citricul. 2, 1051–1061.

Vercher, R., Tadeo, F.R., Almela, V., Zacarìas, L., Primo-Millo, E., Agustí. M., 1994. Rind structure, epicuticular wax morphology and water permeability of 'Fortune' mandarin fruit affected by peel pitting. Ann. Bot. 74, 619–625.

Vitor, R.F., Lidon, F.C., Carvalho, C.S., 1999. Dark stained tissues of the epicarp of 'Encore' mandarin: interactions with the production of hydroxyl radicals. Free Radical Res. 31, S163–S169.

Vitor, R.F., Lidon, M., Barreiro, M.D., Maia, M.I., Medeira, M.C., Guerreiro, A., 2000. 'Peel pitting' of 'Encore' mandarin fruits: Etiology, control and implications in fruit quality. Fruits 56, 27–35.

Wild, B.L., 1991. Postharvest factors governing the development of peteca spot on 'Meyer' lemons. HortScience, 26, 287–289.

Wills, R.H.H., Lee, T.H., Graham, D., McGlasson, W.B., Hall, E.G., 2004. Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables. Australia: New South Wales University Press Limited.

Witney, G.W., Hofman, P.J., Wolstenholme, B.N., 1990. Mineral distribution in avocado trees with reference to calcium cycling and fruit quality. Sci. Hort. 44, 279–291.

Xing, J., Bravo, C., Jancsó, P.T., Ramon, H., De Baerdemaeker, J., 2005. Detecting bruises on 'Golden Delicious' apples using hyperspectral imaging with multiple wavebands. Biosyst. Eng. 90, 27–36.

Xing, J., Landahl, S., Lammertyn, J., Vrindts, E., De Baerdemaeker, J., 2003. Effects of bruise type on discrimination of bruised and nonbruised 'Golden Delicious' apples by Vis-NIR spectroscopy. Postharvest Biol. Technol. 30, 249–258.

Zacarìas, L., Alférez, F., Gariglio, N., Agustí, M., 2000. Rind breakdown in 'Navelate' oranges: influence of the rootstock. Proc. Int. Soc. Citricul. 1, 512.

Zaragoza, S., Almela, V., Tadeo, F.R., Primo-Millo, E., Agustí. M., 1996. Effectiveness of calcium nitrate and GA3 on the control of peel-pitting of fortune mandarin. J. Hort. Sci. 71, 321–326.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort.125, 401–405.

Table 1: An overview of symptomology, time to occurrence and causal factors of different physiological rind disorders on oranges.

Cultivar	Name of disorder	Symptoms	Causal factors	Time to occurrence	Pre-/ post - harvest	Country	Reference
Navel	Peel pitting	Collapsed areas of flavedo & part of albedo that becomes brown with time.	Wide array of preharvest and postharvest factors	ND	Pre- and postharvest	China	Gao et al. (2006)
Navel	Postharvest rind pitting or rind staining	Scattered clusters of pits over the fruit surface that turn dark brown after several weeks	Water loss and sudden changes from low to high RH and waxing	1 to 2 weeks	Postharvest	Spain	Alférez and Zacarìas (2001)
Navel	Peel pitting	Collapsed areas of the flavedo and part of the albedo that becomes brown with time	Wide array of preharvest and postharvest factors	1 week	Postharvest	China	Li et al. (2009)
Navel	SERB	Collapse and browning of the peel at the stem end of the fruit	Water loss and sudden changes from low to high RH	ND	Postharvest	USA	McCornack (1970)
Navel & Valencia	SERB	Collapse and browning of the peel at the stem end of the fruit	Rootstock and storage temperature	9 days	Postharvest	USA	Ritenour et al. (2004)
Navelate	Peel pitting/ Rind breakdown/ rind staining	Collapse, drying and browning of the flavedo	Water loss and sudden changes from low to high RH	7 to 28 days	Postharvest	Spain	Alquezar et al. (2010)
Navelate	Non-chilling peel pitting	ND	Ethylene inhibition by 1-MCP (reduced by external ethylene application)	2 to 14 days	Pre- and postharvest	Spain	Estables-Ortiz et al. (2009)
Navelate	Non-chilling peel pitting	Irregular depressed areas on the flavedo that may turn brown with time	Lack of epicuticular wax and dehydration	7 to 22 days	Postharvest	Spain	Cajuste and Lafuente (2007); Cajuste et al. (2010)
Navelate	Non-chilling peel pitting	ND	Dehydration	14 to 56 days	Postharvest	Spain & Italy	Alférez et al. (2005)
Navelate & Pinalate	Rind breakdown	Sunken areas of the peel which develop into reddish-brown, dry areas on the flavedo.	Water loss and sudden changes from low to high RH	ND	Pre- & post-harvest	Spain	Agustí et al (2001)
Navelate	Non-chilling peel pitting	ND	Lack of epicuticular wax and dehydration. Increased internal ethylene production	14 days	Postharvest	Spain & Italy	Sala et al. (2005)

SERB, Stem end rind breakdown; RH, Relative humidity; ND, No data.

Table 1: (Continued)

Cultivar	Name of disorder	Symptoms	Causal factors	Time to occurrence	Pre-/ post - harvest	Country	Reference
Navelate & Pinalate	Rind staining	Collapsed and dry areas of the flavedo and part of the albedo that becomes dark with time	Abscisic acid, ethylene, RH and storage temperature	6 to 24 days	Postharvest	Spain	Lafuente and Sala (2002)
Navelina	Non-chilling peel pitting	ND	Water loss and sudden changes from low to high RH & increase in ethylene	7 to 28 days	Postharvest	Spain	Sanchez-Ballesta et al. (2008)
Pinalate	Rind breakdown	ND	Increased water loss	5 days	Postharvest	Israel	Porat et al. (2004)
Shamouti	Superficial rind pitting	Collapse and necrosis of flavedo	Water loss	3 to 5 weeks	Pre- & postharvest	Israel	Tamin et al. (2001)
Shamouti	Noxan	Superficial necrosis and pits on flavedo	Low RH, increased water loss	7 to 28 days	Postharvest	Israel	Peretz et al. (2001)
Shamouti	Noxan	Collapsed hypodermis cells in the flavedo	Low RH, increased water loss	10 to 60 days	Postharvest	Israel & South Korea	Ben Yehoshua et al. (2001)
Navel	SERB	Collapse and browning of the peel at the stem end of the fruit	Holding fruit at low RH after picking	2 weeks	Postharvest	USA	McCornack (1970)
Valencia	Pitting	Sunken-necrotic areas on the stylar end of the fruit	Tree water stress at maturity	ND	Pre- & postharvest	USA	Albrigo et al. (1970)
Valencia	SERB	Collapse and browning of the peel at the stem end of the fruit	Lack of epicular wax and dehydration	ND	Postharvest	USA	Albrigo (1972a)

SERB, Stem end rind breakdown; RH, Relative humidity; ND, No data.

Table 2: An overview of symptomology, time to occurrence and causal factors of different non-chilling physiological rind disorders on grapefruit.

Cultivar	Name of disorder	Symptoms	Causal factors	Time to occurrence	Pre-/ post - harvest	Country	Reference
Marsh	Peel pitting	Depressions in the flavedo that ultimately affect oil glands	Low RH during harvest before storage at high RH	2 to 6 days	Postharvest	USA & Spain	Alférez et al. (2005)
Marsh	Pitting	Clusters of collapsed oil glands over the surface of the fruit	Waxing	ND	Postharvest	USA	Sun and Petracek (1999)
Marsh	Peel pitting	Sunken areas on the flavedo followed by browning and dryness	Waxing, warm-temperature storage and sudden changes from low to high RH	2 to 20 days	Postharvest	USA	Alférez and Burns (2004)
Marsh	Peel pitting	Sunken areas on the flavedo followed by browning and dryness	Variation in water, osmotic, & turgor potential.	2 to 20 days	Postharvest	USA & Spain	Alférez et al. (2010)
Marsh	Pitting	Clusters of collapsed oil glands over the surface of the fruit	Waxing coupled with warm-temperature storage	10 days	Postharvest	USA	Petracek et al (1995; 1998)
Ray ruby	SERB	Collapse and browning of the peel at the stem end of the fruit	Rootstock and storage temperature	9 days	Postharvest	USA	Ritenour et al. (2004)

ND, No data; RH, Relative humidity; SERB, Stem end rind breakdown.

Table 3: An overview of symptomology, time to occurrence and causal factors of non-chilling physiological rind disorders reported on mandarin fruit.

Cultivar	Name of disorder	Symptoms	Causal factors	Time to occurrence	Pre-/ post - harvest	Country	Reference
Clementine	Preharvest rind-spotting	Irregular shaped necrotic spots on the rinds	Sudden changes of climatic conditions	ND	Preharvest	Greece	Assimakopoulou et al. (2009)
Encore	Preharvest rind staining	Chlorotic spots	Exposure to solar radiation	ND	Postharvest	Portugal	Maia et al. (2004)
Encore	Peel pitting	Chlorotic spots in the epicarp	Exposure to direct sunlight	ND	Preharvest	Portugal	Vitor et al. (2000)
Encore	Peel pitting	Chlorotic spots in the epicarp	Exposure to direct sunlight	ND	Preharvest	Portugal	Medeira et al. (1999)
Fallglo	Postharvest pitting	Necrosis and scattered collapse of the flavedo	Waxing	2 to 20 days	Postharvest	USA	Petracek et al. (1998)
Fallglo	Peel pitting	Depressions in the flavedo that ultimately affect oil glands	Low RH during harvest before storage at high RH	2 to 6 days	Postharvest	USA & Spain	Alférez et al. (2005)
Fortune	Pitting, necrosis, staining	ND	ND	ND	Postharvest	Spain	Sanchez-Ballesta et al. (2001)
Fortune	Peel pitting	Sunken lesions and darkening of oil glands	Prolonged storage duration and dehydration	15 days	Postharvest	Morroco	El-Hilali and Akhayat (2004)
Fortune	Rind pitting	ND	Exposure to direct sunlight and high maturity	14 to 28 days	Preharvest and Postharvest	Spain	Duarte and Guardiola (1995)
Nova	Rind pitting	ND	Exposure to direct sunlight and high maturity	14 to 28 days	Preharvest and Postharvest	Spain	Duarte and Guardiola (1995)
Nules Clementine	Rind breakdown	Collapse of the oil gland cells leaking into the flavedo causing oxidation and browning of the rind	Higher shipping temperature, fruit from inside of the tree	42 to 84 days	Postharvest	South Africa	van Rensburg and Bruwer (2000)
Nules Clementine	Rind breakdown	Collapse of the oil gland cells leaking into the flavedo causing oxidation and browning of the rind	Direct sunlight, higher shipping temperatures	42 to 84 days	Postharvest	South Africa	van Rensburg et al. (2004)
Nules Clementine	Rind breakdown	Dark/brown spots associated with the collapse of oil glands	Shading and low sunlight	3 to 5 weeks	Postharvest	South Africa	Cronje et al. (2011a)

ND, No data.

Table 4: Review publications on citrus postharvest physiological disorders since 2000

References[a]	Scope of the review
Agustí et al. (2002)	An article reviewing citrus fruit quality, physiological basis and techniques of improvement.
Agustí et al. (2004)	Reviews knowledge about causes, characteristics, water relationships and control measures of the most important postharvest physiological disorders occurring in citrus fruit.
Lafuente and Zacarìas (2006)	An overview of the most important postharvest physiological disorders occurring in citrus fruit.
Ladaniya (2008)	A chapter reviewing causal factors of major physiological disorders in citrus and their management.
Rodov et al. (2010)	A section in a review discussing the effect of modified humidity packaging on fresh produce including physiological disorders of citrus fruit.
El-Otmani et al. (2011)	A book chapter reporting on aspects related to citrus fruit development, maturation in relation to quality and physiological disorders during postharvest

[a] These reviews did not cover rind anatomy, histological characteristics, and molecular biology of fruit affected by the disorders vs. unaffected fruit as well as potential aspects of using non-destructive technology to predict fruit susceptibility to these disorders.

Fig. 1: Visual symptoms of non-chilling postharvest pitting of 'Nova' mandarin (A) and rind breakdown of 'Nules Clementine' mandarin (B) (From: Cronje et al., 2011a, with kind authorisation from Elsevier BV).

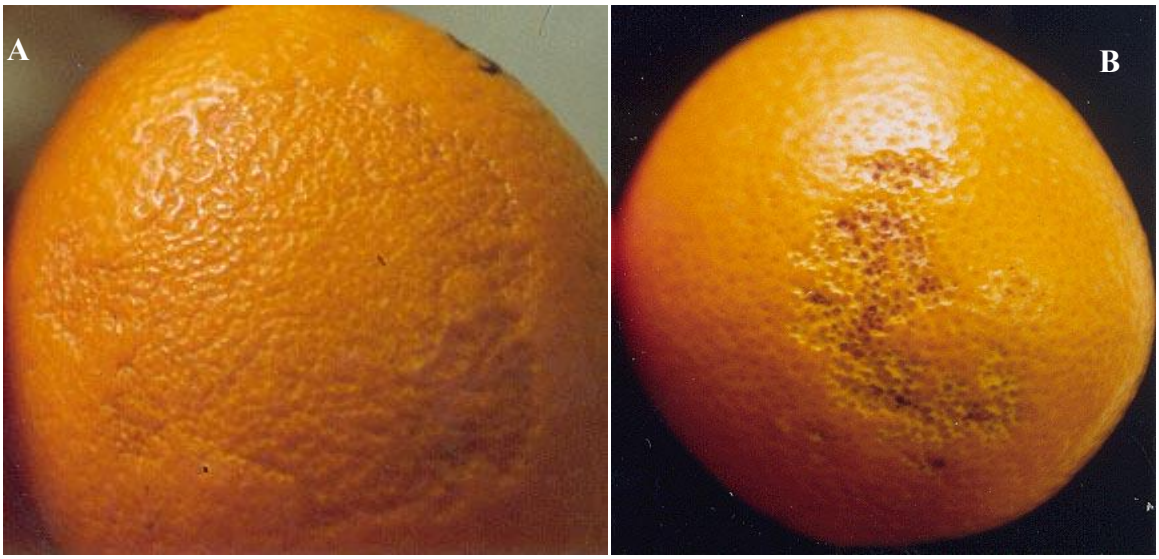

Fig. 2: Rind staining or peel pitting at non-chilling temperature in 'Navelate' (A) and 'Navelina' (B) oranges is aggravated by transference of dehydrated fruits to high RH (From: Alférez et al., 2003, with kind authorisation from Elsevier BV).

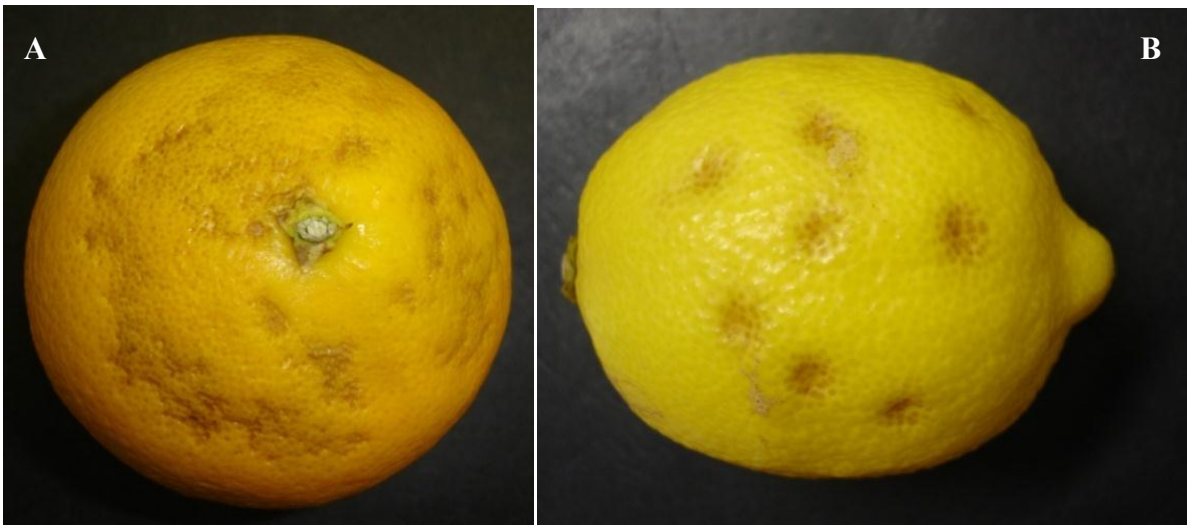

Fig. 3: Rind staining in ‘Navel’ orange (A) and peteca spots of lemon (B) (Photographs by Dr Paul Cronje, unpublished).

Fig. 4: Chilling injury in ‘Navel’ orange (A) and ‘Satsuma’ mandarin (B) (Photographs by Dr Paul Cronje, unpublished).

Fig. 5: Rind breakdown of 'Navelate' sweet orange. A: First symptoms are characterised by depressed colourless areas; B: fruits with severe symptoms of preharvest peel pitting develop reddish-brown and dry areas (From: Agustí et al., 2001, with kind authorisation from Oxford University Press).

Fig. 6: Symptoms of stem end rind breakdown on 'Marsh' grapefruit (From: Ritenour and Dou, 2003, with kind authorisation from Dr M. Ritenour).

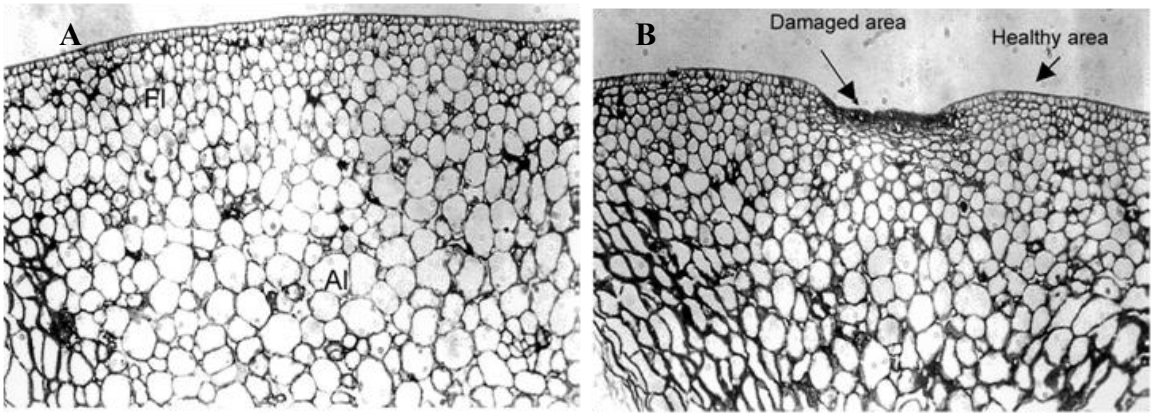

Fig. 7: Cross-sections of rind of 'Navelate' sweet orange. Healthy fruits (A) and fruit with well developed (B) symptoms of rind breakdown. Note on B, epidermal and hypodermal cells are crushed; the surface of the fruit is sunken (From: Agustí et al., 2001, with kind authorisation from Oxford University Press).

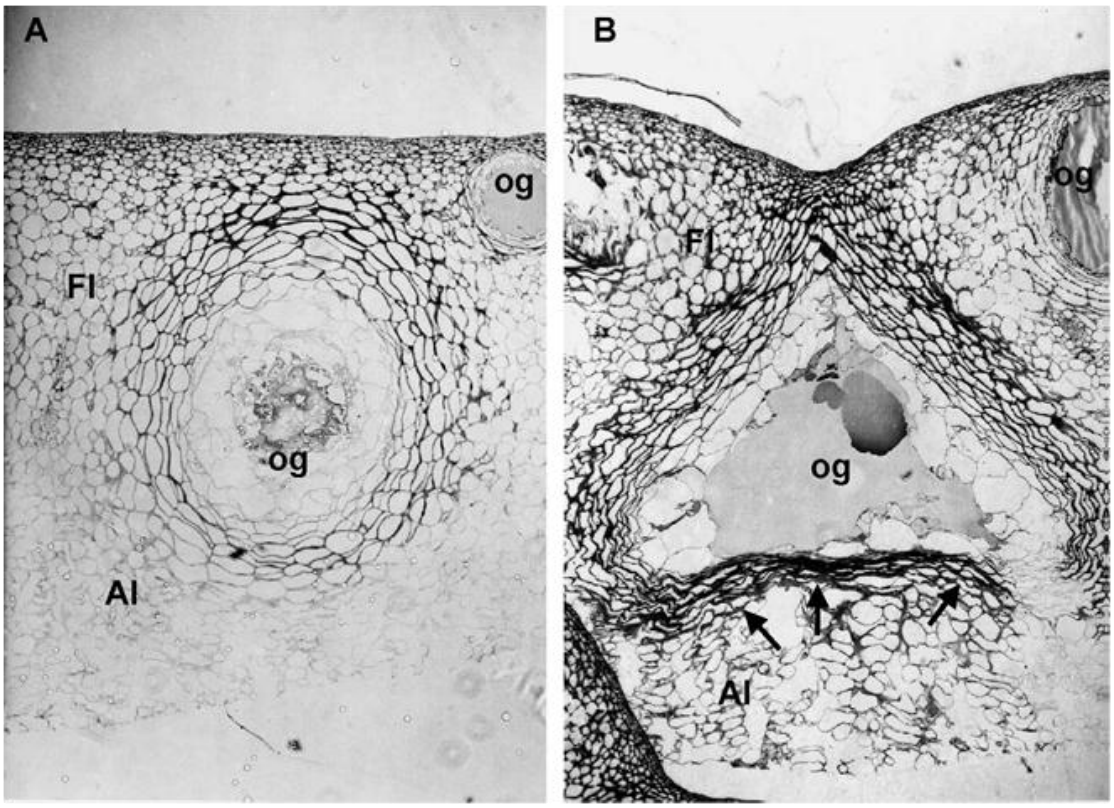

Fig. 8: Light micrographs of cross-sections of 'Marsh' grapefruit peel from healthy (A) and pitted (B) fruit. Al: albedo; Fl: flavedo; og: oil gland (From: Alférez and Burns, 2004, with kind authorisation from Elsevier BV).

CHAPTER 3

LITERATURE REVIEW

PAPER 2:

NIR SPECTROSCOPY APPLICATIONS FOR INTERNAL AND EXTERNAL QUALITY ANALYSIS OF CITRUS FRUIT – A REVIEW*

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NIR SPECTROSCOPY APPLICATIONS FOR INTERNAL AND EXTERNAL QUALITY ANALYSIS OF CITRUS FRUIT – A REVIEW

Abstract

The global citrus industry is continually confronted by new technological challenges to meet the ever increasing consumer awareness and demand for quality assured fruit. To face these challenges, a recent trend in agribusiness is a declining reliance on subjective assessment for quality and increasing adoption of objective, quantitative and non-destructive techniques of quality assessment. Non-destructive instrument-based methods are preferred to destructive techniques because they allow the measurement and analysis of individual fruit, reduce waste and permit repeated measures on the same item over time. A wide range of objective instruments for sensing and measuring the quality attributes of fresh produce have been reported. Among non-destructive quality assessment techniques, visible to near-infrared (Vis/NIR) spectroscopy (Vis/NIRS) is arguably the most advanced with regard to instrumentation, applications, accessories and chemometric software packages. This chapter reviews research progress on Vis/NIRS applications in internal and external quality measurement of citrus fruit, including the selection of NIR characteristics for spectra capture, analysis and interpretation. A brief overview on the fundamental theory, history, chemometrics of Vis/NIRS including spectral pre-processing methods, model calibration, validation and robustness is included. Finally, future prospects for NIRS-based imaging systems such as multispectral and hyperspectral imaging as well as optical coherence tomography as potential non-destructive techniques for citrus quality assessment are explored.

Keywords: Non-destructive evaluation · Near-infrared spectroscopy · Vis/NIRS · Citrus fruit · Internal quality · External quality · Hyperspectral · Multispectral · Optical coherence tomography (OCT) · X-ray computed tomography (CT).

1. Introduction

Assessment of quality parameters is one of the oldest techniques in fruit postharvest management (Butz et al., 2005). Most acceptable citrus fruit quality classification systems, whether manually operated or automated, are based on subjective assessment of visible external aspects of fruit based on surface colour, size, shape and presence of defects (Kader, 2002; Sun et al., 2009). This is mainly because until recently only these attributes could be measured non-destructively at a speed compatible with the typical speed of commercial sorting lines, which may be as high as 10 fruits (Nicolaï et al., 2008). Increasing consumer demand for internal quality attributes such as sweetness and nutritional content, coupled with industry demand for innovative tools for rapid and cost-effective detection and monitoring of physiological disorders, have spurred considerable interest among researchers on the application of visible to near-infrared (Vis/NIR) spectroscopy (Vis/NIRS) for citrus fruit quality monitoring and evaluation. The objective of this paper is to discuss the current knowledge about Vis/NIRS applied to citrus quality analysis and identify other emerging and innovative technologies for the non-destructive investigation of external and internal quality of citrus fruit.

2. Application of Vis/NIRS for citrus fruit quality analysis

Most of the Vis/NIRS research on citrus fruit has focused on assessing internal quality attributes (Liu et al., 2010a, b; Gómez et al., 2006; Fraser et al., 2003). Very limited research work has been conducted on Vis/NIRS applications to assess, monitor, and predict internal and rind physiological disorders (Geecola et al., 1994; Miller and Delwiche, 1991; 2010a, b; Peiris et al., 1998; Clark et al., 2004). This section of the review focuses on general aspects of Vis/NIRS applied to citrus fruit, including the selection of NIR characteristics and wavelength, data acquisition, analysis, and interpretation.

2.1. Selection of Vis/NIR characteristics

Typical Vis/NIR spectra of citrus fruit, ‘Satsuma’ mandarin (Golic et al., 2006) and ‘Valencia’ oranges (Magwaza et al., 2011) are shown in Fig. 1 and Fig. 2, respectively. The

pattern of the absorption curves is similar to that for other fruit such as kiwifruit (McGlone and Kawano, 1998) and mango (Guthrie and Walsh, 1997) although positions and size of peaks are specic specific. From the visible region (400–700 nm) a continuous decrease in absorbance with the minimum at 680 nm is observed. High absorbance observed at 672 nm is indicative of red absorbing pigments, particularly chlorophyll that gives the fruit its characteristically green colour (Gòmez et al., 2006). After this peak, there is a very sharp drop in absorbance as the spectrum enters the NIR region. Gòmez et al. (2006) estimated this drop to be 12 fold. From 680 to ~910 nm the absorbance spectrum stays relatively flat until a prominent peak centred at 975 nm appears. Since a strong water and carbohydrate absorption band exists at 958 nm (Williams and Norris, 1987; McGlone and Kawano, 1998) and 935 nm (Kawano et al., 1993) respectively, this peak is believed to result from water and carbohydrate absorption. Two other peaks related to strong water absorbance exist between 1440 and 1485 nm, and in citrus fruit these water absorption peaks occur at 1184 and 1457 nm.

When working in the NIR region of the spectrum, knowledge of the infrared (IR) absorption wavelengths (3000 to 1000 000 nm) of certain functional groups is critical in knowing where to look in the NIR for overtones (Wetzel, 1983). Compared to NIR, IR radiation is strongly absorbed by organic molecules, with the wavelength of absorption characteristic of the molecule. The absorbance in the NIR region of the spectrum is typically 10–100 times weaker than that of the fundamental absorption bands in the IR region of the electromagnetic spectrum (Walsh et al., 2000). Weak absorptions in the NIR have been cited as a shortcoming by Walsh et al. (2000).

In contrast to IR peaks which are narrow and diagnostic, peaks in NIR spectra are broad, up to 100–150 nm wide (Walsh et al,. 2000). Thus, NIR spectra are comprised of broad band which arise from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C-H, O-H, N-H, and S-H chemical bonds (Osborne, 2000). The molecular vibrations, particularly by O-H and C-H bonds, are responsible for strong absorption bands in the NIR spectral region by biological material, with the absorption band of water O-H bonds dominating in hydrated material (Golic et al., 2003; Nicolaï et al., 2009).

Water constitutes about 80–90% of fruit and vegetables (Goméz et al., 2006) and because of the high water content, water absorption features (bands) dominate the NIR spectrum of fruit and vegetables (Palmer and Williams, 1974). The water absorption bands are fairly broad with centres at approximately 970, 1200, 1450, 1950 and 2250 nm (Williams and Norris, 2001). The

band assignments of the major water and sugar (O-H and C-H) vibrations are summarised in Table 1. Hydrated objects are characterised by complex hydrogen bonding interactions between water, sugar, protein, etc. In NIR spectra of hydrated samples with large molecules such as fruit and vegetables, the effective absorption bands are relatively wide and complex, even at the fundamental or infrared (IR) frequency, due to different chemical environment of each O-H and C-H bonds in water and sugar molecules (Guthrie et al., 2005a). The wavelength drift or shift in informative peaks may be observed due to colour differences, mechanical changes of the monolithic spectrophotometer and temperature changes resulting in the physicochemical properties of fruit tissue (Gaffney, 1973; Zude et al., 2008).

2.2. Data acquisition modes

A host of investigators have extensively evaluated the potential to measure quality attributes of intact citrus fruit by means of Vis/NIRS (e.g. McGlone et al., 2003; Cayuela, 2008). However, application and data acquisition techniques vary among different groups of investigators. In practice, there are five different Vis/NIRS measurement modes fitting different applications. These modes are transmittance, interactance, transflectance, diffuse transmittance and diffuse reflectance (Huang et al., 2008). In citrus, only three types of data acquisition configurations are frequently used for intact fruit quality evaluation and prediction in the Vis/NIR system and those are reflectance, transmittance and interactance (Schaare and Fraser, 2000; Fraser et al., 2003; Cayuela and Weiland, 2010).

Reflectance spectra provide information about the external appearance attributes, which are commonly accepted criteria for the product quality evaluation. Generally, reflectance spectra do not contain sufficient information about the internal quality of the flesh (Krivoshiev et al., 2000). One of the advantages of reflectance mode as reported by Fu et al. (2007) is that measurements are easier to obtain and light levels in this mode are very high, however, variations in superficial and surface properties of the fruit may influence calibrations.

In interactance mode, the field of view of the detector is separated from the illuminated surface by a light seal in contact with the fruit surface (Schaare and Fraser, 2000). Basically, the relative merit of interactance mode is that it provides a compromise between reflection and transmission modes.

Transmission mode is considered the most frequently used and appropriate to assess internal quality attributes such as sugars and acids levels of thick-skinned fruit such as mandarin and orange (Kawano et al., 1993; Miyamoto et al., 1998; Cayuela, 2008). However implementation of transmittance spectra measurement mode is limited by two fundamental factors. The first is the fact that transmittance spectra of fruit with different sizes, thickness of peel and shape have different optical path and optical density (OD) (Krivoshiev et al., 2000). It is therefore expected that calibration model performance of spectra collected using transmission optical geometry will be affected by size, shape and peel thickness (Guthrie et al., 2005a). The second is the fact that in transmittance spectra fruit contains useful information about flesh spectrum and, together with it, the peel spectrum. Since the fruit peel is part of the light path, the spectrum of flesh optical density will vary depending on the changes in peel OD. Basically, fruit should be considered as an object with three-layer structure of "peel-flesh-peel" and the distortion influence of the peel should be adjusted accordingly during the analysis of transmittance spectra (Kawano et al., 1993). However, according to Dull et al. (1989a), it is accepted that for products of the same species and variety the peel thickness is constant and negligible. In a study carried out by the authors, it was concluded that the inclusion of the peel in the light path does not change the fundamental dependencies between the spectra and chemical data. Similar argument was reported in a research paper by Krivoshiev et al. (2000). This team of investigators argued that the transmittance of the flesh or internal quality can be predicted with sufficient accuracy regardless of the peel spectrum.

In a later study of cantaloupe, Dull et al. (1989b) established a higher correlation coefficient (*R or r*) value of 0.97 and lower standard error of calibration (*SEC*) of 0.56 °Brix when determining SSC of slices compared to when intact fruit were measured. On intact fruit measurements *r* decreased to 0.60 and *SEC* increased to 1.67 °Brix. In melons, Dull and Birth (1989) observed lower *r* value (0.87) and higher *SEC* value of 1.6 °Brix. From these studies it would seem that in fruit with thick peel, the distorting influence of the peel is more pronounced than those with thin peel. This argument is in accord with the statement by Kawano et al. (1993) who noted that it would be more difficult to determine internal composition in fruit with a thick peel than in fruit with thin peel. Interactance or body transmittance NIR spectrometric techniques are recommended modes for the internal quality assessment of fruits and vegetables (Peiris et al., 1999).

McGlone et al. (2003) explored the relative merits of three different measurement modes of the Vis/NIRS on 'Satsuma' mandarin fruit. The transmittance mode delivered the most accurate SSC prediction (R^2 = 0.96, *RMSEP* = 0.32 °Brix). Results of the interactance mode were superior (R^2 = 0.85, *RSMEP* = 0.47 °Brix) to those of the reflectance mode (R^2 = 0.75, *RMSEP* = 0.63 °Brix). Irrespective of the slightly higher prediction outcomes using transmittance that were reported by these authors, good results using reflectance (diffuse reflectance) mode have also been reported on oranges (Lu et al., 2006; Cayuela, 2008; Cayuela and Weiland, 2010; Liu et al., 2010a) and mandarin fruit (Gómez et al., 2006). Ou et al. (1997) and Greensill and Walsh (2002) also developed partial least square (PLS) prediction models of mandarins using interactance mode and reported very good calibration and prediction statistics. Another group of investigators that has used interactance mode of Vis/NIRS to assess internal quality attributes of citrus fruit was Guthrie et al. (2005a). These authors reported typical R^2 values of 0.75 and 0.90 for TSS and dry matter (DM), respectively.

Similarly, on kiwifruit, Schaare and Fraser (2000) evaluated the ability of three modes of Vis/NIR spectroscopic measurements to non-destructively estimate internal quality and reported the interactance mode to provide the most accurate estimates of SSC, density, and flesh colour. SSC, for instance was predicted with a standard error of prediction (*SEP*) of 0.80 °Brix and *r*-value of 0.96. Fu et al. (2007) later compared transmission and reflectance modes of Vis/NIRS for detecting brown heart disorder in pears. The results obtained by this group of investigators indicated that transmission mode was better than reflectance mode for internal disorder detection.

2.3. Data acquisition positions

The distribution and level of attributes, such as TSS, within the fruit may differ with maturation and cultivar, growing conditions and size of the fruit (Guthrie et al., 2005a, b). According to Peiris and co-workers (1999), the coefficient of variation of TSS within a single orange fruit was 10.2, 1.8, and 5.6% in proximal to distal ends, around the fruit circumference (equatorial) and radial orientation, respectively; a similar trend was reported by the authors on grapefruit. It is known that titratable acidity (TA) differs with fruit positions in citrus (Moon and Mizutani, 2002). The titratable acidity is higher at the equator than at the stem and stylar ends

(Moon and Mizutani, 2002). However, according to Guthrie et al. (2005a), mandarin fruit are more homogeneous than orange and grapefruit.

In other fruit types, chemical composition has been reported to vary from stem end to blossom end, and from sun to shade sides of the fruit (Guthrie and Walsh, 1997). In big fruit such as pineapple and melons, the variation is more pronounced. For example, the SSC content of the bottom of the pineapple fruit is consistently about 3 °Brix higher than the top of the fruit (Smith, 1984). A very similar trend was reported by Guthrie and Walsh (1997). These investigators further reported that in addition to SSC gradient from top to bottom, the sun-exposed side of the fruit was generally 1 °Brix higher than the shaded side. These authors suggested that in order to minimise the effect of variation within fruit on the obtained spectral data, Vis/NIRS assessment could be made at either a set location on the fruit or repeat the spectral acquisition at several positions around the whole fruit. Likewise, Guthrie et al. (2005a, b) recommended that optical and reference sampling for all citrus fruit should be at any position around the equatorial position of the fruit in order to best represent the entire fruit. However, this is insufficient when, for example, skin disorders are to be detected in a practical application (Nicolaï et al., 2007a).

2.4. Chemometrics

The development of a Vis/NIR spectroscopic technique for assessing a quality trait in fruit or vegetable relies upon collecting spectra of the product samples and developing calibration equations to relate this spectral data with the quality traits ascertained using standard destructive laboratory method (Peiris et al., 1999). One of the main problems encountered in product characterisation from Vis/NIRS signal has been the large volume of primary data (typically between 250 and 2000 points per spectrum, depending on the spectrum used) (Kim et al., 2000). After the door had opened and the potential demonstrated by the pioneers, Vis/NIRS encountered fast development encouraged mainly by instrumental (spectrometer) improvements associated with spectral data acquisition and their treatment (micro-processor) and which, to a great extent depended on the new discipline of chemometrics which supplies the tools for gathering information (Wetzel, 1983; Wang and Paliwal, 2007). Basically, the two techniques are closely related, as Vis/NIRS would never have reached its present stage of development without

chemometrics and Vis/NIRS results are frequently used to illustrate the power of new chemometric algorithms (Blanco and Villarroya, 2002).

The interpretation of the spectra may take place via direct spectrum identification using data bases or increasingly by applying procedures of high speed chemometrics (Butz et al., 2005). Chemometrics is the study of statistical and mathematical methods applied to the field of chemistry (McClure, 2003). Calibration methods are developed using various chemometric tools, such as partial least squares (PLS) regression (PLSR), multivariate linear regression (MLR), principal component regression (PCR) also referred to as principal component analysis (PCA), artificial neural networks (ANN) to mention a few (Blanco and Villarroya, 2002; Nicolaï et al., 2007b).

MLR is the oldest method of calibration developed in the Vis/NIRS (Norris and Hart, 1965). Since its introduction numerous other methods of calibration have been proposed including Fourier Transform (FT) regression (FTR), PCR, PLSR etc. For the Vis/NIRS non-destructive prediction of fruit compositional attributes, MLR or any of the abovementioned analysis procedures are used to relate specific spectral regions to changes in the concentration of a known attribute (Kim et al., 2000). Simple linear multivariate least squares regression models typically do not perform well because of the often high collinearity of the spectra (Saiz-Abajo et al., 2005). For the modelling of complicated data sets in terms of chains of matrices and to overcome the problem of collinearity encountered with linear multivariate least squares regression models, PLSR was introduced by Herman Wold in 1975 (Wold et al., 2001). Guthrie et al. (2005a) compared TSS models of mandarin fruit developed from two regression methods, MLR and PLSR. The authors found that the PLS models were better than MLR models in both calibration development and validation on independent data sets.

PLSR has been used in the development of many prediction models for fruits and vegetables quality parameters, and many applications are reported on citrus (McGlone and Kawano, 1998; Carlini et al., 2000). As stated by Saiz-Abajo et al. (2005), PLSR is today probably the most widely applied method in chemometrics. In ‘Satsuma’ mandarin fruit, the PLS approach was used to evaluate the use of Vis/NIR in measuring the quality characteristics (Gòmez et al., 2006). Using PCR and PLSR to build models, the authors reported the calibration technique to influence the results in a similar fashion that data pre-processing affects the performance of the model. They reported that PLS models were better than PCR models. Besides resulting in better

prediction models, PLS analysis results in models which always have the lowest number of latent variables (LV) since PLS models exclude LVs that are not important to describe the variance of the quality parameter (De Jong, 1993). For instance, in Gòmez et al. (2006) the calibration model for SSC content using PLS had 4 LVs and the following statistics: r = 0.97, *SEC* = 0.160 °Brix while that of PCR had 6 LV, r = 0.969 and *SEP* = 0.163 °Brix.

According to Nicolaï et al. (2007b) the principles of the PCR and PLSR methods are different. The PLSR method is commonly used in quantitative spectroscopy to correlate spectroscopic data (***X***) with related physico-chemical data (***Y***) (Saiz-Abajo et al., 2005). The method is based on LVs like PCA and PCR. Nicolaï et al. (2007a) stated that "unlike PCR in which the LVs are selected so as to explain as much variance of ***X*** as possible with only a limited LV, in PLS the LVs are selected in such a way that the covariance between ***X*** and ***Y*** is maximal". Hence, the variation in ***X*** directly correlating with ***Y*** is extracted. Basically, PLS as described by Cen et al. (2006, 2007), is a bilinear modelling method in which the original independent information (***X*** variable) is projected into a small number of LVs to simplify the relationship between **X** and **Y** for prediction. The authors further elaborate that, "the ***Y*** variable is actively used in assessing the LVs to ensure that the first one is most relevant for predicting the ***Y*** variable".

Various researchers have explored different regression techniques on citrus fruit. Using FT-NIRS on intact citrus fruit to predict SSC, Lu et al. (2006) reported that a PLS model produced better prediction results than PCR. In the work of Cayuela (2008), portable Vis/NIR in reflectance was used to predict SSC, TA and pH of 'Valencia' oranges. The observed spectra were similar to those described by Gómez et al. (2006) in 'Satsuma' mandarins. However, SSC models by Gómez et al. (2006) were superior to that of Cayuela (2008) who obtained r value of 0.95 and RMSEP of 0.51 °Brix. The latter researchers used PLS and modified PLS (MPLS) to develop models for predicting SSC, pH and TA content of mandarin fruit and reported PLS calibration models for SSC to perform better than MPLS models. Due to very low coefficients of calibration obtained for TA and pH, none of the calibration method delivered acceptable prediction model for these parameters. However, a comparison between mandarin (Gómez et al., 2006) and oranges (Cayuela, 2008) is not recommended for oranges are larger in size and have a different structure to mandarin.

Recently, a modification to PLS called interval PLS (iPLS) which searches for spectral interval that is particularly informative with respect to the parameter under consideration has been developed to simplify the model and improve its predictive ability (Nørgaard et al., 2000). The main advantage of the iPLS method is to present a local regression model in a graphical display, to choose a better spectral interval and permit a comparison between interval and the full spectrum models. In addition, wavelength selection enhances the stability of the calibration model resulting from the collinearity in multivariate spectra. Furthermore, wavelength selection helps to interpret the relationship between the model and the sample compositions. In a study aimed at investigating the feasibility of a charge coupled device NIRS (CCD-NIRS), Liu et al. (2010b) simplified and yet improved the predictive ability of an SSC model with *r*-value of 0.92 and *RSMEP* of 0.65 °Brix. In general PLSR has been proved by many researchers to be a better method than the others, such as MLR and PCR.

PLSR method is based on linear models and, consequently, unsatisfactory results may be produced when non-linearity is present (Liu et al., 2010a, b). ANNs are commonly used for modelling non-linearity. Some of the advantages of using ANNs include the flexible learning algorithm, diverse network topology, fast learning algorithm and high error tolerance. Recently, the authors compared PLS to PCA coupled with back propagation neural network (BPNN) (PCA-BPNN) to develop the model for predicting SSC of intact 'Navel' orange fruit. In contrast to results obtained by Lu et al. (2006) comparing PCA-BPNN and PLS, reported PCA-BPNN method to give better prediction model than PLS. Alternative non-linear techniques include kernel based models such as kernel PLS and least squares–support vector machines. This framework allows the PLS calibration to be carried out in a space of nonlinearily transformed input data—the so-called feature space–without actually carrying out the transformation. The advantage is that the feature space has a potentially much richer structure than the space spanned by the original input data set. However, Nicolaï et al. (2006) found that kernel PLS yields comparable results to those obtained with ordinary PLS (covariance kernel), irrespective of the kernels which had been evaluated. It is questionable whether any improvement of accuracy of the calibration models would be worthwhile given the noise due to biological variability and model robustness issues.

2.5. Spectral pre-processing

The spectra of solid and scattering samples such as intact fruit and vegetables are influenced by physical properties such as shape, size, etc. (Leonardi and Burns, 1999). This creates noise when analysing quality parameters for which such physical characteristics are not important (Blanco and Villarroya, 2002). In the analysis of spectra, there are large baseline shifts and noises in the spectra with broad wavelength regions, thus the selection of suitable pre-processing or pre-treatment method is an important step in spectral analysis and calibration (Cen et al., 2006). In order to facilitate handling and to develop more simple and robust models, the complex spectral data are often pre-treated by statistical procedure (Butz et al., 2005).

Some of the more frequently used pre-treatment methods for Vis/NIR spectra include normalisation, derivatives (usually first and second), the multiplicative signal correction also known as multiplicative scatter correction (MSC), Savitsky-Golay first and second derivatives, the standard vector normalisation (SNV), smoothing using moving average, and de-trending (Blanco and Villarroya, 2002). Derivative spectroscopy is a method which has demonstrated success in correcting light scattering contributions provided that the spectrum describes a large wavelength range (Leonardi and Burns, 1999). To reduce the fruit size effect on citrus spectra, Kawano et al. (1993) normalised the second derivative spectra by dividing it by the second derivative value at 844 nm, which had a high correlation to the diameter of the fruit. However, derivative spectroscopy does not correct the pathlength variations. MSC is suggested to correct for pathlength variations.

In Gòmez et al. (2006), two types of pre-processing were employed. First was smoothing using moving average method at different data points and the second was the use of MSC. The later technique was used to correct for additive and multiplicative effects of spectra. From this study it was observed that pre-processing with MSC resulted in models that were better than models without MSC. Working on citrus with Vis/NIRS in reflectance mode, Lu et al. (2006) used three types of data pre-treatments; constant, SNV and MSC. They obtained similar statistical parameters for all three pre-treatment methods and argued that different spectral pre-treatment is not useful for improving quality of models. In contrast, when similar pre-treatment techniques, SNV and MSC, were applied to the spectra of 'Navel' oranges by Liu et al. (2010a), the MSC spectral pre-treatment method showed superior model than SNV.

2.6. Wavelength selection and light penetration depth

Vis/NIRS at different wavelengths ranges extending from the visible spectrum to the NIR has been used and established by many researchers to quantify different quality parameters of fruit and vegetables. However, due to the inherent variability in fruit types and the influence of the growing environment, there is no consensus between researchers on the best wavelength range to study each fruit quality parameter (Peiris et al., 1999). The wavelength region over which Kawano et al. (1993) made the Vis/NIR measurements for sugar content of 'Satsuma' mandarin was 680–1235 nm and reported spectral absorption at 844 nm to be related to fruit size. Similarly, Miyamoto and Kitano (1995) considered spectral absorption at 840–855 to be related to fruit diameter and found the key wavelengths for TSS quantification on peeled and intact mandarin fruit to be 770 and 905 nm, respectively. Miller and Zude (2002) used Vis/NIRS in the region of 400–1100 nm to develop models (r = 0.82) to measure SSC level on grapefruit and tangerine fruit. McGlone et al. (2003) concluded that a wavelength range from 750 to 1100 nm was optimal for the internal quality assessment of mandarin fruit.

In a study aimed at assessing internal quality attributes such as SSC and DM of mandarin fruit, Guthrie et al. (2005a, b) used the wavelength range between 720 and 950 nm. In a research work to evaluate the use of Vis/NIRS in measuring the firmness, SSC and acidity of 'Satsuma' mandarin fruit, Gòmez et al. (2006) developed sound models using the full Vis/NIR spectral range (350–2500 nm). According to McGlone et al. (2002), spectral measurements below 500 nm are usually too noisy to use in the model calibration. Lu et al. (2006) developed calibration models to predict SSC (r = 0.99, *RMSEP* = 0.79 °Brix) of 'Gannan' citrus fruit in the spectrum range of 800–2500 nm. Liu et al. (2010a) developed a SSC prediction model in the wavelength range of 350–1800 nm. In a later study, Liu et al. (2010b) selected the wavelength range between 350 and 1040 nm with a 0.2 nm sampling interval to develop SSC and TA prediction models of 'Nanfeng' mandarin fruit (*RMSEP* = 0.92; *RMSEP* = 0.65).

From the foregoing literature evidence, it is clear that majority of investigators used the short wave spectral range of the NIR radiation (700–1100 nm) to measure citrus fruit internal quality (Guthrie et al., 2005a, Gòmez et al., 2006; Liu et al., 2010a). All these groups of investigators chose this window for its relevance to sugar and water as it include the second and third overtone

of OH stretching and vibrations. In addition, these investigators considered absorption at these wavelengths to be associated with the fourth overtone of CH_2 and the third and overtone of CH and CH_2, respectively (Gòmez et al., 2006). Furthermore, this wavelength range is readily transmitted through citrus fruit irrespective of its thick rind (Fraser et al., 2003). However, McGlone et al. (2003) argued that "whilst it is true that wavelengths in the range 700–1100 nm are readily transmitted through citrus fruit, this does not mean that other wavelengths cannot extract useful information about the fruit". This statement is not uniquely applicable to citrus. Pallav et al. (2009) also noted that NIR signals in the 700 to 900 nm wavelength range are able to penetrate a short distance into most food materials, although the through-transmitted signal is still highly attenuated due to scattering and absorption within the food sample.

Researchers have closely scrutinised the hypothesis that processing the full spectrum can always lead to the best results. However, researchers such as Centner et al. (1996) and Carlini et al. (1999, 2000) argued that best suited models often involve few tens of carefully selected wavelengths. Centner et al. (1996) developed an elimination procedure, called uninformative variable elimination-PLS (UVE-PLS) and tried it on chemical data sets. This method outperformed (RMSEP = 1.61) standard full spectrum PLS (RMSEP = 1.86), because it allows one to eliminate uninformative variables in multivariate data sets before a final modeling is carried out. A forward selection technique for the identification of wavelengths to be included in a PLSR model was also tried by Osborne et al. (1997, 1999) and the calibrations obtained improved. Carlini et al. (1999) presented results on the use of wavelength selection methods for the accurate definition of models for evaluation of SSC in fruit. A selection scheme involving the sub-sampling of the spectral interval from 600 to 1100 nm, with a fixed step, was systemically able to produce better final prediction models than PLS.

The spectral region from 700 to 1100 nm is considered by many spectroscopists to be of particular relevance for predicting fruit quality parameters. According to Carlini et al. (2000) and Golic et al. (2003), this interval has the distinctive advantages of weak absorption by biological material and broad water absorption peaks and does not risk masking spectral information correlated to low concentration of constituents. In addition this light in this range can penetrate much farther in fruit of many species. The iPLS, which was developed by Nørgaard et al. (2000), searches for a spectral interval that is particularly informative with respect to the parameter under consideration. The iPLS is based on the principle of splitting the spectra into smaller equidistant

regions and the regression are developed in every subinterval (Nørgaard et al., 2000; Pereira et al., 2008). This method has been shown to be effective on citrus, mandarin fruit in particular, by improving PLSR models significantly (Liu et al., 2010b). In an attempt to predict SSC and TA on 'Nafeng' mandarin fruit, this group of investigators assessed different wavelength ranges and the optimal (681.36–962.75 nm) was chosen on the basis of the lowest root mean square error of cross validation (*RMSECV*) and root mean square error of prediction (*RMSEP*) values. This would seem logical as this NIR range is strictly known to contain important carbohydrate, sugar and water absorbances.

Due to the light scattering of fresh fruit, the incident light does not always travel the same distance before it is detected. A longer light path corresponds to a lower relative reflectance value, since more light is absorbed (Gòmez et al., 2006). The limited penetration depth restricts the potential of transmittance and interactance modes and decreases the accuracy of Vis/NIRS to measure internal quality attributes of thick-skinned fruit, such as citrus. Guthrie and Walsh (1997) attempted to assess the depth of tissue that contributes to the Vis/NIR spectrum (reflectance mode) of intact citrus fruit. They reported that the depth of penetration was dependant on the wavelength and concluded that all spectral information from the fruit was derived from within 5 mm of the fruit surface, with an exponential decrease in information with increasing depth within the 5 mm zone. It was however interesting to note that when fruit were sequentially sliced from a thickness of 90 to 5 mm no significant change in NIR prediction statistics was reported.

Lammertyn and co-workers (2000) investigated the skin reflectance and skin transmission properties with regard to Vis/NIR radiation using a wavelength range of 500–1900 nm. Penetration depth was calculated from measurements of transmitted light for various thicknesses of the fruit tissue. They discovered that penetration depth of Vis/NIR light through the fruit tissue is strongly dependant on wavelength and varies from 2 to 4 mm in the wavelength range of 700–900 nm and between 2 and 3 mm in the 900–1900 nm range. In a similar study using a less invasive technique, Fraser et al. (2001) reported a penetration depth of 35 mm at 713 nm, reducing to 1 mm in the 1400–1600 nm spectral regions. The big difference between penetration depths obtained by these two groups of investigators could be attributed to a number of issues. Firstly, their definition of penetration depth was different. Fraser et al. (2001) defined penetration depth as "the depth at which the light intensity dropped to 1% of the

incidence". Lammertyn et al. (2000) defined light penetration depth as "the slice thickness for which the diffuse reflectance spectra were significantly different from those of a slice of infinite thickness". It would also seem that the destructive nature of the procedure used by Lammertyn et al. (2000) was another source of variation since light scattering pattern would be affected by tissue/air boundary which significantly alters the optical geometry of investigated fruit. From both these studies it was shown that the penetration depth is much larger in the 700–900 nm range than in the 1600 nm range due to absorption profile of water.

Based on the above information, Fraser and co-workers (2003) selected the wavelength to use in a study of light distribution inside mandarin fruit during internal quality assessment by Vis/NIRS. Using illuminated laser light with 808 nm wavelength they observed a rapid reduction in light levels across the thick skin (~4 mm into the fruit) of mandarin fruit. Compared to the skin, they observed a less rapid reduction as the light continued to pass into the flesh (~14 mm into the fruit). In the fruit tissue regions (~22–32 mm into the fruit) they reported an exponential decay and a rapid drop across the distal skin (~50mm into the fruit). The steep decline in light levels as it passes through the skin of the fruit shows that the thick skin of citrus is highly attenuating compared to the flesh, as was shown by Kawano et al. (1993). The lower penetration depth due to low light intensity above 970 nm was further reported in a study by Kawano et al. (1993). Although the same generic features of absorbance are observed in the reflectance spectrum, much broader bands are observed. In addition, the chlorophyll absorption band appears more prominent as a dip in the spectrum, and the water absorbance is far less attenuating. Given that reflectance is surface dominated, that the skin is a quite dry, pithy material and that white pith (albedo) may have high reflective index and allow appreciable reflected light intensities to be measured, reflectance mode would necessarily be considered as the best measurement option for skin surface defects (McGlone et al., 2003).

McGlone et al. (2003) studied the SSC and TA of mandarin fruit in the wavelength range of 500–1100 nm. They confirmed that transmission mode of the NIR using a spectral window of 700–930 nm gives the most accurate SSC prediction model (R^2 = 0.93, *RMSEP* = 0.32 °Brix). Briefly, for fruit quality measurements, the NIR radiation must sufficiently penetrate into the fruit tissue of interest to allow the determination of the properties of interest (Lammertyn et al., 2000; Fraser et al., 2003). However, due to the high concentration of water present in fresh fruit, it is not possible to get sufficient light penetration outside the 200–1200 nm wavelength region

(Fraser et al., 2001). Previous research also suggests that the use of Vis/NIRS in transmittance mode and the wavelength range of 700–930 nm are better for the accurate prediction of SSC of citrus fruit (Kawano et al., 1993; Miyamoto et al., 1998).

2.7. Model accuracy

The accuracy of Vis/NIRS models for fruit quality prediction is usually described by the value of the *R* (Equation 1), the root mean square error of calibration (*RMSEC*) (Equation 2) and the *RMSECV* or *RMSEP* (Equation 3) (Lu et al., 2006; Sun, 2009; Liu et al., 2010a, b; Bobelyn et al., 2010; Camps and Christen, 2009).

A good model should have a lower *RMSEC*, *RMSECV*, and *RMSEP*, higher correlation coefficient (*r or R*) or coefficient of determination (r^2 *or* R^2), which represent the proportion of explained variance of the response variable in the calibration (R_c^2) or validation dataset (R_v^2). Other statistical parameters explaining a good model are low average difference between predicted and measured values (*Bias*) (equation 4) and a small difference between *RMSEC* and *RMSEP*. In addition, a good model should have as little as possible number of latent variables (*LV*) or principal components (*PC*).

$$R = 1 - \sqrt{\frac{\sum (y_{cal} - y_{act})^2}{\sum (y_{cal} - y_{mean})^2}} \quad (1)$$

$$\mathrm{RMSEC} = \sqrt{\sum (y_{cal} - y_{act})^2 / n} \quad (2)$$

$$\mathrm{RMSECV\ or\ RMSEP} = \sqrt{\sum (y_{pred} - y_{act})^2 / n} \quad (3)$$

$$\mathrm{Bias} = \frac{1}{n}\sqrt{\sum (y_{pred} - y_{act})^2} \quad (4)$$

Where:

n = the number of spectra

y_{act} = the actual value

y_{mean} = the mean value

y_{cal} = the calculated value

y_{pred} = the predicted value of the fruit attribute.

2.8. Model robustness

The steps followed in the development of a calibration equation are most of the time standard, but the conclusions and prediction models obtained are not universally applicable to all fresh fruit or that fruit type because of the effect of fruit surface characteristics and internal chemistry on the NIR reflectance spectra. The application of Vis/NIR technology to a given fruit commodity requires an assessment of the robustness of the developed calibration model across populations of fruit grown under differing conditions (Guthrie et al., 2005b). A robust model was defined by Nicolaï and co-authors (2007a) as a 'model with prediction accuracy that is relatively insensitive towards unknown changes in external factors'. These authors went further to list the within-tree variability (tree age, crop load, fruit position within the tree, light effect), within-orchard variability (location of the tree and light effects), orchard variability, fruit age and seasonal variability as main factors which may affect model performance. In addition, a robust calibration model should be able to perform with similar accuracy when transferred from one instrument to another.

Guthrie et al. (2005b) stated that differing growing conditions may result in differences in physical and chemical properties of the fruit. This change in fruit physico-chemical properties result in altered fruit optical characteristics and spectral band assignment (Golic et al., 2003; Guthrie et al., 2005b). Region and seasonal variation have been considered important to include in the Vis/NIRS experimental design as different growing conditions in different places and seasons affect fruit analyte levels with concomitant effects on NIRS prediction accuracy (McGlone et al., 2002). In support of this notion, Nicolaï et al. (2007a) further stated that external validation is of prime importance for the successful application of multivariate calibration models.

While model robustness has been investigated for other species (Lovász et al., 1994; Guthrie et al., 1998; McGlone and Kawano, 1998; Peirs et al., 2002; Guthrie et al., 2006; Golic and

Walsh, 2006; Bobelyn et al., 2010; Wedding et al., 2013), very little research on citrus models obtained from more than one harvest population has been documented (Miyamoto and Kitano, 1995; Ou et al., 1997; Guthrie et al., 2005b). The latter researchers reported on the robustness of Vis/NIRS models for the evaluation of attributes related to eating quality of intact mandarins. Model performance was validated in terms of fruit from different harvest days from a single tree, different harvest localities and different seasons. They found that model predictions were more variable across seasons than across harvest days and location. Previous studies indicated that model performance should be more stable across seasons, for a given variety, than across varieties, in a given season (Miyamoto and Kitano, 1995; Peiris et al., 1998a, b). Most documented literature did not involve testing the calibration models against fruit from independent populations such as, different harvest dates, localities, temperatures, cultivar, size, shape, etc. Vis/NIRS model developed for a particular cultivar, for instance, is limited to the calibration cultivar. Skin thickness, pulp texture, fruit shape, size and composition are major factors that differ between citrus cultivars (Cayuela, 2008). Therefore, histological differences may influence Vis/NIRS measurements of citrus fruit. One of the weaknesses of Vis/NIRS is that its application in quality evaluation for different fruit species needs to be designed according to the fruit size, the thickness of its skin, and the specific attributes to be tested (Miyamoto and Kitano, 1995). According to Louw and Theron (2010), the best way to develop robust models for biological products is to acquire calibration data over a sufficient period of time to obtain an appropriate range of instrumental and environmental conditions.

Temperature influences are often described as a drawback of Vis/NIRS measurements (Peirs et al., 2003; Zude et al., 2008). Performance of calibration model is affected by sample temperature primarily through the strong effect of temperature on H-bonding and thus on the absorption bands related to O-H (Golic et al., 2003). Calibration models developed across a wide range of temperatures (5.5–23.5 °C) are expected to be more robust in terms of predicting analyte levels of samples at a range of temperatures (Peirs et al., 2003; Zude et al., 2008). However, the accuracy of the model is potentially diminished (Guthrie et al., 2005b). Results obtained in this study were in agreement with those obtained by Kawano et al. (1993) and Miyamoto and Kitano (1995), who recommended that samples scanned at a range of temperatures, should be included in the calibration population in order for the model to be robust in prediction of samples varying in sample temperature. Given the sensitivity of water bands to

temperature, DM calibration models have been previously reported to be more sensitive to temperature than TSS models (Golic et al., 2003).

3. Commercial adoption of Vis/NIRS technology

The important objective governing technology development research in fruit postharvest science is to explore the possibilities of a technology such as Vis/NIRS for in-line assessment of quality. While scientific literature is replete with calibration and prediction statistics for the use of Vis/NIRS for the non-destructive assessment of fruit quality and the technology is in commercial use for a number of commodities including citrus fruit (Golic and Walsh, 2006), Vis/NIRS technology for non-destructive measurement of fruit quality is still in development. A review by Walsh (2005) discussed the application and limitations to adoption of commercially available, low cost, miniaturised NIR spectrophotometers for the assessment of the sugar content of intact fruit, including citrus. Commercial application of Vis/NIRS to fruit sorting was first initiated in Japan in 1990, and has been applied to pack-house sorting lines for TSS of citrus and other fruit since the mid-1990s in Japan, and more recently in other countries. Although the technology has been available for commercial application for over a decade, adoption by the packing industry has been low outside Japan. Similar to most technologies in the agricultural sector, the limitations to Vis/NIRS adoption by commercial packing industry could be attributed to cost, technical limitations, grower resistance, and supply chain limitations. However, the technology is sufficiently well developed, and offered by a sufficient number of manufacturers to overcome 'technical' limitations. It is likely that technology adoption will be rapid once consumers are able to recognise, and consistently source, fruit of superior quality. Although the technology is in commercial use for a number of commodities, commercial operators have not published such information.

4. Specific applications of Vis/NIRS on citrus fruit

4.1. Measurement of internal quality attributes

Citrus quality parameters such as SSC and TA are based on organic molecules which contain C-H, O-H, C-O, and C-C bonds; hence, it is possible to use Vis/NIRS methods to quantify these parameters. Tables 2, 3 and 4 summarise the applications of Vis/NIRS to assess internal quality parameters of citrus fruit. These sources show that some internal properties are more accurately measured with Vis/NIRS than others. For instance, the Vis/NIRS technique has significantly greater accuracy for determining SSC than TA, and the low success could be attributed to the fact that organic acids concentration on intact fruit is relatively low; hence, calibration of this attribute is likely to represent secondary correlation on attributes related to fruit maturity (Guthrie et al., 2005a).

4.2. Measurement of external quality attributes

Appearance is the initial parameter used to evaluate quality of fruit and vegetables and the presence of skin defects is one of the most influential factors in the price of fruit. Under present fruit grading systems, fruit with slight external defects are graded and marketed with unblemished fruit, thereby reducing the quality of the batch. Alternatively, fruit with slight defects are graded and removed together with seriously damaged fruit, thus causing unnecessary economic loses (Blasco et al., 2007a). The challenge is high regarding citrus rind disorders that do not manifest during harvest grading and postharvest treatments but develop about 1-5 weeks after harvest; such as rind breakdown disorder (RBD) of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) (Cronje, 2009), RBD of 'Navel' orange [*C. sinensis* L. (Osb.)] (Alférez et al., 2003) and non-chilling postharvest rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Alférez and Burns, 2004). The challenge is therefore to develop non-destructive technology to determine rind quality in the packing line to assist in the sorting and segregation of fruit into pertinent quality grades. There is therefore a need to develop an objective, fast and non-destructive assessment that can be used to estimate the susceptibility of citrus fruit to rind disorders. Non-visible information, such as that provided by the NIR region of the electromagnetic spectrum can improve grading by detecting specific defects or allowing the detection of non-visible damages (Blasco et al., 2007b).

Most current non-destructive quality measurement technologies have been developed to assess fresh fruit according to their internal quality attributes (Butz et al., 2005). Very limited

research has been conducted to develop a technology that can assess, predict and monitor the physiological disorders and rind physiological disorders of citrus fruit in particular. Nevertheless, NIRS has been used successfully to detect surface bruising in apple (Geeola et al., 1994), surface defects in peach (Miller and Delwiche, 1991), storage disorders in kiwifruit (Clark et al., 2004) and drying internal disorder in Tangerine citrus (Peiris et al., 1998c). The trend has constantly shifted towards developing reliable and cost effective technologies to non-destructively screen fruit physiological disorders at commercial packline speeds. Zheng et al. (2010) used NIR in the reflectance mode to predict oleocellosis sensitivity in citrus fruit. Meglinski et al. (2010) established that internal and external quality defects of onions can be effectively observed and screened by OCT system, and this non-invasive method is suitable for examining intact plants. OCT produces two-dimensional images of plant tissues at a penetration depth of 1–2 mm from the surface (Sapozhnikova et al., 2003). Detailed aspects of OCT theory and applications have been discussed in comprehensive reviews by Tomlins and Wang (2005) and Fercher et al. (2003). NIRS and similar emerging non-destructive technologies such as OCT offer potential for the non-invasive assessment of the chemical composition and changes thereof, such as carbohydrate content in the flavedo of intact fruit that might predispose fruit to the development of rind disorders.

4.2.1. Multi- and hyper-spectral imaging

In recent years, extensive research has been conducted to develop techniques for detecting defects in fruit and vegetables (Xing et al., 2005). Systems based on various types of electromagnetic methods have been investigated. The analytical spectral regions of these systems include the X-ray, ultraviolet, visible, NIR, mid-infrared and far infrared regions. Imaging techniques have been developed and successfully applied as inspection tools for quality assessment of a range of fruit (Xing et al., 2003, 2005; Mehl et al., 2004). Multispectral imaging involves making images using more than one spectral component of the electromagnetic energy from the same region of an object and at the same scale. Hyperspectral imaging on the other hand, integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object. Hyperspectral images are made up of hundreds of contiguous wavebands for each spatial position of a sample studied and each pixel in an image contains the

spectrum for that specific position (Gómez-Sanchis et al., 2008). The development of multi-spectral and hyper-spectral imaging provides the possibility to capture images not only in the visible range, but also extend to capture multiple images at different wavelengths in the Vis/NIR regions (Mehl et al., 2004; Qin et al., 2008). These analytical technologies, which combine Vis/NIRS with digital imaging, now allow both spatial and spectral information to be obtained simultaneously. Further information on the principles of these technologies could be found in a review by Gowen et al. (2007).

Several spectroscopic studies demonstrated the possibility of using multispectral and hyperspectral imaging techniques for identifying external skin damages in citrus fruit (e.g. Blasco et al., 2007a; Qin et al., 2008). A recent review on advances in machine vision applications for automatic inspection and the application of hyperspectral and multispectral imaging techniques was done by Cubero et al. (2010). Tables 5 and 6 present an overview of applications of multispectral and hyperspectral imaging systems to assess external quality parameters of citrus fruit, including different peel disorders, such as citrus canker (Balasundaram et al., 2009), anthracnose, stem-end injury, green mould, oleocellosis, and chilling injury (Blasco et al., 2007a) and common peel defects (Blasco et al., 2007b). Results by Blasco et al. (2007a, b) showed that the inclusion of non-visible information detected in the NIR region can improve the detection and identification of some defects. Blasco et al. (2009) showed that their system could successfully discriminate between 11 defects in 86% of cases. Using only visible information, the results were also acceptable (82%) but the confusion between serious and slight defects increased since green mould could be detected using ultraviolet (UV) in 97% of cases and anthracnose was detected using NIR in 95% of cases. Although very limited work has been reported on citrus rind disorders, spectrophotometric surface detection offers an alternative or complementary method to machine vision in fruit and vegetable research (Xing et al., 2003). The vast accumulation of data with multispectral imaging creates novel opportunities and applications. However, there are still many unexplored applications to be investigated. For instance, reducing the processing time is still a challenge, since it is too high to allow defect prediction algorithm to be implemented in a real-time system. However, further advances in technology can help this to be achieved.

5. Prospects for future research

Vis/NIRS technology is one of the most advanced non-destructive quality evaluation systems available for a wide range of applications. There are considerable research activities into the investigation of NIR-based sensors for determining various quality factors in a range of fruit and vegetables, including citrus. The move to tomographic imaging would seem the next logical step as prercusor to the development of true 3D imaging of citrus and other fresh food products (Hebden et al., 2002). Already, attempts have been undertaken to implement non-destructive techniques of acquiring tomographic images of plant tissues (Lammertyn et al., 2003a, b; Kemsley et al., 2008). Due to the poor transparency of biological tissues in the Vis/NIR region, optical methods were previously regarded inapplicable for the visualisation of structural features of such tissues. As stated earlier in this review and by Sapozhnikova et al. (2003) that although biological tissues are optically turbid media, they do not strongly absorb radiation in the NIR region, 830 nm≤λ≤1300 nm in particular. In addition, biological tissues exhibit sufficientlystrong scattering properties.

Optical tomography (OT) is one such non-invasive analytical technique currently available, at least to researchers, that is suitable for examining the internal structure of plant and animal tissues. OT reconstructs 3D images of objects based on transmission or scattering images. Since its first inception in the biological field, OT has been utilising two fundamental techniques, which are optical diffuse tomography and OCT. Although a wide range of tomographic imaging techniques and sensors has been explored, based on merits, OCT has been advocated to be suitable for examining the internal structure of biological tissues (Fercher et al., 2003; Huang et al., 1991; Lu et al., 2004). OCT provides a means to visualise the internal structure of biological objects with high resolution in real-time (Meglinski et al., 2010). The image acquisition time is 1–3 s per tomogram. The method is based on the interferometric measurements of NIR light back-scattering from the fruit (Sapozhnikova et al., 2003). The radiation in the NIR region used in OCT systems is suitable and safe for examining intact plants because of its non-ionising characteristics (Fercher et al., 2003; Kemsley et al., 2008). In addition to its non-destructive properties, another advantage of OCT include larger depth in scattering media such as fruit, provides the means to observe and monitor physiological activity and changes of plant tissues during growth and development. Other advantages include the fact that depth resolution is

decoupled from transverse resolution. OCT images are synthesised from a series of adjacent interferometric depth scans performed by a straight propagation low-coherence probing beam. The main parameters of an OCT instrument are its optical power and wavelength, its penetration depth, resolution and sensitivity, and its image acquisition rate. The choice of wavelength is highly dependent on the nature of the sample. An important feature of an OCT system is the weakest sample reflectivity yielding to a signal power equal to noise of the system (Fercher et al., 2003).

Recently, Kutis et al. (2005) conducted a comparative analysis of two optical methods, namely, OCT and optical coherence microscopy (OCM) to visualise plant tissues. The latter method combines the advantages of the confocal microscope and OCT, the two reputed instruments to observe objects with strong light scattering properties. The authors reported a higher spatial resolution of the OCM method compared with the OCT method. Although OCM provided higher spatial resolution and appeared more reliable than OCT in the identification of plant morphological structures, it should be noted that both methods have their advantages and drawbacks. In summary, both OCM and OCT as non-destructive techniques could open new perspectives in plant physiology and morphology studies.

Because of the relatively low value of horticultural products compared to biomedical and pharmaceutical products, the horticultural industry had been users instead of developers of advanced technologies including sensors. Similarly, the OCT method has been previously used exclusively by the biomedical research for differential diagnosis of tumours (Sapozhnikova et al., 2003; Kutis et al., 2005). The feasibility of OCT for imaging of plants and related food materials such as fruit is currently receiving attention among researchers. Sapozhnikova et al. (2004) reported that OCT can be used to visualise not only plant tissues and tissue boundaries but also the structure of individual cells. This non-invasive method is suitable for examining intact plants and it is capable of producing 2D images of plant tissues at a penetration depth of 1–2 mm from the surface (Sapozhnikova et al., 2003). The technique has opened a new avenue of research towards non-invasive prediction and monitoring of peel disorders such as peel pitting that do not manifest visually at harvest but after 1–5 weeks postharvest.

6. Conclusion

Over the years, much technological progress has been made in the development of non-destructive quality evaluation systems for the food industry, including fruit. Among non-destructive quality assessment techniques, Vis/NIRS is arguably the most advanced with regard to instrumentation, applications, accessories, and availability of suitable chemometric software packages. In addition to monitoring and predicting external quality attributes of citrus fruit, Vis/NIRS also offers considerable potential for the non-invasive measurement of the chemical composition, and changes thereof, such as carbohydrate content, in the flavedo of intact citrus fruit that might predispose fruit to the development of rind disorders. Very limited research has been reported which apply Vis/NIRS technology to assess, predict and monitor the presence of physiological disorders in the rind and flesh of citrus fruit. Recently, the trend has shifted towards developing reliable and cost effective technologies to non-destructively screen fruit for physiological disorders. Innovative technologies such as OCT and OCM offer new prospects. One of the challenges facing future applications of Vis/NIRS technology is the inherent variability in fruit and similar biological products. Consequently, researchers have applied different Vis/NIRS settings and analytical frameworks to improve prediction, but it is conceivable that the choice of these parameters could affect both model accuracy and robustness and this deserves attention for future research.

Increasing consumer demand for consistent supply of a wide range of safe, nutritious, and traceable food products, including citrus and other horticultural fresh produce, assures the need for the development of innovative non-destructive tools for field and laboratory measurement, in-line sorting and grading based on both external and internal product attributes. In addition to consumer demand for information on fruit internal quality attributes related to the nutritional value, citrus fruit also develop physical defects and physiological disorders which are exacerbated during postharvest handling and marketing. Vis/NIRS and similar technological tools reviewed in this chapter offer the potential for the non-destructive measurement and prediction of these quality problems. Equipment cost and consumer willingness to pay for fresh fruit graded in terms of internal quality attributes such as sugar content, acidity and vitamins remains a major challenge for future development and commercialisation of Vis/NIRS and similar non-invasive tools in citrus and other fruit sectors.

References

Aleixos, N., Blasco, J., Navarrón, F., Moltó, E., 2002. Multispectral inspection of citrus in real-time using machine vision and digital signal processors. Comp. Electron agric. 33, 121–137.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Antonucci, F., Pallottino, F., Paglia, G., Palma, A., D'Aquino, S., Menesatti, P., 2011. Non-destructive estimation of mandarin maturity status through portable Vis-NIR spectrophotometer. Food Bioprocess Tech. 4, 809–813.

Balasundaram, D., Burks, T.F., Bulanon, D.M., Schubert, T., Lee, W.S., 2009. Spectral reflectance characteristics of citrus canker and other peel condition of grapefruit. Postharvest Biol. Technol. 51, 220–226.

Blanco, M., Villarroya, I., 2002. NIR spectroscopy: a rapid-response analytical tool. Trends in Anal. Chem. 21, 240-250.

Blasco, J., Aleixos, N., Moltó, E., 2007a. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. J. Food Eng. 81, 535–543.

Blasco, J., Aleixos, N., Gómez, J., Moltó, E., 2007b. Citrus sorting by identification of the most common defects using multispectral computer vision. J. Food Eng. 83, 384–393.

Blasco, J., Aleixos, N., Gómez-Sanchís, J., Moltó, E., 2009. Recognition and classification of external skin damage in citrus fruits using multispectral data and morphological features. Biosyst. Eng.10, 137–145.

Bobelyn, E., Serban, A., Nicu, M., Lammertyn, J., Nicolaï, B.M., Saeys, W., 2010. Postharvest quality of apple predicted by NIR spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55,133–143.

Bulanon, D.M., Burks, T.F., Alchanatis, V., 2010. A multispectral imaging analysis for enhancing citrus fruit detection. Envir. Control. Biol. 48, 81–91.

Butz, P., Hofmann, C., Tauscher, B., 2005. Recent developments in non-invasive techniques for fresh fruit and vegetable internal quality analysis. J. Food Sci. 70, 131–141.

Camps, C., Christen, D., 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. LWT - Food Sci. Technol. 42, 1125–1131.

Carlini, P., Massantini, R., Mencarelli, F., 1999. Wavelength selection methods for PLS-based Vis-NIR evaluation of SSC in fresh fruits. In: Proceedings of the NIR'99, 9th International Conference on Near-Infrared Spectroscopy, Verona, Italy, 13-18 June 1999.

Carlini, P., Massantini, R., Mencarelli, F., 2000. Vis-NIR measurement of soluble solids in cherry and apricot by PLS regression and wavelength selection. J. Agric. Food Chem. 48, 5236–5242.

Cayuela, J.A., 2008. Vis-NIR soluble solids prediction in intact oranges (*Citrus sinensis* L.) cv. Valencia Late by reflectance. Postharvest Biol. Technol. 47, 75–80.

Cayuela, J.S., Weiland, C., 2010. Intact orange quality prediction with two portable NIR spectrometers. Postharvest Biol. Technol. 58, 113–120.

Cen, H., He, Y., Huang, M., 2006. Measurements of soluble solids contents and pH in orange juice using chemometrics and Vis-NIRS. J. Agric. Food Chem. 54, 7437–7443.

Cen, H., Bao, Y., He, Y., Sun, D.-W., 2007. Visible and near infrared spectroscopy for rapid detection of citric and tartaric acids in orange juice. J. Food Eng. 82, 253–260.

Centner, V., Massart, D.L., de Noord, O.E., de Jong, S., Vandeginste, M.B., Sterna, C., 1996. Elimination of uninformative variables for multivariate calibration. Anal. Chem. 68, 3851–3858.

Clark, C.J., McGlone, V.A., DeSilva, H.N., Manning, M.A., Burdon, J., Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit (*Actanidia cinensis*) based on visible-NIR spectral characteristics at harvest. Postharvest Biol. Technol. 32, 147–158.

Cronje, P.J.R., 2005. Peteca spot of lemons. S. African Fruit J., Feb/March issue, 26–28.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cubero, S., Aleixos, N., Moltó, E., Gómez-Sanchis, J., Blasco, J., 2011. Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. Food Bioprocess Tech. 4, 487–504.

De Jong, S., 1993. PLS fits closer than PCR. J. Chemometr. 7, 551–557.

Dull, G.G., Birth, G.S., 1989. Nondestructive evaluation of fruit quality: Use of near infrared spectrophotometry to measure soluble solids in intact honeydew melons. HortScience 24, 754.

Dull, G., Birth, G., Leffler, R., 1989a. Use of near infrared analysis for the non-destructive measurement of dry matter in potatoes. Am. Potato J. 66, 215–225.

Dull, G.G., Birth, G.S., Smittle, D.A., Leffler, R.G., 1989b. Near infrared analysis of soluble solids of intact cantaloupe. J. Food Sci. 54, 393–395.

Fercher, A.F., Drexler, W., Hitzenberger, C.K., Lasser, T., 2003. Optical coherence tomography-principles and applications. Rep. Prog. Phys. 66, 239–303.

Fraser, D.G., Künnemeyer, R., McGlone, V.A., Jordan, R.B., 2001. Letter to the editor. Postharvest Biol. Technol. 22, 191–195.

Fraser, D.G., Jordan, R.B., Künnemeyer, R., McGlone, V.A., 2003. Light distribution inside mandarin fruit during internal quality assessment by NIR spectroscopy. Postharvest Biol. Technol. 27, 185–196.

Fu, X., Ying, Y., Lu, H., Xu, X., 2007. Comparison of diffuse reflectance and transmission mode of visible-near infrared spectroscopy for detecting brown heart of pear. J. Food Eng. 83, 317–323.

Gaffney, J.J., 1973. Reflectance properties of citrus fruit. Trans. Am. Soc. Agric. Eng. 310–314.

Geeola, F., Geeola, F., Peiper, U.M., 1994. A spectrophotometric method for detecting surface bruises on 'Golden Delicious' apples. J. Agric. Eng. Res. 58, 47–51.

Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

Golic, M., Walsh, K.B., 2006. Robustness of calibration models based on near infrared spectroscopy for the in-line grading of stonefruit for total soluble solids contents. Analytica Chimica Acta 555, 286–291.

Gómez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Gómez-Sanchis, J., Gómez-Chova, L., Aleixos, N., Camps-Valls, G., Montesinos-Herrero, C., Moltó, E., Blasco, J., 2008. Hyperspectral system for early detection of rottenness caused by *Penicillium digitatum* in mandarins. J. Food Eng. 89, 80–86.

Gowen, A.A., O'Donnell, C.P., Cullen, P.J., Downey, G., Frias, J.M., 2007. Hyperspectral imaging-an emerging process analytical tool for food quality and safety control. Trends Food Sci. Technol. 18, 590–598.

Greensill, C.V., Walsh, K.B., 2002). Calibration transfer between miniature photodiode array-based spectrometers in the near infrared assessment of mandarin soluble solids content. J. Near Infrared Spectrosc. 10, 27–35.

Guthrie, J., Walsh, K., 1997. Non-invasive assessment of pineapple and mango fruit quality using near infrared spectroscopy. Aust. J. Exp. Agric. 37, 253–263.

Guthrie, J.A., Wedding, B., Walsh, K.B., 1998. Robustness of NIR calibrations for soluble solids in intact melon and pineapple. J. Near Infrared Spectrosc. 6, 259–265.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005a. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Aust. J. Exp. Agric. 56, 405–416.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005b. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. Aust. J. Exp. Agric. 56, 417–426.

Guthrie, J.A., Liebenberg, C.J., Walsh, K.B., 2006. NIR model development and robustness in prediction of melon fruit total soluble solids. Aust. J. Agric. Res. 57, 1–8.

Hebden, J.C., Gibson, A., Yusof, R.M., Everdell, N., Hillman, E.M.C., Delpy, D.T., Arridge, S.R., Austin, T., Meek, J.H., Wyatt, J.S., 2002. Three-dimensional optical tomography of the premature infant brain. Phys. Med. Biol. 47, 4155–4166.

Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., Hee, M.R., Flotte, T., Gregory, K., Puliafito, C.A., Fujimoto, J.G., Optical coherence tomography. Science 254, 1178–1181.

Huang, H., Yu, H., Xu, H., Ying, Y., 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. J. Food Eng. 87, 303–313.

Kader, A.A., 2002. Opportunities in using biotechnology to maintain postharvest quality and safety of fresh produce. HortScience 37, 24–25.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Non-destructive determination of sugar content in 'Satsuma' mandarins using NIRS transmittance. J. Japan. Soc. Hort. Sci. 62, 465–470.

Kemsely, E.K., Tapp, H.S., Binns, R., Mackin, R.O., Peyton, A.J., 2008. Feasibility study of NIR diffuse optical tomography on agricultural produce. Postharvest Biol. Technol. 48, 223–230.

Kim, J., Mowat, A., Poole, P., Kasabov, N., 2000. Linear and non-linear pattern recognition models for classification of fruit from visible–near infrared spectra. Chemometr. Intell. Lab. Syst. 51, 201–216.

Krivoshiev, G.P., Chalucova, R.P., Moukarev, M.I., 2000. A possibility for elimination of the interference from the peel in nondestructive determination of the internal quality of fruit and vegetables by Vis-NIR spectroscopy. Lebensm-Wiss Univ. Technol. 33, 344–353.

Kutis, I.S., Sapozhnikova, V.V., Kuranov, R.V., Kamenskii, V.A., 2005. Study of the morphological and functional state of higher plant tissues by optical coherence microscopy and optical coherence tomography. Russ. J. Plant Physiol. 52, 559–564.

Lammertyn, J., Peirs, J., De Baerdemaeker, J., Nicolaï, B.M., 2000. Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121–132.

Lammertyn, J., Dressalaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003a. MRI and X-ray CT study of spatial distribution of core breakdown in 'Conference' pears. Magn. Reson. Imag. 21, 805–815.

Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003b. Analysis of the time course of core breakdown in 'Conference' pears by means of MRI and X-ray CT. Postharvest Biol. Technol. 29, 19–28.

Leonardi, L., Burns, D.H., 1999. Quantitative multiwavelength consistuent measurements using single-wavelength photo time-of-flight correction. Appl. Spectrosc. 53, 637–646.

Liu, Y., Sun, X., Ouyang, A., 2010a. Non-destructive measurements of soluble solid content of navel orange fruit by visible-NIR spectrometric technique with PLSR and PCA-BPNN. LWT - Food Sci. Technol. 43, 602–607.

Liu, Y., Sun, X., Zhang, H., Aiguo, O., 2010b. Nondestructive measurement of internal quality of Nanfeng mandarin fruit by charge coupled device near infrared spectroscopy. Comp. Electron. Agric. 71(S1), S10–S14.

López-García, F., Andreu-García, A., Blasco, J., Aleixos, N., Valiente, J. M., 2010. Automatic detection of skin defects in citrus fruits using a multivariate image analysis approach. Comp. Electron. Agric. 71, 189–197.

Louw, E.D., Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina* L.) using NIR spectroscopy. Postharvest Biol. Technol. 58, 176–184.

Lovász, T., Merész, P., Salgó, A., 1994. Application of near infrared transmission spectroscopy for the determination of some quality parameters of apples. J. Near Infrared Spectrosc. 2, 213–221.

Lu, Q., Gan, X., Gu, M., Luo, Q., 2004. Monte Carlo modelling of optical coherence tomography imaging through turbid media. Appl. Optics 43, 1628–1637.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. 7, 794–799.

Magwaza L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., 2011. Non-destructive quality assessment of 'Valencia' orange using FT-NIR spectroscopy. Proceedings of the 15th international conference on NIR spectroscopy, 13-20 May 2011, Cape Town, RSA. Pp. 375.

McClure, W.F., 2003. Review: 204 years of near infrared technology: 1800-2003. J. Near Infrared spectrosc. 11, 487–518.

McGlone, V.A., Kawano, S., 1998. Firmness, dry-matter and soluble solids assessment of postharvest kiwifruit by NIR spectroscopy. Postharvest Biol. Technol. 13, 131–141.

McGlone, V.A., Jordan, R.B., Martinsen, P.J., 2002. Vis-NIR estimation at harvest of pre- and post-storage quality indices for 'Royal Gala' apple. Postharvest Biol. Technol. 25, 135–144.

McGlone, V.A., Fraser, D.G., Jordan, R.B., Kunnemeyer, R., 2003. Internal quality assessment of mandarin fruit by Vis-NIR spectroscopy. J. Near Infrared spectrosc. 11, 323–332.

Meglinski, I.V., Buranachai, C., Terry, L.A., 2010. Plant photonics: Application of optical tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Mehl, P.M., Chen, Y-R., Kim, M.S., Chan, D.E., 2004. Development of hyperspectral imaging technique for the detection of apple surface defects and contaminations. J. Food Eng. 61, 67–81.

Menesatti, P., Antonucci, F., Pallottino, F., Rocuzzo, G., Allegra, M., Stagno, F., Intrigliolo, F., 2010. Estimation of plant nutritional status by Vis-NIR spectroscopic analysis on orange leaves [*Citrus sinensis* (L.) Osbeck *cv* Tarocco]. Biosyst. Eng. 105, 448–454.

Miller, B.K., Delwiche, M.J., 1991. Spectral analysis of peach surface defects. Trans. Am. Soc. Agric. Eng. 34, 2509–2515.

Miller, W.M., Zude, M., 2002. Non-destructive brix sensing of Florida grapefruit and honey tangerines. Proc. Fla. State Hort. Soc. 115, 56–60.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3, 227–237.

Miyamoto, K., Kawauchi, M., Fukuda, T., 1998. Classification of high acid fruits by PLS using the near infrared transmittance spectra of intact Satsuma mandarins. J. Near Infrared Spectrosc. 6, 267–271.

Moon, D.G., Mizutani, F., 2002. Relationship between fruit shape acid content in different parts of citrus fruits. J. Japan. Soc. Hort. Sci. 71, 56–58.

Nicolaï, B.M., Lötze, E., Peirs, A., Scheerlinck, N., Theron, K.I., 2006. Non-destructive measurement of bitter pit in apple fruit using NIR hyperspectral imaging. Postharvest Biol. Technol. 40, 1–6.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I.K., Lammertyn, J., 2007a. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Nicolaï, B.M., Theron, K.I., Lammertyn, J., 2007b. Kernel PLS regression on wavelet transformed NIR spectra for prediction of sugar content of apple. Chemometr. Intell. Lab. Syst. 85, 243–252.

Nicolaï, B.M., Verlinden, B.E., Desmet, M., Saevels, S., Saeys, W., Theron, K., Cubeddub, R., Pifferi, A., Torricelli, A., 2008. Time-resolved and continuous wave NIR reflectance spectroscopy to predict soluble solids content and firmness of pear. Postharvest Biol. Technol. 47, 68–74.

Nicolaï, B.M., Bulens, I., De Baerdemaker, J., De Ketelaere, B., Hertog, M.L.A.T.M., Verboven, P., Lemmertyn, J., 2009. Non-destructive evaluation: detection of external and internal attributes frequently associated with quality and damage. In: Florkowiski et al. (eds).

Postharvest Handling: A Systems Approach, pp 421-442. Academic Press, Elsevier, Amsterdam.

Nørgaard, L., Saudland, A., Wagner, J., Nielsen, J.P., Munck, L., Engelsen, S.B., 2000. Interval partial least-square (iPLS): a comparative chemometric study with an example from near infrared spectroscopy. Applied Spectrosc. 54, 413–419.

Norris, K.H., Hart, J.R., 1965. Direct spectrophotometric determination of moisture content of grain and seeds. In: Humidity and Moisture, 4, Principles and Methods of Measuring Moisture in Liquids and Solids, Reinhold Publishing Corp., New York.

Osborne, S.D., Jordan, R.B., Kunnemeyer, R., 1997. Method of wavelength selection for partial least squares. Analyst 122, 1531–1537.

Osborne, S.D., Kunnemeyer, R., Jordan, R.B., 1999. A low-cost system for the grading of kiwifruit. J. Near Infrared Spectrosc. 7, 9–15.

Osborne, B.G., 2000. Near infrared spectroscopy in food analysis, pp. 1–14. BRI Australia Ltd, North Ryde, Australia.

Ou, A.S., Lin, S., Lin, T., Wu, S., Tiarn, M., 1997. Studies on the determination of quality-related constituents in 'Ponkan' Mandarin by near infrared spectroscopy. J. Chinese Agric. Chem. Soc. 35, 462–474.

Pallav, P., Diamond, G.G., Hutchins, D.A., Green, R.J., Gan, T.J., 2009. A near infrared (NIR) technique for imaging food materials. J. Food Sci. 74, 23–33.

Palmer, K.F., Williams, D., 1974. Optical properties of water in the near infrared. J. Opt. Soc. Am. 64, 1107–1110.

Peirera, A.F.C., Pontes, M.J.C., Neto, F.F.G., Santos, S.R.B., Galvaõ, R.K.H., Araújo, M.C.U., 2008. NIR spectrometric determination of quality parameters in vegetable oils using iPLS and variable selection. Food Res. Int. 41, 341–348.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1998a. Near-infrared spectrometric method for nondestructive determination of soluble solids content of peaches. Am. Soc. Hort. Sci. 123, 989–905.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1998b. Near-infrared (NIR) spectrometric technique for non-destructive determination of soluble solids content in processing tomatoes. Am. Soc. Hort. Sci. 123, 1089–1093.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., 1998c. Nondestructive detection of selection drying, an internal disorder in tangerine. HortScience 33, 310–312.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. HortScience 34, 114–118.

Peirs, A., Tirry, J., Verlinden, B., Darius, P., Nicolaï, B.M., 2002. Effect of biological variability on the robustness of NIR-models for soluble solids content of apples. Postharvest Biol. Technol. 28, 269–280.

Peirs, A., Scheerlinck, N., Nicolaï, B.M., 2003. Temperature compensation for near infrared reflectance measurement of apple fruit soluble solids contents. Postharvest Biol. Technol. 30, 233–248.

Qin, J., Burks, T.F., Kim, M.S., Chao, K., Ritenour, M.A., 2008. Citrus canker detection using hyperspectral reflectance imaging and PCA-based image classification method. Sens. Instr. Food Qual. 2, 168–177.

Qin, J., Burks, T.F., Ritenour, M.A., Bonn, W.G., 2009. Detection of citrus canker using hyperspectral reflectance imaging with spectral information divergence. J. Food Eng. 93, 183–191.

Saiz-Abajo, M.J., Mevick, B-H., Segtnan, V.H., Naes, T., 2005. Ensemble methods and data augmentation by noise addition applied to the analysis of spectroscopic data. Anal. Chim. Acta 533, 147–159.

Sapozhnikova, V.V., Kamenskii, V.A., Kuranov, R.V., 2003. Visualization of plant tissues by optical coherence tomography. Russ. J. Plant Physiol. 50, 282–286.

Sapozhnikova, V.V., Kamensky, V.A., Kuranov, R.V., Kutis, I., Snopova, L.B., Myakov, A.V., 2004. In vivo visualization of Tradescantia leaf tissue and monitoring the physiological and morphological states under different water supply conditions using optical coherence tomography. Planta 219, 601–609.

Schaare, P.N., Fraser, D.G., 2000. Comparison of reflectance and transmission modes of visible-near infrared spectroscopy for measuring internal properties of kiwifruit (*Actinidia chinensis*). Postharvest Biol. Technol. 20, 175–184.

Smith, L.G., 1984. Pineapple specific gravity as an index of eating quality. Trop. Agric. (Trinidad), 61, 196–199.

Steuer, B., Schulz, H., Läger, E., 2001. Classification and analysis of citrus oils by NIR spectroscopy. Food Chem. 72, 113–117.

Sun, X., Zhang, H., Liu, Y., 2009. Nondestructive assessment of quality of 'Nanfeng' mandarin fruit by a portable near infrared spectroscopy. Int. J. Agric. Biol. Eng. 2, 65–71.

Tewari, J.C., Dixit, V., Chi, B-K., Malik, K.A., 2008. Determination of origin and sugars of citrus fruit using genetic algorithm, correspondence analysis and partial least square combined with fiber optic NIR spectroscopy. Spectrochim. Acta Mol. Biomol. Spectrosc. 71, 1119–1127.

Tomlins, P.H., Wang, R.K.W., 2005. Theory, developments and applications of optical coherence tomography. J. Phys. D: Appl. Phys. 38, 2519–2535.

Tsuchikawa, S., Sakai, E., Inoue, K., Miyamoto, K., 2003. Application of time-of-flight near-infrared spectroscopy to detect sugar and acid content in Satsuma mandarin. J. Am. Soc. Hort. Sci. 128, 391–396.

Walsh, K.B., Guthrie, J.A., Burney, J.W., 2000. Application of commercially available, low cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. Austr. J. Plant Physiol. 27, 1175–1186.

Walsh, K.B., 2005. Commercial adoption of technologies for fruit grading, with emphasis on NIRS. Information and technology for sustainable fruit and vegetable production, FRUTIC 05, 12-16 September, 2005, Montpellier France.

Wang, W., Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. Sens. Instr. Food Chem. 1, 193–207.

Wedding, B.B., Wright, C., Grau, S., White, R.D., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biol. Technol. 75, 9–16.

Wetzel, D.L., 1983. Near infrared reflectance analysis: Sleeper among Spectroscopic Techniques. Anal. Chem. 55, 1165–1176.

Williams, P.C., Norris, K.H., 1987. Qualitative applications of near-infrared reflectance spectroscopy. In: Williams, P.C., Norris, K.H. (eds), Near-Infrared Technology in the Agricultural and Food Industries, pp 241–246. American Association of Cereal Chemistry, St Paul, MNL.

Williams, P., Norris, K.H., 2001. Variable affecting near infrared spectroscopic analysis. In: Williams, P., Norris, K.H. (eds). Near infrared technology in the agriculture and food industries, 2nd edition, pp. 171–185. The American Association of Cereal Chemists, St Paul, MNL.

Wold, S., Sjostrom, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemometr. Intell. Lab. Syst. 58, 109–130.

Xia, J., Li, X., Li, P., Ma, Q., Ding, X., 2007. Application of wavelet transform in the prediction of 'Navel' orange vitamin C content by near-infrared spectroscopy. Agric. Sci. China 6, 1067–1073.

Xing, J., Landahl, S., Lammertyn, J., Vrindts, E., De Baerdemaeker, J., 2003. Effects of bruise type on discrimination of bruised and nonbruised 'Golden Delicious' apples by Vis-NIR spectroscopy. Postharvest Biol. Technol. 30, 249–258.

Xing, J., Bravo, C., Jancsó, P.T., Ramon, H., De Baerdemaeker, J., 2005. Detecting bruises on 'Golden Delicious' apples using hyperspectral imaging with multiple wavebands. Biosyst. Eng. 90, 27–36.

Zhao, X., Burks, T.F., Qin, J., Ritenour, M.A., 2010. Effect of fruit harvest time on citrus canker detection using hyperspectral reflectance imaging. Sens. Instr. Food Qual. 4, 126–135.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort. 125, 401–405.

Zude, M., Pflanz, M., Kaprielian, C., Aivazian, B., 2008. NIRS as a tool for precision horticulture in citrus industry. Biosyst. Eng. 99, 455–459.

Table 1: The band assignments of the major vibration bonds (Golic et al., 2003).

		Vibrational frequency overtones			
Tentative assignment		Fundamental	1st	2nd	3rd
OH stretching	nm	2860-3120	1410-1440	970	738
	cm^{-1}	3200-3500	6950-7100	10300	13550
OH combinations	nm	1920-2068	1100	840	
	cm^{-1}	14800-5200	9090	11900	
CH stretching	nm	3300-3470	1600-1800	1100-1230	910
	cm^{-1}	2880-3000	5550-6250	8100-9100	11000
CH combination	nm	2100-2352			
	cm^{-1}	4250-4750			
CH_2 stretching	nm	3460-3500	1720-1765	1215	930
	cm^{-1}	2880-2910	5670-5820	8230	10750
CH_2 combination	nm	2310-2325			
	cm^{-1}	4300-4330			
OH, CH, and CH_2 deformations	nm	6900-8330 11111-25000	2250-2320	2400-2600	1850-2120
	cm^{-1}	1200-1450 400-900	4310-4440	3840-4170	4720-5400

Table 2: Overview of applications of NIR spectroscopy to measure fruit quality of mandarin fruit.

Cultivar	Parameter	Mode	Wavelength	Preprocess	Validation	Analysis	R/R^2	Prediction error	Reference
Imperial	SSC DM TA % Juice	Inter.	306-1130 nm	SVN detrending 1st der. 2nd der.	Cross validation	MLR MPLS	$R^2 = 0.75$ $R^2 = 0.90$ $R^2 = 0.30$ $R^2 = 0.20$	RMSEC = 0.4 RMSEC = 0.6 RMSEC = 0.2 RMSEC = 5.0	Guthrie et al. (2005a; b)
Nanfeng	SSC TA Vit. C Colour	Transm.	400-1040 nm	Smoothing MSC 1st der. 2nd der.	ND	BPNN PLS	R = 0.93 R = 0.66 R = 0.81 R = 0.57	RMSEP = 0.65 RMSEP = 0.09 RMSEP = 2.7 RMSEP = 0.81	Sun et al. (2009)
Nafeng	SSC TA	Transm.	350-1040 nm	ND	Cross & External	iPLS	R = 0.92 R = 0.64	RMSEP = 0.65 RMSEP = 0.09	Liu et al. (2010b)
Page Tangelo	SSC TA	Refl.	400-1000 nm	ND	Cross validation	PLS	R = 0.85 R = 0.88	RMSECV = 0.45 RMSECV = 0.09	Antonucci et al. (2010)
Sastuma	SSC TA	Refl.	400-1000 nm	Savitzky Golay	Cross validation	PLS	R = 0.84 R = 0.81	RMSECV = 0.59 RMSECV = 0.07	Antonucci et al. (2010)
Satsuma	SSC TA	Refl. Int. & Transm.	500-1100 nm	2nd der. SVN	Cross & external	PLS	$R^2 = 0.93$ $R^2 = 0.65$	RMSEP = 0.32 RMSEP = 0.15	McGlone et al (2003b)
Satsuma	SSC pH Firm.	Refl.	350-2500 nm	Smoothing, MSC	ND	PLS and PCA	R = 0.94 R = 0.80 R = 0.83	RMSEP = 0.33 RMSEP = 0.18 RMSEP = 8.53	Gómez et al. (2006)
Satsuma	SSC TA	Transm.	800-1100 nm	Normalisa-tion	ND	ND	ND	ND	Tsuchikawa et al. (2003)
Satsuma	SSC	Transm.	680-1235 nm	2nd der.	ND	MLR	R = 0.99	SEP = 0.32	Kawano et al. (1993)

SSC, Soluble solids content; DM, Dry matter; TA, Titratable acids; Vit. C, Vitamin C; Firm., Firmness; Inter., Interactance; Transm.; Transmittance; Refl., Reflectance; SVN, Standard vector normalisation; 1st der., First derivative; 2nd der., Second derivative; MSC, Multiple scatter correction; ND, No data provided; PLS, Partial least squares; MPLS, Modified PLS; RMSEC, Root mean square error of calibration; RMSECV, Root mean square error of cross validation; RMSEP, Root mean error of prediction; SEP, Square error of prediction; MLR, Multiple linear regression; BPNN, Back propagation neural network; R, Correlation coefficient; R^2, Regression coefficient.

Table 3: Overview of applications of NIR spectroscopy to measure fruit quality of orange fruit.

Cultivar	Parameter	Mode	Wavelength	Preprocess	Validation	Analysis	R/R^2	Prediction error	Reference
Gannan	SSC	Refl.	800-2500nm	SVN MSC	–	PLS PCR	R = 0.995	RMSEP = 0.75	Lu et al. (2006)
Navel	SSC	Refl.	350-1800nm	MSC and SVN	Cross and external validation	PLS and PCA-BPNN	R = 0.90	RMSEP = 0.68	Liu et al. (2010a)
Navel	Vit. C	Refl.	800-2500nm	COE, SLS, SVN, MMN, MSC, 1st der and 2nd der	Cross validation	PLS	R = 0.96	RMSECV = 3.9	Xia et al. (2007)
Sanguinelli Valencia, Salustiana, Navelate	SSC pH TA MI FW F JV JV/FW RW FCI JCI	Refl.	350-2500nm	MN MSC	External validation	PLS	R = 0.92 R = 0.90 R = 0.86 R = 0.85 R = 0.97 R = 0.76 R = 0.92 R = 0.89 R = 0.97 R = 0.90 R = 0.86	RMSEP = 0.74 RMSEP = 0.15 RMSEP = 0.17 RMSEP = 1.92 RMSEP = 16.52 RMSEP = 1.05 RMSEP = 7.05 RMSEP = 0.04 RMSEP = 12.98 RMSEP = 2.65 RMSEP = 66.78	Cayuela and Weiland (2010)
Valencia Late	SSC TA pH	Refl.	578-1850nm	SVN and detrending	Internal and external validation	PLS and MPLS	R^2 = 0.91 R^2 = 0.56 R^2 = 0.45	RMSEP = 0.51 RMSEP = 0.33 RMSEP = 0.49	Cayuela (2008)
Trovita	Oleo. rate Oleo. Degree	Dif. Refl.	325-1075nm	Moving average, MSC	Cross validation	PLS	R = 0.98 R = 0.99	SEP = 0.0079 SEP = 0.0056	Zheng et al. (2010)

SSC, Soluble solids content; TA, Titratable acids; Vit. C, Vitamin C; F, Flesh firmness; FW, Fresh weight; JV, Juice volume; RW, Rind weight; FCI, Fruit colour index; JCI, Juice colour index; Dif. Refl., Diffuse reflectance; MI, Maturity index; SVN, Standard vector normalisation; 1st der., First derivative; 2nd der., Second derivative; BPNN, Back propagation neural network; Oleo, Oleocellosis; COE, Constant offset elimination; SLS, Straight line subtraction; MSC, Multiple scatter correction; ND, No data provided; PLS, Partial least squares; MMN, Min-max normalisation; PCR, Principal component regression; RMSECV, Root mean square error of cross validation; RMSEP, Root mean square error of prediction; SEP, Square error of prediction; MPLS, Modified PLS; R, Correlation coefficient; R^2, Regression coefficient.

Table 4: Overview of applications of NIR spectroscopy in other citrus fruit and citrus products.

Application	Parameter	Mode	Wavelength	Preprocess	Validation	Analysis	R/R^2	Prediction error	Reference
Twenty two varieties	Fructose Glucose Sucrose	Transm.	1100-2500nm	1st der., 2nd der., SVN	Cross validation	PLS	$R^2 = 0.95$ $R^2 = 0.94$ $R^2 = 0.98$	ND	Tewari et al. (2008)
Grapefruit	Limonene Myrcene α-Pinene β-Pinene Sabinene γ-Terpinene Optical rotation Aldehyde	Refl. Transm	1100-2500nm	ND	Cross validation	PLS	$R^2 = 0.99$ $R^2 = 0.87$ $R^2 = 0.98$ $R^2 = 1.00$ $R^2 = 0.95$ $R^2 = 0.99$ $R^2 = 0.99$ $R^2 = 0.79$	SECV = 0.65 SECV = 0.06 SECV = 0.10 SECV = 0.28 SECV = 0.15 SECV = 0.25 SECV = 1.18 SECV = 0.33	Steuer et al. (2001)
Indian River red	SSC	Refl.	400-1100nm	ND	ND	PLS, NN	$R < 0.7$	ND	Miller and Zude (2002)
Dif. brands orange juice	SSC pH	Refl.	325-1075nm	SVN, Savitzky-Golay	Cross validation	PLS	$R = 0.98$ $R = 0.96$	RMSEP = 0.73 RMSEP = 0.06	Cen et al. (2006)
Dif. brands orange juice	Citric acid Tartaric acid	Refl.	325-1075nm	MSC	Cross validation	PLS	$R = 0.94$ $R = 0.93$	RMSEP = 0.596 RMSEP = 0.013	Cen et al. (2006)
Trovita	Oleo. rate Oleo. Degree	Dif. Refl.	325-1075nm	Moving average, MSC	Cross validation	PLS	$R = 0.98$ $R = 0.99$	SEP = 0.0079 SEP = 0.0056	Zheng et al. (2010)
Tarocco citrus leaves	N P K Ca Mg Fe Mn Zn	Transm.	400-1000nm	Baseline, MSC	Cross validation	PLS	$R = 0.91$ $R = 0.43$ $R = 0.99$ $R = 0.95$ $R = 0.94$ $R = 0.92$ $R = 0.93$ $R = 0.89$	SEP = 0.049 SEP = 0.018 SEP = 0.058 SEP = 0.304 SEP = 0.048 SEP = 6.054 SEP = 1.637 SEP = 0.972	Menesatti et al. (2010)

SSC, Soluble solids content; N, Nitrogen; P, Phosphorus; K, Potassium; Ca, Calcium; Mg, Magnesium; Fe, Iron; Mn, Manganese; Zn, Zinc; Oleo., Oleocellosis; Transm., Transmittance; Refl., Reflectance; Dif. Refl., Diffuse reflectance; SVN, Standard vector normalisation; 1st der., First derivative; 2nd der., Second derivative; MSC, Multiple scatter correction; ND, No data provided; PLS, Partial least squares; SECV, Square error of cross validation; RMSEP, Root mean square error of prediction; SEP, Square error of prediction; NN, Neural network; R, Correlation coefficient; R^2, Regression coefficient.

Table 5: Summary of applications of multispectral imaging system to identify external defects and diseases of citrus fruit.

Commodity	Quality parameter	Imaging system	Wavelength	Data processing	Optimal wavelength	Accuracy	Reference
Valencia	Defects	CCD (1392x1040 pixels)	Vis/NIR (550-800nm)	PCA, LDA & ANNs	650nm	93.20%	Bulanon et al (2010)
Clemenules Marisol, Fortune & Valencia	Defects	3-CCD (768x576 pixels & 0.17 mm/pixel resolution)	- - - -	PCA & MIA	- - - -	93.30% 93.30% 96.60% 93.30%	Lopez-Garcia et al. (2010)
Grapefruit	Canker	3-CCD RGB (640x320 pixels)	UV, Vis/NIR (200-2500nm)	DA	500-800nm	100%	Balasundaram et al. (2009)
Mandarin & orange	Defects	Vis/NIR & monochromatic RGB camera (768-576 pixels & 0.17 mm/pixel resolution)	RGB (350-400nm) Vis/NIR (400-1800nm	LDA	-	86%	Blasco et al. (2009)
Mandarin & orange	Defects	Vis/NIR, UV, Fl (3-CCD 768x576 pixels &.017 mm/pixel resolution)	RGB (350-400nm) Vis/NIR (400-1800nm)	DA	-	95%	Blasco et al., 2007b
Lemons, mandarins & oranges	Defects	Vis/NIR & 2-CCD NIR & RGB (768x576 pixels & 0.17 mm/pixel resolution)	Centered at 750nm	- - -	- - -	94% 93% 94%	Aleixos et al. (2002)

CCD, Charge-coupled device; DA, Discriminate analysis; UV, Ultraviolet; LDA, Linear discriminate analysis; PCA, Principal component analysis; ANNs, Artificial neural networks; RGB, Red green blue colour camera; MIA, Multiple image analysis.

Table 6: Summary of applications of hyperspectral imaging system to identify external defects and diseases of citrus fruit.

Commodity	Quality parameter	Imaging system	Wavelength	Image pre-processing	Data processing	Optimal wavelength	Accuracy	Reference
Mandarin	Green mould (*Penicillium digitatum*)	Monochrome camera (551x551 pixels & 3.75 mm/pixel resolution)	Vis/NIR 320-1100 nm	Faster geometrical correction	CA & LAD	ND	91%	Gomez-Sanchis et al., 2008
Mandarin & Orange	Defects	RGB CCD (768x576 & 1.17 mm/pixel resolution)	ND	Smoothing using peer group filtering	ND	ND	95%	Blasco et al. (2007a)
Ruby red grapefruit	Canker	EMCCD (658-496 pixels)	Vis/NIR 400-900 nm	Flat-field correction	PCA	553, 677, 718, 858nm	92.7%	Qin et al. (2008)
Ruby red grapefruit	Canker	EMCCD (1004x1002 pixels)	Vis/NIR 450-930 nm	Flat-field correction	CA, SID	ND	95.7%	Zhao et al. (2010)
Ruby red grapefruit	Canker	EMCCD (1004x1002 pixels)	Vis/NIR 450-930 nm	Flat-field correction	SID	ND	96.2	Qin et al. (2009)

CA, Correlation analysis; CCD, Charge-coupled device; DA, Discriminate analysis; EMCCD, Electron multiplying charge-couple device; LDA, Linear discriminate analysis; PCA; Principal component analysis; SID, Spectral information divergence; RGB, Red green blue colour camera.

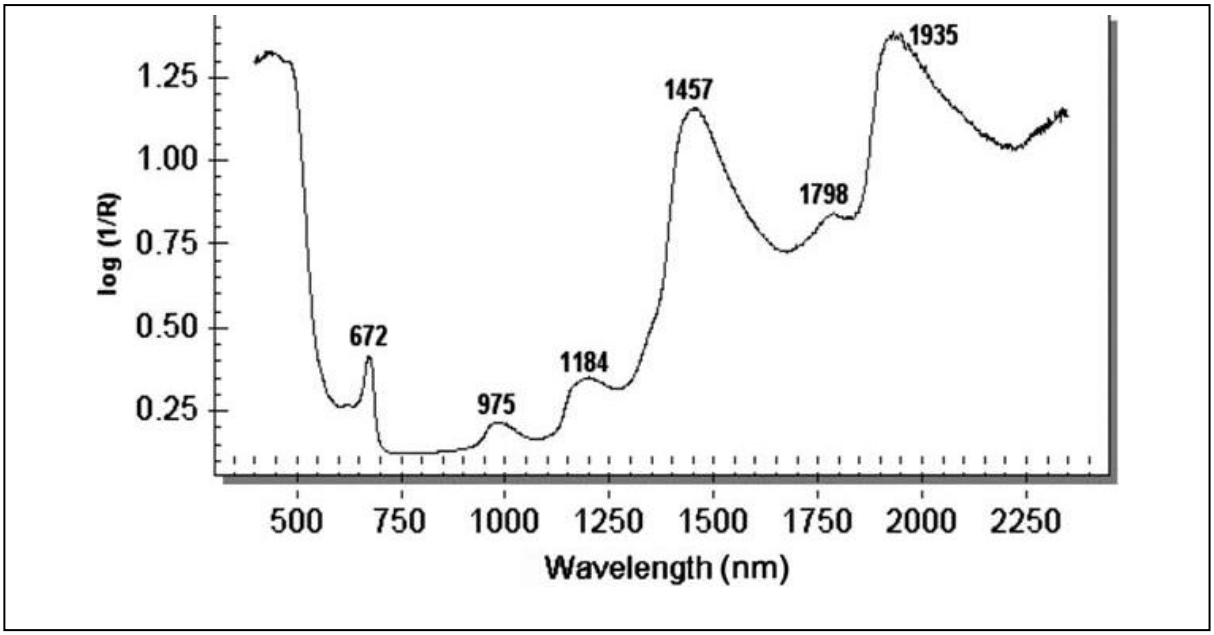

Fig 1: A typical absorbance (log(1/R) spectrum of Satsuma mandarin in the wavelength from 400-2350 nm (Gòmez et al., 2006).

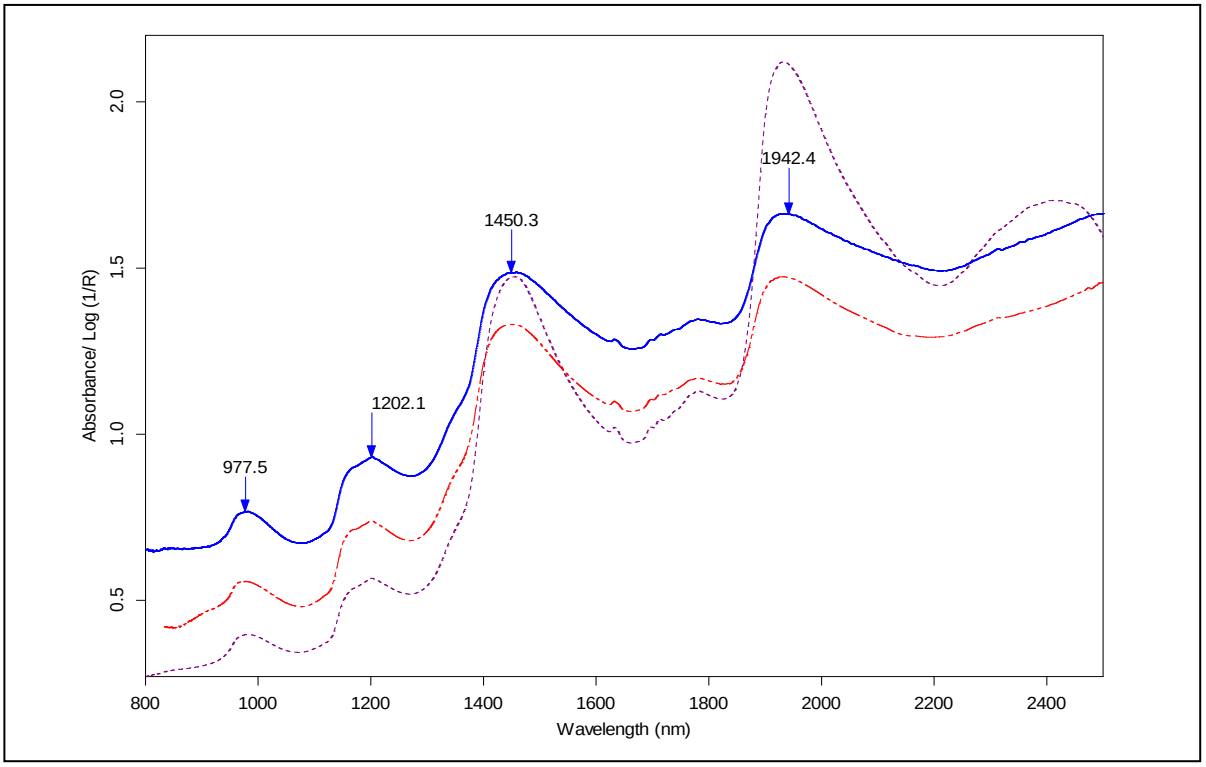

Fig. 2: Typical absorbance spectra of 'Valencia' oranges obtained using three different spectral acquisition modes, emission head (Matrix F) (blue solid line), fibre optic probe for solid samples (purple broken line) and integrating sphere (red broken line) (Magwaza et al. 2011).

SECTION II

NON-DESTRUCTIVE EVALUATION OF POSTHARVEST QUALITY OF CITRUS FRUIT

A. Paper 3: Evaluation of Fourier transform-NIR spectroscopy for integrated external and internal quality assessment of 'Valencia' oranges
B. Paper 4: Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy
C. Paper 5: The use of Vis/NIR spectroscopy and chemometrics to predict fruit defects and postharvest behaviour of 'Nules Clementine' mandarin fruit
D. Paper 6: Robust Vis/NIR spectroscopy models for the prediction of postharvest rind quality on 'Nules Clementine' mandarin fruit
E. Paper 7: Application of optical coherence tomography to non-destructively monitor rind breakdown disorder of 'Nules Clementine' mandarins

CHAPTER 4

RESEARCH RESULTS

PAPER 3:

EVALUATION OF FOURIER TRANSFORM-NIR SPECTROSCOPY FOR INTEGRATED EXTERNAL AND INTERNAL QUALITY ASSESSMENT OF 'VALENCIA' ORANGES*

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EVALUATION OF FOURIER TRANSFORM-NIR SPECTROSCOPY FOR INTEGRATED EXTERNAL AND INTERNAL QUALITY ASSESSMENT OF VALENCIA ORANGES

Abstract

Diffuse reflectance near infrared (NIR) spectroscopy was explored as a non-destructive method to assess the external and internal quality of 'Valencia' oranges. The study compared three different Fourier transform NIR acquisition methods, namely, a fibre-optic probe for solid samples (SP), an integrating sphere (IS) and an emission head (EH). Fruit quality attributes measured included mass, colour index, total soluble solids (TSS), titratable acidity (TA), and vitamin C concentration. Partial least squares regression was applied to spectral data to develop prediction models for each quality attribute and by randomly dividing the data into calibration and independent validation sets. To test robustness, a set of fruit harvested from another location was used for external validation. Fruit mass, colour index, TSS, and vitamin C were predicted with significant accuracy showing RPD-values of 3.53, 1.99, 1.87 and 1.33, respectively. The spectral acquisition method had a significant influence on the calibration regression statistics and accuracy of prediction. The models developed using the EH gave the best prediction statistics for mass (R = 0.96, RMSEP = 10.45 g), colour index (R = 0.83, RMSEP = 0.82) and vitamin C (R = 0.66, 8.01 mg/100 mL), while the IS gave the best prediction for TSS (R = 0.83 and RMSEP = 0.58). The model parameters remained fairly constant when the models were validated using fruit from another location, indicating a high level of model robustness. The EH demonstrated the potential of this spectrometer as a non-destructive tool to holistically evaluate external and internal quality parameters.

Keywords: Valencia orange · *Citrus sinensis* L. · Near infrared spectroscopy · NIR · FT-NIR · Citrus fruit · Vitamin C · Food composition · Integrated non-destructive food analysis techniques.

1. Introduction

Citrus fruit quality classification is currently based on the evaluation of external attributes of fruit including colour, size, shape and defects which are evaluated by humans or by machine vision systems (Sun et al., 2009). Although these features are only rough indicators of the internal quality, fruit quality inspection is limited to these external assessment, because until recently only these attributes could be measured non-destructively at a speed compatible with the typical speed of commercial sorting lines, which may be as high as 10 fruit per second (Nicolaï et al., 2008). Internal quality of citrus fruit is usually evaluated by destructive methods using a 'representative' sample. Although helpful, the results of these tests only reflect the properties of the specific fruit being evaluated. Fruit quality determined using this approach may exhibit significant variation in both external and internal fruit quality due to variations in maturity stage, position in the canopy and other environmental factors (Peiris et al., 1999; Guthrie et al., 2005).

Recent trends in agribusiness and analytical chemistry are moving away from subjective to objective, quantitative and non-destructive techniques for quality assessment (Shiroma and Rodriguez-Saona, 2009). The high variability in fruit batches and increased market demand for quality segmentation has spurred the search for rapid and non-destructive tools for objective quality assessment of individual fruit (Tijskens and Schouten, 2009).

Non-destructive instrument-based methods are preferred to destructive techniques because they allow the measurement and analysis of individual fruit, reduce waste and permit repeated measures on the same item over time (Nicolaï et al., 2007). A wide range of objective instruments for sensing and measuring the quality attributes of fresh produce have been summarised in Chapter 3. Among non-destructive quality assessment techniques, such as hyperspectral imaging and multispectral imaging (Blasco et al., 2009), magnetic resonance imaging (MRI) (Lammertyn et al., 2003), X-ray-computed tomography (CT) (Verboven et al., 2008), optical coherence tomography (OCT) (Meglinski et al., 2010), near infrared (NIR) spectroscopy (NIRS) is arguably the most advanced with regard to instrumentation, applications, accessories, and chemometric software packages (Nicolaï et al., 2007, Magwaza et al., 2012). The large amount of research has demonstrated the usefulness of dispersive NIR (Peirs et al., 2002a).

Fourier-transform NIR (FT-NIR) spectroscopy was developed to overcome some limitations encountered with the dispersive NIR such as low signal-to-noise ratio and low resolution. Advantages of FT-NIR spectroscopy include higher speed, wavelength accuracy,

self-calibrating with a HeNe laser and wavelength and simplified mechanics (Shiroma and Rodriguez-Saona, 2008).

NIRS-based non-destructive measurements of different quality attributes have been extensively investigated on citrus fruits. Most of the previous NIRS investigations on citrus fruit have focused on assessing specific internal quality attributes and do not integrate quantitative assessment of external and internal quality attributes in one system (Gómez et al. 2006; Lu et al., 2006; Liu et al., 2010; Jamshidi et al., 2012). Very limited studies have sought to assess the suitability of NIRS for an integrated assessment of internal and external quality parameters of intact oranges (Sánchez et al., 2013). The objective of this study was to develop a new FT-NIR method for integrated non-destructive measurements of external and internal composition of 'Valencia' oranges.

2. Materials and methods

2.1. Fruit samples

'Valencia' oranges (*Citrus sinensis* L.) used in this study were collected from a commercial citrus pack house and immediately transported to the Postharvest Technology Laboratory of Stellenbosch University, Western Cape Province, South Africa. A total of 180 fruit were selected from a batch of about 1000 fruit, using the criteria mentioned to the two experiments listed below:

2.1.1. Experiment 1: Effect of pre-sorting by size on model performance

In this experiment, sixty (60) fruit were sorted into three size classes (mass: small – 113.56±9.28 g, medium – 149.48±12.32 g and large – 227.25±27.55 g).

2.1.2. Experiment 2: Effect of pre-sorting by colour on model performance

In experiment 2, the remaining fruit (n = 120) were visually sorted into three colour groups on a subjective scale of 1 to 3, where 1 was a green fruit, 2 was a light green to yellow fruit and 3, a deep orange fruit. The chronological experimental design and distribution of samples during model development and validation is illustrated in Fig. 1.

Upon arrival in the laboratory, each fruit was individually numbered and a circle was drawn on two opposite equatorial positions of the fruit using a permanent marker to ensure that NIR spectroscopic, colour and firmness measurements were obtained at the same positions.

2.2. Spectral acquisition

FT-NIR absorbance spectra were obtained using three different reflectance acquisition methods. The first set of NIR spectra was obtained using the fibre-optic probe for solid samples (SP) (Fig. 2A). The fiber optic probe for solid samples is an accessory of the Multi-Purpose Analyser (MPA) spectrometer (Bruker Optics, Ettlingen, Germany) and measures spectra in reflectance mode. The probe consists of a 60 cm long fiber optic module consisting of a pistol grip and external trigger. The probe has a head length of 80 mm and a diameter of 5 mm. The MPA is equipped with a high energy air cooled NIR source (20 W tungsten-halogen lamp) and uses a permanently aligned and highly stable RockSolid^{TM} interferometer. The interferometer has a wavenumber reproducibility better than 0.04 cm^{-1} and a wavenumber accuracy better than 0.1 cm^{-1}. The mirrors in the interferometer are gold coated (high reflective surface and inert), the beam splitter is made of a quartz substrate with proprietary coating. A He-Ne class 1 laser is used to calculate the correct position and velocity of the movable mirror. The fiber optic probe has a bifurcated optical configuration, which guides the light to the sample by the source fibers and receives the reflected light with the detector fibers. The detector is a thermoelectrically cooled, high sensitivity InGaAs-detector. The fiber optic probe was placed in direct contact with the fruit. The wavelength region scanned was from 780 nm to 2500 nm with a resolution of 8 cm^{-1}. For each fruit, 32 scans were taken per spectrum. This took about 15.32 s. After every 20 samples a reference spectrum (Spectralon®) was taken.

The second set of spectra was also obtained by the integrating sphere (IS) (Fig. 2B). The IS is also an accessory of the MPA used to measure diffuse reflectance of highly scattering solid media. This accessory has a 50 mm width sample cup holder for measurements of heterogenous samples. The oranges were placed on the sample cup holder. Diffuse reflectance measurements are simplified by using an integrating, gold coated sphere. The NIR beam is directed into the sphere and travels directly through the centre of the sphere and the optical window into the orange sample. The beam scatters off the sample and the reflected light beams re-enter the sphere. Due to the gold coating, all light beams are collected and

directed towards the detector. The integrating sphere uses the same spectroscopic elements as for the fiber optic probe, except for the detector. The integrating sphere makes use of a high sensitivity PbS detector with non-linearity correction. The resolution, the scanning region and the number of scans are the same as the fiber optic probe. An internal gold reference spectrum was obtained by mechanically closing the optical window with a gold reference plate.

The third set of spectra was obtained from almost half of the fruit surface using a *MATRIX^{TM}-F (Q410/A)* FT-NIR spectrometer equipped with a fiber optic detecting emission head (EH) (230 mm diameter, 185 mm height) for contactless measurement (Bruker Optics, Ettlingen, Germany) (Fig. 2C). The fiber optic NIR illumination and detection head contains 4 air cooled tungsten NIR light sources (tungsten halogen, 12 V, 20 W) which illuminate the sample. The diffusely reflected light is collected and guided via a fiber optic cable to the spectrometer equipped with a high sensitivity, thermoelectric cooled and temperature-controlled InGaAs diode detector.

With each of the setups diffuse reflectance spectra in the wavelength range 780 – 2500 nm (resolution of 2 cm^{-1}, scanner frequency of 10 kHz) were obtained from the same two opposite equatorial positions of each fruit. At each position on the fruit 32 scans from exactly the same position were averaged to obtain a single spectrum, which was converted into absorbance using the Log (1/R) transformation. Acquired spectra were processed using OPUS chemometric software for Microsoft Windows (OPUS version 6.1, Bruker Optics, Ettlingen, Germany) and saved automatically.

2.3. Reference measurements

Fruit were weighed individually using a calibrated balance (Mettler Toledo, ML3002E / 01, Switzerland). Rind colour components were measured in L*a*b* colour space using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203). From the L*, a* and b* colour parameters, the colour index (CI) was calculated according to Jimenez-Cuesta et al. (1981) using equation (1).

$$CI = \frac{1000 \times a}{L \times b} \qquad (1)$$

Prior to the biochemical measurements, fruit pulp was homogenised using a laboratory blender (Assistant® all-in-one, AEG Elecrolux, South Africa), filtered through a 1 mm tea sieve, and divided into two portions. One portion was used immediately for total soluble solids (TSS), and titratable acidity (TA) analysis. The other portion was put into plastic specimen jars and stored in an ultra low temperature freezer (Innova U725-86, New Brunsswick Scientific, England) at -80 °C for subsequent analysis of vitamin C.

TSS was measured with a digital hand-held refractometer (Palette, PR-32α, Brix 0.0-32.0, Atago, Co. LTD, Japan) using 1 mL of freshly filtered homogenate and expressed as SSC. TA was measured by titrating 2 mL of filtered homogenate against 0.1 N sodium hydroxide to an end point of pH = 8.2 using an automatic TitroSampler (862 Compact TitroSampler®, Metrohm Ion Analysis, Switzerland). MI was expressed as the ratio between TSS and TA.

The vitamin C assay was based on the methods of Klein and Perry (1982) and Barros et al. (2007) with slight modification. Briefly, 1 mL of the homogenate was diluted with 9 mL of 1 % metaphosphoric acid, agitated for 30 s using a vortex (Vortex Genie2® G560E, Scientific Industries, Inc, Fla, USA), sonicated at 4 °C for 5 min using an ultrasonic bath (Ultrasonic Cleaner DC4004, MRC LTD, Israel) and centrifuged at 10000 rpm for 5 min in a 4 °C pre-cooled centrifuge (Centrifuge 5810R, Eppendorf AG, Germany). One mL of the supernatant was transferred into a test tube and incubated in the dark for 10 min at room temperature after adding 9 mL of 0.005% 2, 6 dichloroindophenol dye (Sigma Aldrich, Cape Town, South Africa). Absorbance was measured at 515 nm in triplicate samples using a UV-Vis spectrophotometer (Helios Omega, Thermo Fisher Scientific, Madison, USA) against the 1% metaphosphoric acid solution blank. The amount of vitamin C was calculated from a linear standard curve (0–100 µg/ml) (R^2 = 0.99) of analytical grade L-ascorbic acid (Sigma Adrich). Fresh chemical solutions and a new standard curve were prepared for each day of analysis. The chemicals used were of analytical grade.

2.4. Data analysis

In order to determine effective wavelength, discriminate fruit from three size and colour groups using FT-NIR and to detect outliers, principal component analysis (PCA) was performed using full cross validation. Partial least squares regression (PLSR) was applied to spectral data to develop prediction models for mass, colour index, TSS, TA and vitamin C concentration.

The average of the spectra taken on two opposite sides of the oranges was used for the PLS calibrations. The averaged spectra were subjected to several spectral pre-processing and multivariate analysis using the Unscrambler chemometric software (The Unscrambler Version 9.2, Camo Process SA, Trondheim, Norway) to develop calibration models for predicting the different quality attributes from the spectral data. The dataset was randomly separated into two subsets, 60% for calibration and 40% for validation. Different spectral pre-processings including multiplicative scattering correction (MSC), min-max normalisation (MMN), first derivative, second derivative, standard vector normalisation (SNV), straight line subtraction (SLS), constant offset elimination (COE) were applied to the raw spectral data prior to the PLS regression. Each pre-processing method was first applied individually and later tested in combination with others. The spectral variables which contributed the most to the model were determined from its regression coefficients curve. Wavelength bands with high regression coefficient values indicate that the variable is important to the model while the regression coefficient with a value close to zero does not contribute to the model. Outliers were evaluated using the X-residuals and leverage on the PLS and PCA models (Shiroma and Rodriguez-Saona, 2009). After PLS regression, the spectral pre-processing method which gave the lowest root mean square error of prediction (RMSEP) value for a certain quality attribute was used to develop the calibration model for that quality attribute.

The results were compared on the basis of the regression statistics of the calibration models described by the value of the root mean square error of calibration (RMSEC), *R*-value, the number of latent variables (LV), selected based on the lowest RMSEP value in the RMSEP vs. LV plot, and the residual predictive deviation (RPD), described by Williams and Sobering (1993) and Davey et al. (2009) as the ratio of the standard deviation of the reference data for the validation set to RMSEP. Data analysis was divided into three stages as illustrated in Fig. 1. The first data evaluation stage was comparing the three spectral acquisition modes using reference and spectral data obtained from fruit pooled from the two experiments. From this stage, the best spectral acquisition instrument was selected and the spectra from that instrument were used in the second stage to evaluate the effect of pre-sorting by colour (experiment 1) and size (experiment 2) on model performance. The third stage of data evaluation aimed to develop a robust model using the spectra obtained using the superior instrument and spectra and reference data collected from a wide range of fruit in experiment 1 and 2 (n = 180). Model robustness was tested using independent validation set of 70 fruit picked on the 26th of August 2010 from a farm in Citrusdal, South Africa (32° 25' 22" South, 19° 0' 53" East).

3. Results and discussion

3.1. Spectral characteristics of three FT-NIR acquisition modes

Typical absorbance average spectra (second derivative) obtained from the same batch of 20 'Valencia' oranges using the three different spectral acquisition modes are presented in Fig. 3. There were slight differences in absorbance values obtained using different modes with overall higher absorbance values for the EH of *MATRIX*^{TM}*-F* followed by IS and SP of the MPA spectrometer. These differences could be attributed to the light penetration depth as well as the surface area illuminated by each of the NIR energy. EH, which had the largest surface area illuminated by NIR had higher absorbance values. The shapes of the spectral curves for the respective acquisition modes are similar, all having peaks at 977, 1200, 1450, and 1942 nm. The peaks at 970 and 1450 nm corresponded to the second and first vibrational overtones of OH stretching associated with water absorption, while the peaks at 1200 and 1942 nm correspond to the second and first overtones of CH stretching as well as the third overtone of OH, CH and CH_2 deformation associated with sugar solution reported by Golic et al. (2003). The water peaks for SP at 1450 and 1900 nm were more pronounced than for the other acquisition methods. Considering that SP illuminates a small surface area and reflected light enters through small aperture fibers, having these water peaks more pronounced in the SP than other acquisition methods could suggest that a large amount of NIR radiation was scattered.

3.2. Reference data distribution

In Table 1 the distributional statistics for the reference data of the calibration and validation sets are summarised. The reference data of measured quality parameters in this study were all fairly normally distributed around the mean. The interpretation of calibration results depends greatly on the precision of the determined reference data (Lu et al., 2006). As is apparent in the data presented, wide ranges of variation of all parameters analysed were included in calibration and validation populations. The determination values of the sample quality parameters cover a large range, which is helpful for developing calibration models for NIRS (Clément et al., 2008). The overall mean values of the mass measurements for calibration and validation data sets were, respectively, 171.26 and 164.30 g, while the corresponding standard deviation values were 37.13 and 34.09 g. It is noteworthy that there

were no significant differences in maturity stages between different size and colour classes. However, the MI of different colour classes shows that green fruit corresponded to a slightly less advanced stage of maturation, yellow and orange fruit being at intermediate and most advanced stages of maturation, respectively. The overall mean MI measurements were 10.66 with standard deviation 1.74, while those for green, yellow and orange fruit were 9.63, 10.48 and 11.15, respectively with corresponding standard deviations of 1.79, 1.68 and 1.50.

3.3. Comparison of three FT-NIR acquisition modes

The spectral acquisition mode had a major influence on the calibration regression statistics and the accuracy of prediction (Table 2). The model developed for the spectra acquired by the EH gave the best prediction of mass (R = 0.96, RMSEP = 10.45 g and RPD = 3.53), followed by the IS (R = 0.86, RMSEP = 18.75 g and RPD = 1.97). Louw and Theron (2010) have stated that mass is a physical parameter that cannot be directly measured by NIRS, but can be quantified indirectly measuring water contents. However, in this study, the hypothesis behind predicting mass with high accuracy using the EH and IS compared to direct-contact mode of SP is based on the curvature of the fruits. Curvature of the fruit and size are closely related to mass. EH and IS irradiated the orange fruit from a distance and thus have the better chances to collect the information about curvature.

In the two contactless spectral acquisition modes (EH and IS), smaller fruit reflect less light towards the detector due to a rapidly changing angle of reflection. In this regard, EH had an added advantage, since it irradiates a bigger surface of the fruit and thus receives more information, resulting in a better model with lower RMSEP.

Similarly, the best stable model for colour index prediction was obtained using the EH spectral acquisition mode (R = 0.83, RMSEP = 0.82 and RPD = 1.99). The models were selected based on stability as defined by Gómez et al. (2006) and Fu et al. (2008). These two groups of researchers stated that “The small differences between RMSEC and RMSEP or RMSECV indicate that the model was robust not only for the observations in the calibration dataset but also for external samples”. A large difference between RMSEC and RMSEP indicates the possibility of overfitting and noise is probably modelled (Gómez et al., 2006). Vitamin C was also best predicted using the EH (R = 0.66, RMSEP = 8.01 mg/100mL and RPD = 1.33), while the IS gave the best prediction of TSS (R = 0.83, RMSEP = 0.58 °Brix and RPD = 1.87).

For fruit and vegetables models, Saeys et al. (2005), Davey et al. (2009), Bellon-Maurel et al. (2010) and Cozzolino et al. (2011), suggested that RPD values below 1.5 are considered unusable, those between 1.5 and 2.0 are suitable for rough prediction, those between 2.0 and 2.5 are suitable for quantitative predictions, while RPD values between 2.5 and above 3.0 are respectively considered good and excellent prediction models. In the case of colour index in this study, the RPD value for models developed using IS was 2.21 and higher than that of EH. However, the difference between RMSEC and RMSEP for the emission head was the lowest. The good agreement between the values of RMSEC and RMSEP in the EH model indicated that overfitting noise modelling were not evident (Gómez et al., 2006; Fu et al., 2008) despite the relative higher number of LVs. In addition, the use of RPD has been under some criticism in recent years, because no statistical basis was used to determine these thresholds (Bellon-Maurel et al., 2010; Cozzolino et al., 2011). This demonstrates the importance of using the combination of statistical parameters to evaluate calibration model performance.

Similar to the results obtained by Cayuela (2008) the prediction performance for TA was poor, with R-values for prediction lower than 0.30 (Table 2). The poor model performance observed for TA could be attributed to the fact that citric acid; a major organic acid in citrus fruit is present in relatively lower concentrations in intact fruit, such that their contribution to the NIR spectra is too small to provide good predictions (Guthrie et al 2005).

The high predictive power for TSS of the models developed using IS and vitamin C using EH suggest the possibility of high penetration depth of the NIR radiation. In apples using FT-NIR spectroscopy, the penetration depth is estimated to vary between 1 and 5 mm, depending on the wavelength and the instrument (Lammertyn et al., 2000; Peirs et al., 2002b). In the case of oranges which have thicker rind and rough surfaces, this penetration depth might be different to those of apples, although no recorded literature was found which recorded this on oranges. Furthermore, considering that the oranges were only partially irradiated by the NIR radiation, it is not surprising that the emission head, a spectral acquisition device which irradiates a bigger surface and higher light intensity, had best predictive power for the three out of four quality parameters measured. The prediction statistics for the TSS obtained using EH were also comparable (R = 0.79; RMSEP = 0.65; RPD = 1.68) to those of IS. It is important to note that the mass, colour index and vitamin C models developed in the succeeding sections are based on NIR spectra obtained from the EH while TSS models were developed from spectra obtained from the IS.

3.4. Spectral region selection and spectral pre-processing

The optimal spectral wavelength bands best reflecting the quality parameters were determined after averaging spectra from two opposite equatorial positions of the fruit and testing different spectral pre-processing. The spectral variables which contribute the most to the model were determined from its regression coefficients curve. A wavelength bands with high regression coefficient values indicated that the variable is important to the model while the regression coefficient with a value close to zero does not contribute to the model (Williams and Norris, 2001). Typical regression coefficient curves obtained during prediction model development for TSS in 'Valencia' oranges using spectra obtained by three acquisition methods is portrayed in Fig. 4. In addition, the suitable spectral range was identified by computing the correlation spectrum from the constituent of interest (Tewari et al., 2008).

The optimal waveband for calibrating fruit TSS of 'Valencia' oranges, using EH, was determined to be 900–2000 nm with distinct peaks (high absolute regression coefficients) at 930, 990, 1100, 1200, 1300, 1380, and 1945 nm. According to the table assigning different major vibrational/absorption bonds to positions in the NIR spectrum (Williams and Norris, 2001; Golic et al., 2003; Clément et al., 2008; Shiroma and Rodriguez-Saona, 2009), the high regression coefficient at 930 and 1200 nm can be assigned to the third overtone of CH_2 stretching while 1100 nm to the second overtone of CH stretching related to sugars. The high regression coefficient at 990 and 1100 nm corresponded to OH stretching bands for water absorption. With these results it is possible the informative wave band for predicting TSS using IS was between 900 and 1800 nm. Sugars are known to exhibit bands in the 1100–1600 nm and 1700–2300 nm regions (Tewari et al., 2008). These results are in agreement with the interpretation of these bands as combinations of first, second, and third overtones of OH and CH stretching vibrations of water hydrogen bonds with sugar molecules (Golic et al., 2003). The best vitamin C model obtained by EH was developed based on the 1050-2000 nm wavebands, which is in agreement with the wavebands reported by Xia et al. (2007). Colour was best predicted in the wavelength range from 801 nm to 2355 nm. Since colour is less likely to be directly detected outside the visible range, quantification of CI within the 1000-1900 nm range used in this experiment is likely to represent secondary correlation on attributes related to fruit maturity. Considering the large number of LVs (10) used to develop the model, there is also a very high chance of overfitting the data to the model.

In addition to selecting suitable wavelengths, several NIR pre-processing methods were tried to enhance the prediction ability of the models. The results showing a comparison of

these pre-processing methods in the CI, mass, TSS and vitamin C models are shown in Table 3. In comparison to other pre-processing methods, MSC gave the best calibration and prediction model for CI and vitamin C while TSS was best predicted with models developed after Savitsky-Golay first derivative spectral pre-processing with the second order polynomial. The best model for mass prediction was developed using spectral data without pre-processing.

3.5. Effect of pre-sorting by colour on model performance

The models of green, yellow, and orange fruit pooled together as well colour-specific models for the three colours are presented in Table 4. Scatter plots of the relationships between measured quality parameters and model predictions developed from a combination of all colours are presented in Fig. 5. High correlation between NIR spectra and measured mass, colour index, TSS and vitamin C were observed in all colour fruit. However, calibration models developed on multi-coloured fruit, in most cases gave relatively better prediction accuracy than models developed on a single colour population. This is evident on the mass model where the pooled colour samples had relatively higher prediction statistics (R = 0.93, RMSEP = 9.96, RPD = 2.70) compared to, for instance, the yellow fruit (R = 0.83, RMSEP = 10.09, RPD = 1.80). Similarly, the colour index prediction model of pooled fruit was superior (RPD = 2.08) to those for green, yellow, and orange fruit which had RPD values of 1.55, 1.03, and 1.02, respectively. Higher colour variation in the pooled fruit group than in individual colour groups is responsible for this high prediction power, as previously demonstrated by Clément et al. (2008), namely that a parameter that shows a large sample variation is likely to be better predicted by NIR spectroscopy.

In this study, the spectral wavelength used was ranging from 800 to 2500 nm. Therefore colour effect on absorbance and high predictability of colour index in this region could either result indirectly through its correlation with some biochemical components which are active in the NIR region or that the pigments are not only active in the visible region but also in the NIR region. Ruiz et al. (2008) showed that the spectral range from 1111 to 2500 nm was the most suitable wavelength interval used for developing NIR models for evaluating carotenoid content. It has previously been demonstrated that extension of the wavelength range down to the visible area is beneficial to the determination of colour parameter and pigments, including chlorophyll, carotenoids and lycopene (Williams and Sobering, 1993). These authors reported that results for pigments obtained using an NIR instrument, consisting of the visible range of

the spectrum were better than those without the visible range. A recent study by Davey et al. (2009), evaluating the NIR prediction potential of carotenoids indicated that the visible region between 500 and 600 nm is influential in the developing predicting models. Similarly, Cléments et al. (2008) evaluated the use of NIR to predict lycopene pigment by separating spectral regions into visible to short wave NIR (400–1000 nm) and long wave NIR (900–1500 nm). Results obtained by these authors indicated that the visible and short-wave NIR region (400–1000 nm) was necessary for predicting pigment content and colour variables. A correlation between fruit colour parameters and carotenoid content has previously been reported by Ruiz et al. (2008). In the visible region, pigments would produce some influences on the variation of absorbance in the spectra and this could be directly related to the colour index.

3.6. Effect of pre-sorting by size on model performance

The prediction performance of the models developed using only samples from a certain size group as well those developed by combining the spectra from the different size groups is illustrated in Fig. 6 and summarised in Table 5. The prediction performances slightly higher for the models based on all sizes compared to those for the individual size groups, except for vitamin C for yellow fruit, which was higher than all fruit combined. Briefly, regression statistics showed that pre-sorting fruit by size did not improve the prediction accuracy. A typical example is shown on TSS, where the RPD value of the overall model was 2.20 while that for small, medium and large fruit were 1.51, 1.70 and 1.82, respectively. A similar declining trend in accuracy with size grouping could also be observed for other quality parameters such as mass and colour index. This reduction in model accuracy with narrow range samples further demonstrated the importance of having enough variability when developing prediction model.

3.7. Models covering a wide range of colours and sizes

Models covering the whole range of colours and sizes were developed by using a combination of fruit from colour and size experiments. Table 6 summarises the calibration and prediction performance of the calibration models developed using fruit from all colour and size groups as well as a robust model that included all fruit validated by fruit harvested from another location, Citrusdal. The models developed using fruit from different size classes

gave better prediction statistics for mass and TSS compared to models developed using a wide range of colours and those developed from all fruit combined. For instance, the mass prediction model developed from a wide range of colours gave a correlation coefficient of 0.96, an RMSEP of 10.45 g and an RPD of 3.53 compared to the model developed for size classes which had R = 0.98, RMSEP = 9.79 g and RPD = 5.22. It is noteworthy that the statistical values for the models that covered all size and colour groups were calculated using different calibration and test set data randomly selected from the combination of the three individual colour and size groups. In Fig. 7 the values for mass, colour index, TSS and vitamin C predicted for the external validation set by a vigorous model including all fruit colour and size classes are plotted against the analysed values. The better models developed across population with larger range of the attribute in question demonstrates the importance of enough variability in the reference samples as stated by Davey et al. (2009).

4. Conclusion

In this study, the usefulness of FT-NIR spectroscopy in combination with different sample presentation accessories for the prediction of some major quality properties of 'Valencia' oranges has been investigated. Acceptable predictions of fruit mass, colour index, TSS, and vitamin C were obtained from the NIR reflectance spectra. Clear effects of the spectral acquisition mode on the prediction performance were observed. The emission head of *MATRIX*^{TM}*-F* spectrometer gave the best results for mass, colour index and vitamin C, while the integrating sphere of Multiple Purpose Analyser gave the best results for TSS. Good correlation between spectral information obtained by the Emission Head of *MATRIX*^{TM}*-F* spectrometer and measured parameters (mass, colour index, TSS and vitamin C) demonstrated the potential of this FT-NIR instrument as a non-destructive tool to holistically evaluate external, and internal fruit quality parameters. Calibration models developed across a range of sizes performed well in predicting quality parameters compared to models developed from a single size group. Generally, better calibration and validation results were achieved for models developed using all fruit than for models based on an individual colour or size group. It was no surprise that the acquisition methods with bigger irradiated surface gave the best results for skin colour and mass. Modifying the lamps and detectors of the instruments to match colour measurement range, would allow colour parameters to be measured from principle, rather than indirectly through PLS models against Minolta measured colour space.

References

Barros, L., Ferreira, M. J., Queiros, B., Ferreira, I. C. F. R., Baptista, P., 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidants activities. Food Chem. 103, 413–419.

Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J-M., McBratney, A., 2010. Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. Trends Anal. Chem. 29, 1073–1081.

Blasco, J., Aleixos, N., Gómez-Sanchís, J., Moltó, E., 2009. Recognition and classification of external skin damage in citrus fruits using multispectral data and morphological features. Biosyst. Eng. 10, 137–145.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56, 9813–9818.

Cozzolino, D., Cyankar, W.U., Shah, N., Smith, P., 2010. Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. Food Res. Int. 44, 1888–1896.

Davey, M. W., Saeys, W., Hof, E. Ramon, H., Swennen, R. L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. J. Agric. Food Chem. 57, 1742–1751.

Fu, X., Ying, Y., Zhou, Y., Xie, L., Xu, H., 2008. Application of NIR spectroscopy for firmness evaluation of peaches. J. Zhejiang Univ. Sci. B. 9, 552–557.

Golic, M., Walsh, K. B., Lawson, P., 2003. Short-wavelength near infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

Gómez, A. H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Austr. J. Agric. Res. 56, 405–416.

Jamshidi, B., Minaei, S., Mohajerani, E., Ghassemian, H., 2012. Reflectance Vis/NIR spectroscopy for nondestructive taste characterization of Valencia oranges. Comp. Electron. Agric. 85, 64–69.

Jimenez-Cuesta, M., Cuquerella, J., Martinez-Javega, J.M., 1981. Determination of a colour index for citrus degreening. In Proceeding of the 4th International Society for Citriculture (Vol. II, pp. 750-753). International Citrus Congress: Tokyo, Japan.

Klein, B.P., Perry, A.K., 1982. Ascorbic Acid and Vitamin A Activity in Selected Vegetables from Different Geographical Areas of the United States. J. Food Sci. 47, 941–945.

Lammertyn, J., Peirs, J., De Baerdemaeker, J., Nicolaï, B.M., 2000. Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121–132.

Lammertyn, J., Dressalaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolaï, B.M., 2003. MRI and X-ray CT study of spatial distribution of core breakdown in 'Conference' pears. Magn. Reson. Imag. 21, 805–815.

Liu, Y., Sun, X., Ouyang, A., 2010. Non-destructive measurements of soluble solid content of navel orange fruit by visible-NIR spectrometric technique with PLSR and PCA-BPNN. LWT-Food Sci. Technol. 43, 602–607.

Louw, E.D., Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina* L.) using NIR spectroscopy. Postharvest Biol. Technol. 58, 176–184.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application of Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. 7, 794–799.

Magwaza, L. S., Opara, U. L., Nieuwoudt, H., Cronje, P. Saeys, W., Nicolaï, B., 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food Bioprocess Tech. 5, 425–444.

Meglinski, I.V., Buranachai, C., Terry, L.A., 2010. Plant photonics: Application of optical coherence tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I.K., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Nicolaï, B.M., Verlinden, B.E., Desmet, M., Saevels, S., Saeys, W., Theron, I.K., Cubeddub, R., Pifferi, A., Torricelli, A., 2008. Time-resolved and continuous wave NIR reflectance

spectroscopy to predict soluble solids content and firmness of pear. Postharvest Biol. Technol. 47, 68–74.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. HortScience 34, 114–118.

Peirs, A., Scheerlinck, N., Touchant, K., Nicolaï, B.M., 2002a. Comparison of fourier transform and dispersive near-infrared reflectance spectroscopy for apple quality measurements. Biosyst. Eng. 81, 305–311.

Peirs, A., Tirry, J., Verlinden, B., Darius, P., Nicolaï, B.M., 2002b. Effect of biological variability on the robustness of NIR-models for soluble solids content of apples. Postharvest Biol. Technol. 28, 269–280.

Ruiz, D., Reich, M., Bureau, S., Rebard, C.M.G.C., Audergon, J-M., 2008. Application of reflectance colorimeter measurements and infrared spectroscopy methods to rapid and nondestructive evaluation of carotenoids content in apricot (*Prunus armeniaca* L.). J. Agric. Food Chem. 4916–4922.

Saeys, W., Mouazen, A.M., Ramon, H., 2005. Potential onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst. Eng. 91, 393–402.

Sánchez, M-T., Dela Haba, M-J., Serrano, I., Pérez-Marín, D., 2013. Application of NIRS for nondestructive measurement of quality parameters in intact oranges during on-tree ripening and at harvest. Food Anal. Method. 6, 826–837.

Shiroma, C., Rodriguez-Saona, S., 2009. Application of NIR and MIR spectroscopy in quality control of potato chips. J. Food Comp. Anal. 22, 596–605.

Sun, X., Zhang, H., Liu, Y., 2009. Nondestructive assessment of quality of 'Nanfeng' mandarin fruit by a portable near infrared spectroscopy. Int. J. Agric. Biol. Eng. 2, 65–71.

Tewari, J.C., Dixit, V., Chi, B-K., Malik, K.A., 2008. Determination of origin and sugars of citrus fruit using genetic algorithm, correspondence analysis and partial least square combined with fiber optic NIR spectroscopy. Spectrochim. Acta Mol. Biomol. Spectrosc. 71, 1119–1127.

Tijskens, L.M.M., Schouten, R.E., 2009. Modelling quality attributes and quality related product properties. In W.J. Florkowski, R.L. Shewfelt, B., Brueckner, S.E. Prusia (Eds.), Postharvest Handling: A Systems Approach (1st ed.), pp. 283–312). Amsterdam, Netherlands: Academic Press, Elsevier.

Verboven, P., Kerckhofs, G., Mebatsion, H. K., Ho, Q. T., Temst, K., Wevers, M., Cloetens, P., Nicolaï, B.M., 2008. Three-dimensional gas exchange pathways in pome fruit characterized by synchrotron x-ray computed tomography. Plant Physiol. 147, 518–527.

Williams, P., Norris, K.H., 2001. Variables affecting near infrared spectroscopic analysis. In P. Williams, K.H. Norris, K.H. (Eds.), Near Infrared Technology in the Agriculture and Food Industries (2nd ed.) (pp. 171–185). The American Association of Cereal Chemists, St. Paul, MNL.

Williams, P.C., Sobering, D.C., 1993. Comparison of commercial near infrared transmittance and reflectance instruments for the analysis of whole grains and seeds. J. Near Infrared spectrosc. 1, 25–32.

Xia, J., Li, X., Li, P., Ma, Q., Ding, X., 2007. Application of wavelet transform in the prediction of 'Navel' orange vitamin C content by near-infrared spectroscopy. Agric. Sci. China 6, 1067–1073.

Table 1: Mean, standard deviation (SD) and range for calibration and validation subsets of green (n = 40), yellow (n = 40), orange (n = 40), big (n = 20), medium (n = 20) and small (n = 20) 'Valencia' oranges.

Quality parameter	Fruit class	Calibration			Validation			Overall CV %
		Mean	SD	Range	Mean	SD	Range	
Mass (g)	**All**	171.26	37.13	91.33-296.33	164.30	34.09	99.87-280.81	21.75%
	Colour	171.48	26.19	123.87-243.61	172.91	27.98	114.29-227.29	11.81%
	Green	159.07	32.39	114.29-243.61	165.17	34.22	116.77-227-29	20.33%
	Yellow	168.32	16.78	133.45-206.15	173.36	20.14	138.53-200.87	10.64%
	Orange	182.47	20.09	147.60-231.62	189.13	25.69	159.05-227.12	12.05%
	Size	164.99	51.96	91.33-296.33	161.08	50.78	99.87-280.81	31.27%
	Small	113.92	10.27	91.33-131.37	113.00	8.21	99.87-121.83	8.17%
	Medium	151.51	13.68	127.04-171.50	146.68	10.54	131.87-164.17	8.24%
	Large	229.45	30.14	194.48-296.33	225.05	26.14	194.99-280.81	12.12%
Colour index	**All**	4.10	1.66	-2.48-7.54	3.72	1.63	-0.48-6.94	40.96%
	Colour	3.80	1.72	-2.49-6.94	3.42	1.61	-0.48-6.94	30.74%
	Green	2.01	1.46	-2.49-5.40	2.44	1.14	-0.48-4.00	61.54%
	Yellow	3.27	0.52	2.15-4.47	3.31	0.60	2.39-4.40	16.64%
	Orange	5.62	0.64	4.69-6.94	5.50	0.75	4.58-6.87	12.40%
	Size	4.58	1.37	1.98-7.54	4.46	1.22	2.06-6.57	28.78%
	Small	3.20	0.85	1.98-5.41	3.15	0.64	2.06-4.28	23.79%
	Medium	5.50	0.74	4.35-6.57	5.52	0.64	4.28-6.04	13.20%
	Large	5.02	1.51	2.85-7.54	4.96	0.46	4.27-5.92	21.73%
TSS (°Brix)	**All**	9.41	1.00	6.80-11.80	9.40	1.19	6.80-11.60	11.38%
	Colour	9.31	0.98	6.80-11.60	9.28	1.25	6.80-11.60	9.75%
	Green	8.69	1.11	6.80-11.50	8.61	1.10	7.30-11.50	12.61%
	Yellow	9.85	1.01	8.00-11.60	9.69	0.93	8.20-11.40	9.89%
	Orange	9.52	0.83	7.60-11.40	9.36	0.97	8.00-11.30	9.24%
	Size	9.78	0.98	7.50-11.80	9.35	1.02	7.70-11.50	10.54%
	Small	9.67	1.33	7.50-11.80	9.25	1.10	7.80-11.10	12.94%
	Medium	9.17	0.54	8.30-9.90	10.08	0.83	9.20-11.20	9.33%
	Large	9.70	1.06	7.70-11.20	9.55	0.79	8.40-11.00	9.49%
Vit. C (mg/100mL)	**All**	46.62	10.32	23.82-77.86	48.52	11.10	25.47-74.24	22.49%
	Colour	46.00	9.88	23.82-74.24	47.75	11.15	28.32-74.24	19.29%
	Green	42.96	12.78	23.82-69.02	43.10	9.11	32.80-67.53	26.33%
	Yellow	52.30	9.49	31.07-68.78	50.46	10.24	34.31-65.76	18.83%
	Orange	44.79	8.20	36.17-74.24	46.46	7.87	35.20-59.80	17.64%
	Size	48.81	12.51	25.47-77.86	48.65	8.93	29.80-64.96	22.84%
	Small	49.87	17.79	25.47-73.02	49.15	9.02	39.12-63.75	29.47%
	Medium	45.96	3.65	38.50-50.59	49.79	4.57	42.30-55.27	12.72%
	Large	47.18	14.60	29.80-77.86	48.59	7.95	35.41-63.63	23.94%
Maturity index	**All**	10.79	1.69	6.53-16.44	10.47	1.82	6.55-14.32	16.33%
	Colour	10.57	1.78	6.53-16.44	10.16	1.82	6.55-14.32	15.82%
	Green	9.39	1.93	6.53-14.14	9.99	1.55	7.19-13.38	18.62%
	Yellow	10.42	1.77	6.55-14.32	10.43	1.64	7.82-13.33	16.31%
	Orange	11.44	1.49	9.10-16.44	10.84	1.71	7.41-13.58	14.10%
	Size	11.16	1.51	7.71-14.03	11.16	1.54	8.78-13.91	13.53%
	Small	11.45	1.79	7.71-13.81	10.19	1.34	8.78-12.33	15.63%
	Medium	10.99	1.54	8.91-13.91	11.46	1.56	9.03-13.85	13.73%
	Large	11.43	1.68	8.99-14.03	11.36	0.89	10.21-13.38	11.47%

CV %, Coefficient of variation; TSS, Total soluble solids; Vit. C, Vitamin C.

Table 2: Model performance for each quality parameter using different FT-NIR probes.

Quality parameter	NIR probe	LV	Pre-pr	Calibration model			Validation Model				Waveband (nm)
				R	RMSEC	Slope	R	RMSEP	RPD	Slope	
Mass (g)	EH	7	None	0.97	9.72	0.93	0.96	10.45	3.53	0.92	900-1550
	IS	7	None	0.89	16.84	0.79	0.86	18.75	1.97	0.76	
	SP	7	None	0.60	29.50	0.35	0.54	31.04	1.19	0.33	
Colour Index	EH	10	MSC	0.88	0.69	0.78	0.83	0.82	1.99	0.73	1000-1900
	IS	10	MSC	0.93	0.51	0.87	0.86	0.74	2.21	0.77	
	SP	10	MSC	0.86	0.79	0.73	0.73	1.04	1.57	0.59	
TSS (°Brix)	EH	6	FD	0.84	0.57	0.70	0.79	0.65	1.68	0.65	900-1800
	IS	6	FD	0.89	0.48	0.79	0.83	0.58	1.87	0.73	
	SP	6	FD	0.42	0.98	0.17	0.17	1.10	0.99	0.07	
Vitamin C (mg/100 mL)	EH	8	MSC	0.73	7.21	0.53	0.66	8.01	1.33	0.48	1050-2000
	IS	8	MSC	0.76	6.88	0.57	0.59	8.69	1.23	0.44	
	SP	8	MSC	0.38	9.83	1.45	0.04	11.28	0.94	0.02	
TA (%)	EH	7	MSC	0.43	0.12	0.43	0.22	0.14	1.14	0.30	801-2175
	IS	7	MSC	0.29	0.16	0.02	0.12	0.15	1.06	0.01	
	SP	7	MSC	0.46	0.13	0.46	0.27	0.15	1.09	0.31	

Pre-pr, pre-processing; LV, latent variables; R, correlation coefficient; RMSEC, root mean square error of calibration; RPD, residual predictive deviation; RMSEP, root mean square error of prediction; EH, emission head; IS, integrating sphere; SP, fibre-optic probe for solid samples; None, no pre-processing; MSC, multiplicative scattering correction; FD, first derivative; TSS, total soluble solids; TA, titratable acidity.

Table 3: Performance of models developed using different spectral pre-processing methods. Mass colour index, and vitamin C were analysed using Matrix-F emission head, while TSS was predicted using integrating sphere of the multipurpose analyser.

Quality			Calibration model			Validation model				Waveband
parameter	Pre-Pr	LV	R	RMSEC	Slope	R	RMSEP	RPD	Slope	(nm)
Mass	None	7	0.97	9.72	0.93	0.93	10.45	2.57	0.92	
(g)	MSC	7	0.91	15.68	0.82	0.82	17.08	1.57	0.80	900-1550
	FD	7	0.97	8.78	0.94	0.94	10.95	2.45	0.89	
	SD	7	0.95	10.67	0.91	0.91	17.00	1.58	0.73	
Colour	None	10	0.84	0.78	0.71	0.71	0.90	1.89	0.64	
Index	MSC	10	0.88	0.69	0.78	0.78	0.82	2.08	0.73	1000-1900
	FD	10	0.87	0.69	0.79	0.79	0.86	1.98	0.70	
	SD	10	0.89	0.69	0.79	0.79	0.85	2.00	7.00	
TSS	None	6	0.84	0.58	0.70	0.70	0.61	1.78	0.68	
(°Brix)	MSC	6	0.86	0.53	0.73	0.73	0.59	1.84	0.70	900-1800
	FD	6	0.89	0.48	0.79	0.79	0.58	1.87	0.73	
	SD	6	0.80	0.64	0.64	0.64	0.69	1.58	0.60	
Vitamin C	None	8	0.69	7.71	0.47	0.47	8.30	1.25	0.44	
(mg/100 mL)	MSC	8	0.73	7.21	0.53	0.53	8.01	1.30	0.48	1050-2000
	FD	8	0.71	7.52	0.50	0.50	8.74	1.19	0.41	
	SD	8	0.61	8.42	0.37	0.37	8.58	1.21	0.36	

Pre-pr, pre-processing; LV, latent variables; R, correlation coefficient; RMSEC, root mean square error of calibration; RPD, residual predictive deviation; RMSEP, root mean square error of prediction; None, no pre-processing; MSC, multiplicative scattering correction; FD, first derivative; SD, second derivative; TSS, total soluble solids.

Table 4: Model performance of all colours grouped together and for each of the colour groups individually. Mass colour index, and vitamin C were analysed using Matrix-F emission head, while TSS was predicted using integrating sphere of the multipurpose analyser.

Quality Parameter	Fruit class	Pre-Pr	LV	Calibration			Validation				Info. Region (nm)
				R	RMSEC	Slope	R	RMSEP	RPD	Slope	
Mass (g)	All	None	7	0.94	8.825	0.89	0.93	9.962	2.70	0.87	
	Green	None	7	0.98	7.170	0.95	0.95	9.714	3.38	0.92	900-1550
	Yellow	None	7	0.92	7.130	0.84	0.83	10.09	1.80	0.79	
	Orange	None	7	0.93	7.840	0.87	0.91	9.275	2.41	0.85	
Colour Index	All	MSC	10	0.89	0.701	0.78	0.84	0.818	2.08	0.74	
	Green	MSC	10	0.87	0.469	0.76	0.54	0.864	1.55	0.45	1000-1900
	Yellow	MSC	10	0.64	0.412	0.41	0.37	0.530	1.03	0.26	
	Orange	MSC	10	0.70	0.473	0.49	0.35	0.676	1.03	0.26	
TSS (°Brix)	All	FD	4	0.87	0.529	0.75	0.83	0.593	1.83	0.71	
	Green	FD	4	0.87	0.524	0.75	0.79	0.643	1.70	0.66	900-1800
	Yellow	FD	4	0.87	0.471	0.76	0.81	0.570	1.70	0.70	
	Orange	FD	4	0.98	0.156	0.96	0.88	0.370	2.36	0.74	
Vit. C (mg/ 100mL)	All	MSC	8	0.81	6.072	0.66	0.72	7.290	1.42	0.61	
	Green	MSC	8	0.57	9.307	0.33	0.46	10.12	1.12	0.26	1050-2000
	Yellow	MSC	8	0.93	3.589	0.87	0.81	5.918	1.64	0.74	
	Orange	MSC	8	0.71	5.586	0.50	0.56	6.686	1.20	0.39	

Pre-pr, pre-processing; LV, latent variables; R, correlation coefficient; RMSEC, root mean square error of calibration; RPD, residual predictive deviation; RMSEP, root mean square error of prediction; None, no pre-processing; MSC, multiplicative scattering correction; FD, first derivative; TSS, total soluble solids.

Table 5: Model performance for each of the size groups and all sizes grouped together. Mass colour index, and vitamin C were analysed using Matrix-F emission head, while TSS was predicted using integrating sphere of the multipurpose analyser.

Quality Parameter	Fruit class	Pre-Pr	LV	Calibration			Validation				Info. Region (nm)
				R	RMSEC	Slope	R	RMSEP	RPD	Slope	
Mass (g)	All	None	7	0.99	8.36	0.97	0.98	9.79	5.22	0.97	
	Small	None	7	0.76	5.85	0.58	0.65	6.91	1.34	0.47	900-1550
	Medium	None	7	0.96	3.25	0.93	0.93	4.41	2.79	0.90	
	Large	None	7	0.96	7.14	0.93	0.93	10.17	2.71	0.96	
Colour Index	All	MSC	10	0.89	0.59	0.79	0.79	0.81	1.62	0.68	
	Small	MSC	10	0.72	0.51	0.52	0.54	0.64	1.18	0.42	1000-1900
	Medium	MSC	10	0.75	0.46	0.55	0.39	0.70	1.02	0.32	
	Large	MSC	10	0.67	0.81	0.45	0.23	1.19	0.91	0.17	
TSS (°Brix)	All	FD	6	0.94	0.33	0.88	0.86	0.48	2.20	0.78	
	Small	FD	6	0.79	0.68	0.62	0.68	0.82	1.51	0.53	900-1800
	Medium	FD	6	0.99	0.16	0.97	0.82	0.53	1.70	0.68	
	Large	FD	6	0.86	0.47	0.73	0.77	0.58	1.82	0.63	
Vit. C (mg/ 100mL)	All	MSC	8	0.71	7.59	0.51	0.59	8.78	1.27	0.42	
	Small	MSC	8	0.98	2.51	0.96	0.71	9.94	1.47	0.69	1050-2000
	Medium	MSC	8	0.71	4.27	0.50	0.46	5.64	1.10	0.35	
	Large	MSC	8	0.77	7.10	0.60	0.61	8.96	1.28	0.45	

Pre-pr, pre-processing; LV, latent variables; R, correlation coefficient; RMSEC, root mean square error of calibration; RPD, residual predictive deviation; RMSEP, root mean square error of prediction; None, no pre-processing; MSC, multiplicative scattering correction; FD, first derivative; TSS, total soluble solids.

Table 6: Model performance for all fruit grouped together, all colour fruit grouped together and all sizes grouped together. Mass colour index, and vitamin C were analysed using Matrix-F emission head, while TSS was predicted using integrating sphere of the multipurpose analyser.

Quality	Fruit			Calibration			Validation				Info. Region
Parameter	class	Pre-pr	LV	R	RMSEC	Slope	R	RMSEP	RPD	Slope	(nm)
Mass	All	None	7	0.97	9.72	0.93	0.96	10.45	3.53	0.92	
(g)	All Colour	None	7	0.94	8.83	0.89	0.93	9.96	2.7	0.87	900-1550
	All size	None	7	0.99	8.36	0.97	0.98	9.79	5.22	0.97	
Colour	All	MSC	10	0.88	0.69	0.78	0.83	0.82	1.99	0.73	
Index	All Colour	MSC	10	0.89	0.7	0.78	0.84	0.82	2.08	0.74	1000-1900
	All size	MSC	10	0.89	0.59	0.79	0.79	0.81	1.62	0.68	
TSS	All	FD	6	0.89	0.48	0.79	0.83	0.58	1.87	0.73	
(°Brix)	All Colour	FD	6	0.87	0.53	0.75	0.83	0.59	1.83	0.71	900-1800
	All size	FD	6	0.94	0.33	0.88	0.86	0.48	2.2	0.78	
Vit. C	All	MSC	8	0.73	7.21	0.53	0.66	8.01	1.33	0.48	
(mg/	All Colour	MSC	8	0.81	6.07	0.66	0.72	7.29	1.42	0.61	1050-2000
100mL)	All size	MSC	8	0.71	7.59	0.51	0.59	8.78	1.27	0.42	

Pre-pr, pre-processing; LV, latent variables; R, correlation coefficient; RMSEC, root mean square error of calibration; RPD, residual predictive deviation; RMSEP, root mean square error of prediction; None, no pre-processing; MSC, multiplicative scattering correction; FD, first derivative; TSS, total soluble solids.

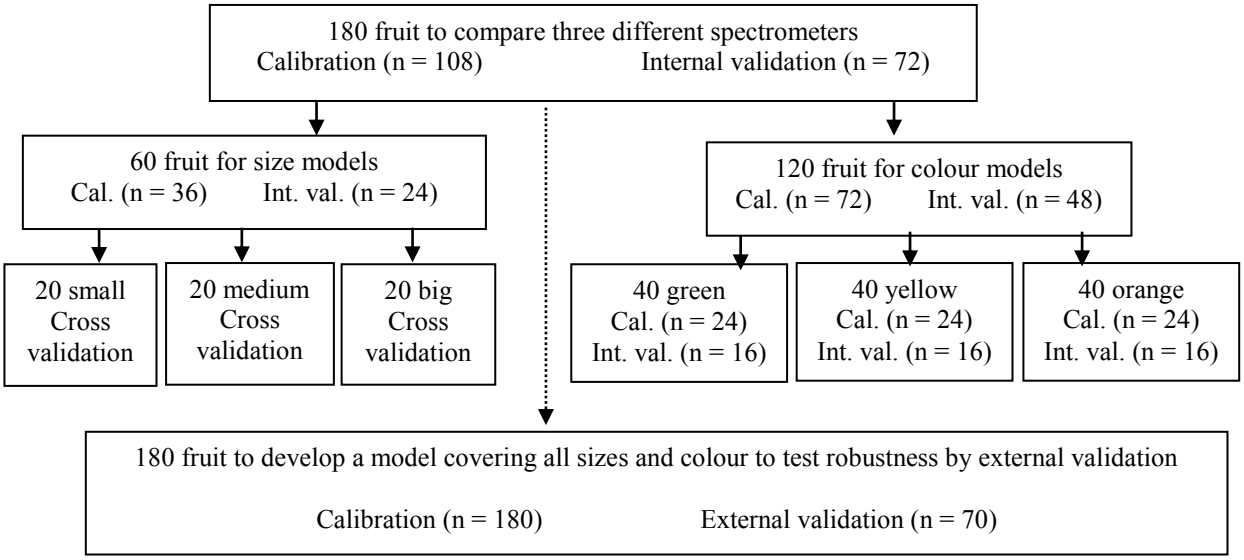

Fig. 1: Chronological sequence of experimental design and distribution of samples during model development.

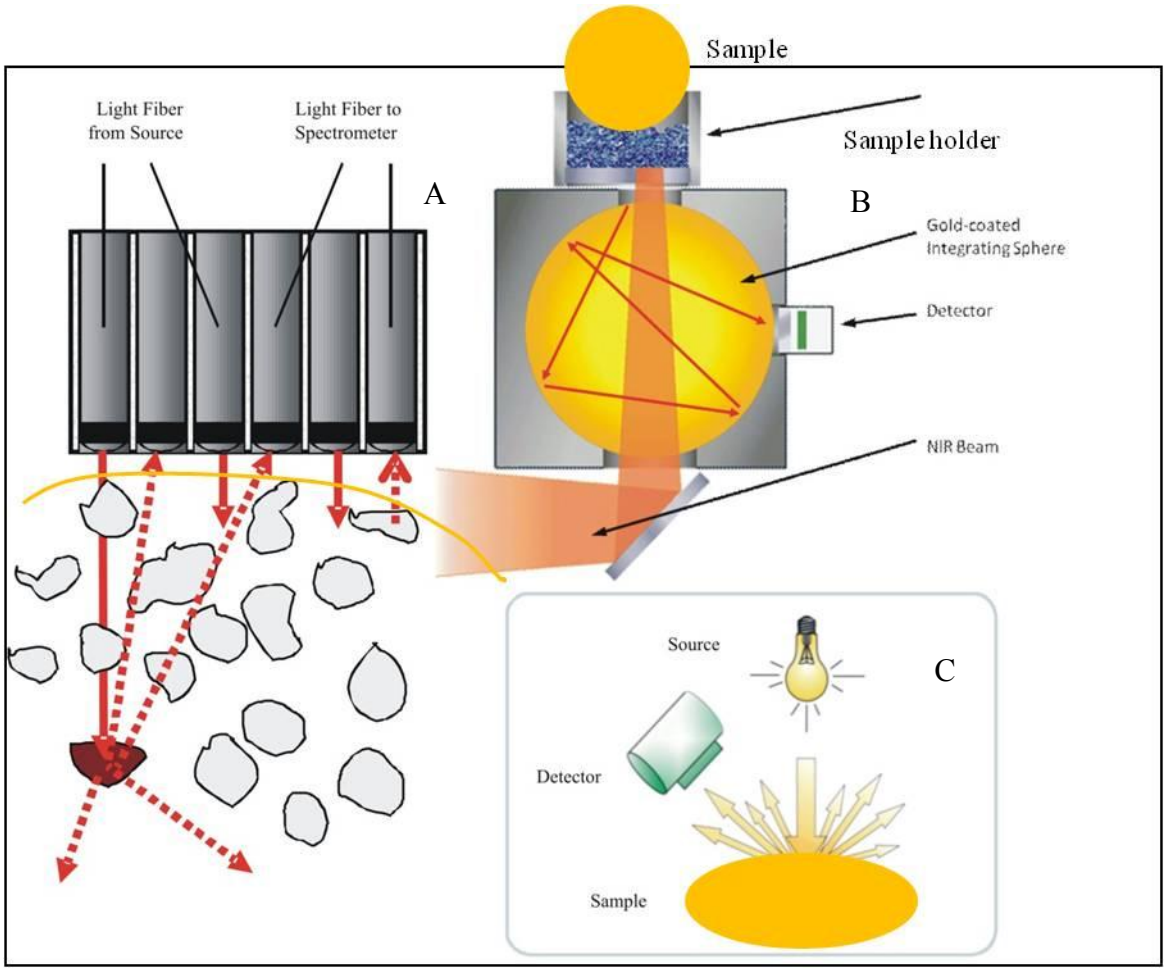

Fig. 2: Schematic diagrams of FT-NIR reflectance acquisition set-up in the fibre-optic probe for solid samples (A), integrating sphere (B) and *MATRIX*^{TM}*-F (Q410/A)* (C).

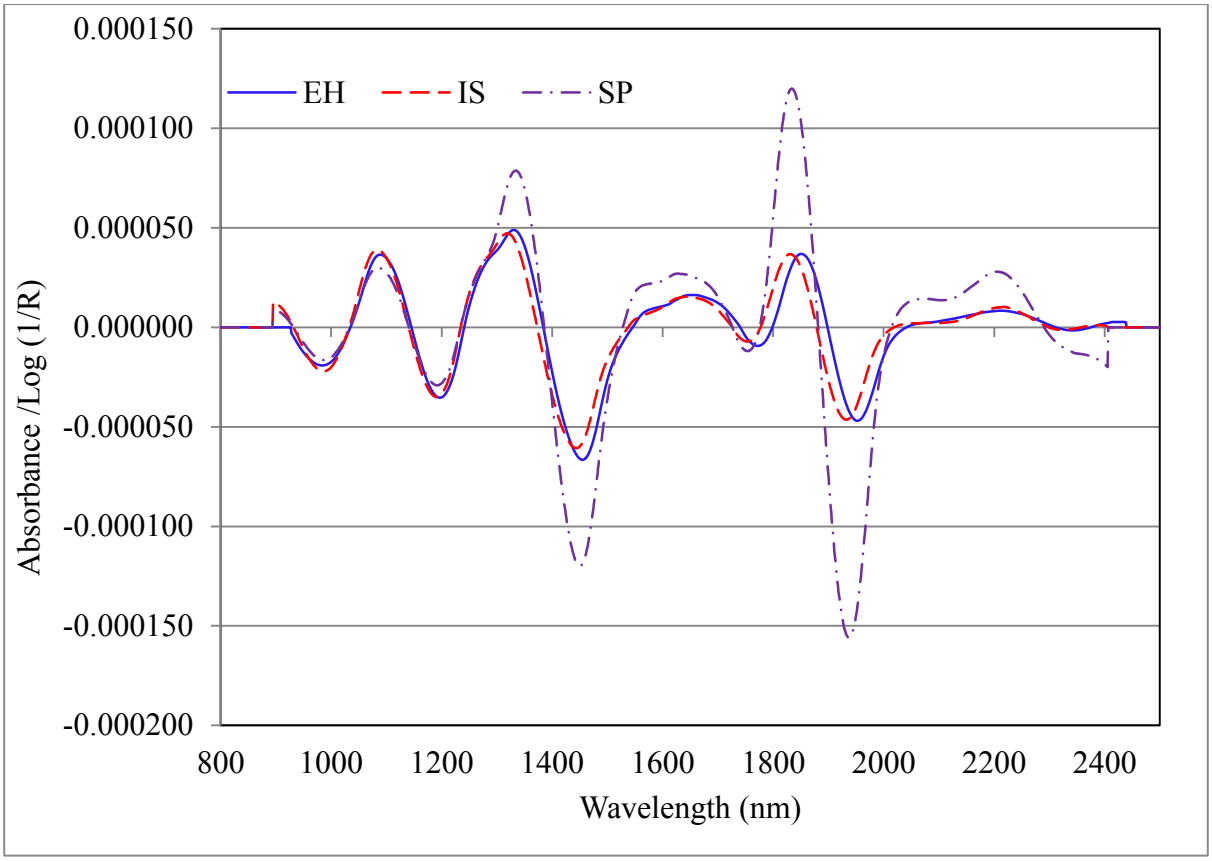

Fig. 3: Typical second derivative absorbance spectra of 'Valencia' oranges obtained using three different spectral acquisition modes, emission head (EH, Matrix F, blue solid line), fiber optic probe for solid samples (SP, purple broken line) and integrating sphere (IS, red broken line).

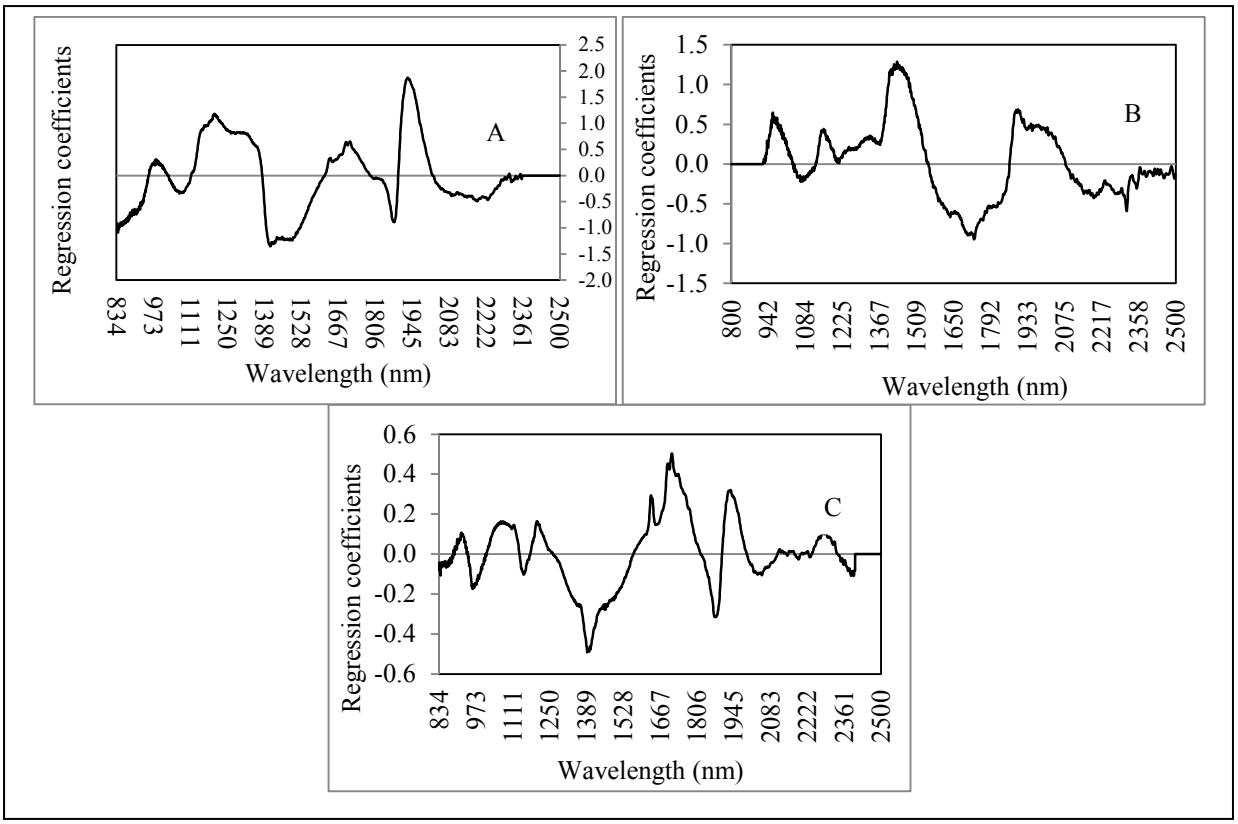

Fig. 4: Typical regression coefficients curve of the TSS models of intact 'Valencia' oranges with 6 latent variables. Spectra were acquired using emission head (A), integrating sphere (B) and fiber optic probe for solid samples (C).

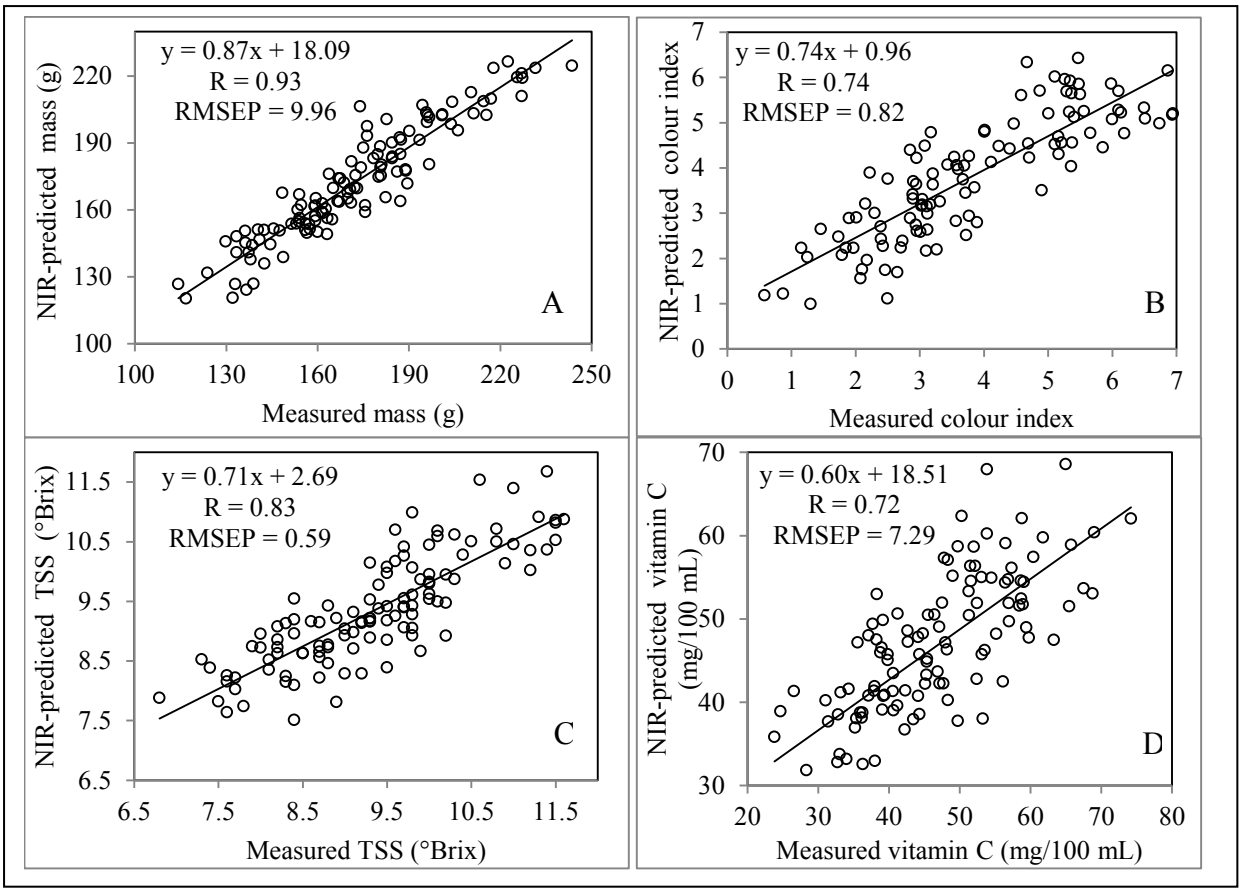

Fig. 5: Scatter plots for of NIR predicted mass (A), TSS (B), vitamin C (C) and colour index (D) against conversional measured parameters on pooled fruit from different colour groups.

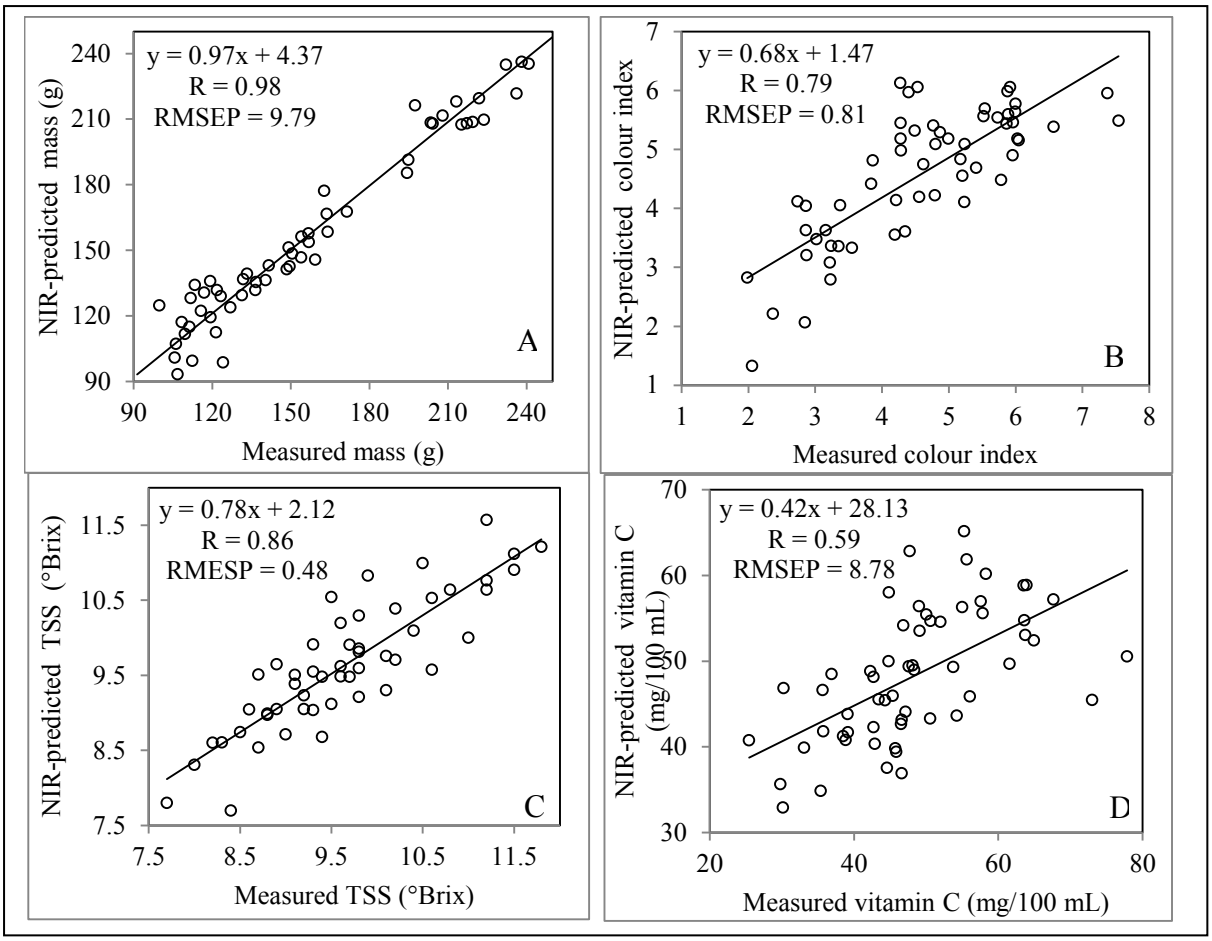

Fig. 6: Scatter plots for of NIR predicted mass (A), TSS (B), vitamin C (C) and colour index (D) against conversional measured parameters on pooled fruit from different size groups.

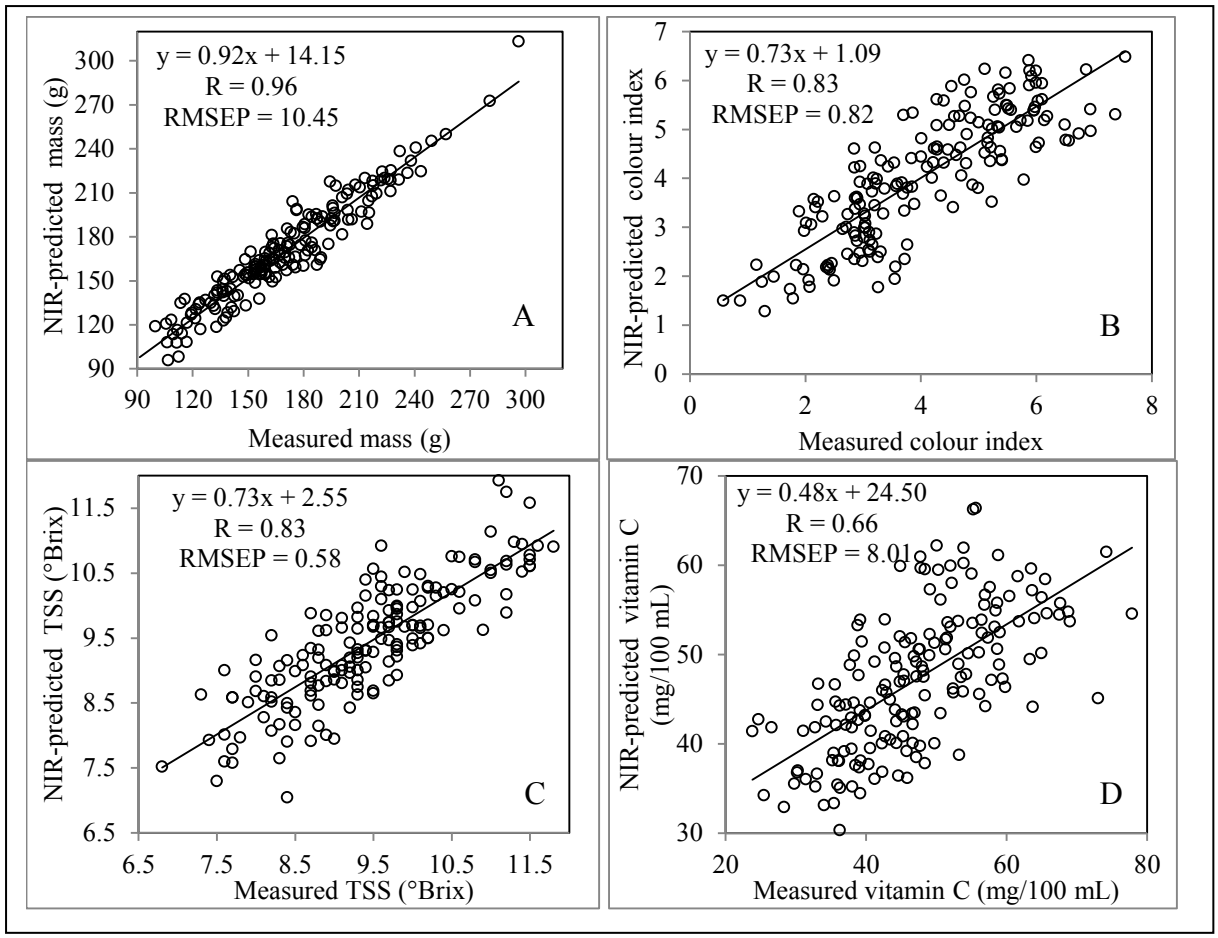

Fig. 7: Scatter plots for of NIR predicted mass (A), TSS (B), vitamin C (C) and colour index (D) against conversional measured parameters on pooled fruit from all size and colour groups.

Chapter 5

RESEARCH RESULTS

PAPER 4: PREDICTION OF 'NULES CLEMENTINE' MANDARIN SUSCEPTIBILITY TO RIND BREAKDOWN DISORDER USING VIS/NIR SPECTROSCOPY*

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PREDICTION OF 'NULES CLEMENTINE' MANDARIN SUSCEPTIBILITY TO RIND BREAKDOWN DISORDER USING VIS/NIR SPECTROSCOPY

Abstract

The use of diffuse reflectance visible and near infrared (Vis/NIR) spectroscopy was explored as a non-destructive technique to predict 'Nules Clementine' mandarin fruit susceptibility to rind breakdown (RBD) disorder by detecting rind physico-chemical properties of 80 intact fruit harvested from different preharvest canopy positions and bagging treatments. Vis/NIR spectra were obtained using a LabSpec® spectrophotometer. Reference physico-chemical data of the fruit were obtained after 8 weeks of storage at 8°C using conventional methods and included RBD, hue angle, colour index, mass loss, rind dry matter, as well as carbohydrates, and total phenolic acid concentrations. Principal component analysis (PCA) was applied to analyse spectral data to identify clusters in the PCA score plots and outliers. Partial least squares regression (PLS) was applied to spectral data after PCA to develop prediction models for each quality attribute. The spectra were subjected to a test set validation by dividing the data into calibration (n = 48) and test validation (n = 32) sets. An extra set of 40 fruit harvested from a different part of the orchard was used for external validation. PLS-discriminant analysis (PLS-DA) models were developed to sort fruit based on canopy position and RBD susceptibility. Fruit position within the canopy had a significant influence on rind biochemical properties. Outside fruit had higher concentrations of carbohydrates in the rind, phenolic acids and dry matter content and lower RBD index than inside fruit. The data distribution in the PCA and PLS-DA models displayed four clusters that could easily be identified. These clusters allowed distinction between fruit from different preharvest treatments. NIR calibration and validation results demonstrated that colour index, dry matter, total carbohydrates and water loss were predicted with significant accuracy, with residual predictive deviation (RPD) for prediction of 4.13, 3.57, 3.15 and 2.34, respectively. The good prediction statistics for carbohydrate concentration demonstrated the potential of Vis/NIR as a non-destructive tool to predict fruit susceptibility to RBD.

Keywords: Non-destructive technique · Vis/near infrared spectroscopy · Rind breakdown disorder (RBD) · Citrus · 'Nules Clementine' Mandarin · Canopy position.

1. Introduction

Citrus fruit is the primary fruit crop in international trade, with current annual worldwide production estimated at over 122 million tons (Moltó and Blasco, 2008; FAOSTAT, 2010). However, the development of various types of physiological disorders limits postharvest storage potential and causes commercial losses. A lack of understanding of the physiological mechanism underlying these disorders affects both supply and profits. The challenge is significant because postharvest disorders such as RBD of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.), do not manifest during harvest or grading in the packhouse but develop about 3 to 5 weeks after harvest.

RBD is initially manifested on the equatorial plane as small, irregular, slightly sunken and colourless patches of about 3 to 6 mm in diameter scattered about the flavedo (the outer-most, pigmented part of citrus rind) of the fruit (Fig. 1) (Cronje et al., 2011). These sunken areas, occurring directly above and among the oil glands of the flavedo, coalesce producing larger affected areas, turning reddish to dark-brown, and become dry and necrotic in the severe stages of the disorder with extended storage period (Cronje, 2007, 2009). According to Agustí et al. (2001) browning of the affected rind surface appears to be the result of oxidative processes.

Intensive research has been conducted towards determining factors triggering RBD. As a result, it has been established by several research groups that different microclimates are influencing sensitivity of fruit to RBD and similar rind disorders such as rind pitting on oranges (Alférez and Zacarìas, 2001) and grapefruit (Alférez and Burns, 2004) and peteca spot of lemon (Wild, 1991). In a study conducted in Spain (Northern Hemisphere), Almela et al. (1992) reported that these microclimatic fluctuations could also be observed among mandarin fruits from the same tree and the incidence being enhanced by exposure of individual fruit to the sun. These investigators further showed that fruit oriented to the north-west (NW) in canopy were most affected by the disorder. In a later study conducted in the same country, Agustí et al. (2001) corroborated that orange fruit positioned in the NW face of the tree to be more prone to develop the rind pitting disorder, a similar disorder, and this was reported to be consistent over five consecutive seasons. Similar observations were reported in 'Fortune' (Duarte and Guardiola, 1995) and 'Encore' (Chikaizumi, 2000) mandarin fruit, where it was maintained that the various rind disorders affect mainly the exposed fruit from the NW quadrant of the tree.

In a study conducted in South Africa (Southern Hemisphere), fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affect the flavedo concentration of carbohydrates during fruit development (Cronje, 2009; Cronje et al., 2011). The latter authors reported the flavedo from fruit borne on the outside of the canopy to have significantly higher sucrose, glucose and fructose content than the fruit borne inside the canopy. Interestingly, the results obtained by this group of investigators revealed a correlation between fruit position, rind carbohydrate concentration and ultimately development of RBD. The incidence of rind breakdown was higher on inside fruit compared with the outside fruit and this was consistent from season to season and could be attributed to their exclusion from adequate sunlight during their fruit development. In addition, fruit borne inside the canopy had lower chlorophyll and carotenoid contents, and therefore poorer rind colour, and lower carbohydrates which might contribute on fruit susceptibility to the disorder (Khumalo, 2006; Cronje, 2009). Although intensive research aimed at elucidating the causal mechanism of RBD has been conducted, the disorder still occurs frequently and unpredictably, reducing the quality of the fruit (Almela et al., 1992; Cronje, 2005).

There is therefore a need to develop an objective, fast and non-destructive assessment that can be used to accurately determine/predict citrus fruit susceptibility to RBD. Non-visible information, such as that provided by the near infrared (NIR) region of the electromagnetic spectrum can be used to quantify rind biochemical profile and hence lead to the possible identification of non-visible physiological disorder (Blasco et al., 2007). Most current non-destructive quality measurements using NIR spectroscopy (NIRS) have been developed to assess fresh fruit according to their internal quality attributes (Butz et al., 2005). Limited research has been conducted to develop technology that can assess, predict and monitor physiological disorders in general and rind disorders of citrus fruit in particular. Nevertheless, NIRS has been used successfully to detect surface bruising in apple (Geeola et al., 1994), surface defects in peach (Miller and Delwiche, 1991), storage-related disorders in kiwifruit (Clark et al., 2004) and drying internal disorder in ‘Tangerine’ citrus (Peiris et al., 1998). Recently, Teerachaichayut and co-workers (2011) successfully used NIR spectroscopy to non-destructively predict pericarp hardening disorder in mangosteen fruit. The recent trend has shifted towards developing reliable and cost effective technologies to non-destructively screen fruit based on their susceptibility to physiological disorders. Recently, Zheng et al. (2010) used NIRS in the reflectance mode to predict oleocellosis sensitivity in citrus fruit. A review by Magwaza et al. (2012) (Chapter 3) discusses the recent developments and

application of Vis/NIR spectroscopy to non-destructively evaluate internal and external fruit quality.

In summary, the knowledge of biochemical changes in the rind of citrus fruit that could be used to precisely predict fruit rind condition and therefore susceptibility to rind disorders is limited. However, previous research by Cronje et al. (2011) indicated that fruit position within the canopy affects the rind biochemical profile, particularly carbohydrate concentration and hence susceptibility of fruit to RBD. This study aims to explore the use of diffuse reflectance Vis/NIR spectra collected at harvest to predict susceptibility of intact fruit to RBD, which shows symptoms about 3-8 weeks postharvest, by detecting rind physico-chemical properties of individual intact fruit from different canopy positions.

2. Materials and methods

2.1. Site, fruit sampling and postharvest handling

A total of 15 'Nules Clementine' mandarin (*Citrus reticulata* Blanco.) trees in an orchard at Stellenbosch University experimental farm, Western Cape Province, South Africa (33°53'04.56"S, 18°37'36.84"E) were identified as being similar in health and fruit setting. On each tree, five fruit of uniform size from sun-exposed and five from shaded canopy positions, were randomly selected and tagged. To increase our success of having fruit with RBD, a method of inducing the disorder demonstrated by Cronje (2009) was adopted. During January 2011 (after physiological drop in December and four months before commercial maturity), half of the selected fruit from each position were covered with brown paper bags without removing or covering subtending leaves. The study, therefore consisted of four preharvest treatments, viz., outside, outside bagged, inside and inside bagged. Of the total of 150 fruit that were tagged, 80 fruit were selected on the basis of size uniformity and external appearance and used for a calibration set (48) and a validation set (32) during model development. An additional set of 40 fruit from a different part of the orchard was selected as external validation set.

At commercial maturity (16 May 2011) individual fruit were harvested, coded according to treatment and canopy position before receiving all commercial postharvest practices, including drenching (Thiabenzole, 500 mg/L; Imazalil, 500 mg/L and 2,4-dichlorophenoxyacetic acid, 125 mg/L) and waxing (polyethylene citrus wax, Citrashine®, Johannesburg, South Africa). After which, fruit were sorted to remove any defective fruit and

weighed. A total of 80 blemish free fruit (20 fruit from each treatment) were selected to provide fruit samples for non-destructive and destructive measurements. After phytosanitary inspection and certification, fruit were separately packed in boxes marked, and sent at ambient temperature via a courier service to Cranfield University (CU) in the United Kingdom, where experiments were conducted. Fruit arrived at CU after 48 hours and were stored for 24 hours at 20 °C and 80% relative humidity to equilibrate, prior to taking NIR measurements.

2.2. Spectral acquisition

Vis/NIR spectra were obtained upon arrival at CU using a method described by Bobelyn et al. (2010) with modification. Spectral acquisition from intact fruit samples was carried out using a mobile fibre-optic Vis/NIR spectrophotometer (350-2500 nm) (LabSpec2500®) Near Infrared Analyser, Analytical Spectral Devices Inc., USA) in diffuse reflectance mode equipped with one Si array (350-1000 nm) and two Peltier cooled InGaAs detectors (1000-1800 nm and 1800-2500 nm). The sampling interval of the instrument was 1 nm. However, the spectral resolution was 3 nm at 700 nm and 10 nm at 1400 nm and 2100 nm. The integration time was less than 500 ms per sample. However, to maintain constant light level and avoid variable sunlight through the window, the probe was housed in an opaque wooden box. A high intensity probe with an in-built light source (quartz-halogen bulb of 3000 K) and detection fibre at 35° angle to the light source was used (Kuang and Mouazen, 2011).

Prior to scanning the fruit, and periodically at intervals of 30 min, white reference measurements were taken. The fruit were placed in direct contact with the high intensity probe. A total of 10 scans were obtained from exactly the same position and were automatically averaged to obtain a single spectrum per fruit position. Reflectance spectra were acquired from eight positions on the fruit; four from equatorial spots of the fruit and two from the stem-end and two from the stylar-end of the fruit, all of which were averaged and used for spectral pre-processing and multivariate analysis.

2.3. Physico-chemical measurements to obtain reference values

2.3.1. Storage conditions, RBD rating, mass and rind colour

After scanning, fruits were stored for 8 weeks in a cold room with delivery air temperature of 8° C, a temperature which is known to cause the highest degree of RBD incidence (Khumalo, 2006). During cold storage, fruit were scored weekly for the incidence of RBD for the duration of 8 weeks. RBD was scored by visual inspection on a subjective scale from 0 = no breakdown to 3 = severe breakdown. RBD was expressed as RBD index, after calculations according to the following formula previously reported for chilling injury and rind pitting by (Lafuente et al., 1997; Lafuente and Sala, 2002; Alférez et al., 2003):

$$RBDindex = \frac{\sum\{RBD(0-3) \times \text{No.of fruit in each class}\}}{\text{Total number of fruit}} \qquad (1)$$

Fruit were weighed weekly using a calibrated balance (Mettler Toledo, ML3002E / 01, Switzerland). Rind colour components were measured in three values, the CIE X, Y and Z colour space using a Minolta CR-400 colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203). The values were transformed to CIE L*, a*, and b*, which is more visually uniform than the XYZ colour space (Abbot, 1999). The mean of the three readings at three equidistant points around the equatorial axis of the fruits was automatically calculated (Terry et al., 2007). From L*, a* and b* colour parameters, colour index (CI) was calculated according to Jimenez-Cuesta et al. (1981) using equation 2.

$$CI = \frac{1000 \times a}{L \times b} \qquad (2)$$

2.3.2. Sample preparation

After 8 weeks in storage, fruit samples were destructed where the rind was peeled from the rest of the fruit. The pulp was juiced and the juice used for fresh total soluble solids (TSS) analysis. TSS was measured with a digital hand-held refractometer (Palette, PR-32α, Brix 0.0-32.0, Atago, Co. Ltd, Japan) using 1 mL of freshly squeezed juice and expressed as

soluble solid content units (SSC). The rind from each sample was snap frozen in liquid nitrogen and stored at -40° C until further analysis.

Frozen rind samples were freeze-dried in a Labogene ScanVac CoolSafe Freeze Dryer System (CS55-4, Lynge, Denmark) for 7 days at 0.015 kPA and -55° C. Lyophilised samples were weighed and water content was calculated from freeze dried samples and expressed as a percentage of fresh mass. Samples were then ground using a pestle and mortar into fine powder and returned into the freezer prior to being used for carbohydrate and phenolic acids determination by high performance liquid chromatography (HPLC).

2.3.3. Extraction and HPLC quantification of non-structural sugars

Non-structural sugars were extracted from 150 mg of fruit rind powder using 62.5% (v/v) aqueous methanol as described by Terry et al. (2007). Following extraction, the concentrations of fructose, glucose, and sucrose were determined using an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent refractive index detector (RID) G1362A, based on the method described by Crespo et al. (2010). Briefly, sample extracts were diluted (1:10), and injected into a Rezex RCM monosaccharide Ca^{+} (8%) column of 300 mm x 7.8 mm diameter (Phenomenex, Torrance, CA) with a Carbo-Ca^{2+} guard column of 4 mm x 3 mm diameter (Phenomenex). The temperature of the column was set at 80° C using a G1316A temperature regulated column compartment. The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min (Giné Bordonaba and Terry, 2008). The presence and abundance of the selected sugars was calculated from a standard curve of known concentration using ChemStation Rev. B.3.01.

2.3.4. Extraction and HPLC quantification of phenolic compounds

Phenolic acids were extracted and quantified using the method described by (Crespo et al., 2010). Briefly, freeze-dried citrus rind powder (150 mg) was dissolved into 3 mL of 70:29.5:0.5 (methanol:H_2O:HCL). Samples were extracted for 30 minutes in an ultrasonic water bath (Ma et al., 2008) at 35° C, agitated for 30 s every 5 min and the flocculate filtered through a 0.2 µm syringe filter. Phenolic acid concentrations were determined using an HPLC system equipped with an Agilent diode array detector (DAD) G1315B/G1365G photodiode array with multiple wavelength detection. Citrus rind extracts (20 µL) were injected into a Poroshell 120 column of 4.6 x 150 mm and 2.7 µm particle size column (Part

Number 693975-902, Agilent Technologies, Berks., UK) which was held at 30 °C. The mobile phase consisted of two solvents, 0.1% (v/v) formic acid in HPLC-grade water (A) and in HPLC-grade acetonitrile (B). The concentration of polyphenols was quantified by comparing sample peak area to standards, ranging from 0.1 to 1.0 mg/mL for all standards except for hesperidin which ranged from 0.1 to 1.8 mg/mL using ChemStation Rev. B.02.01. Total phenolics were calculated by adding all measured polyphenols.

2.3.5. Determination of antioxidant capacity

Antioxidant capacity was measured on sample extracts obtained from phenolic extraction following the method by Crespo et al. (2010). The absorbance of prepared sample solutions was measured spectrophotometrically at 517 nm using a Camspec M501 UV/vis spectrometer. The antioxidant capacity determination with 2,2-diphenyl-1-picrylnhydrazyl (DPPH) is based on the properties of DPPH, which in its radical form has an absorption band at 517 nm and disappears upon reduction by an antiradical compound.

2.4. Data analysis

2.4.1. Statistical analysis

Statistical analyses were carried out using SPSS 10.0 for Windows (SPSS Inc. Chicago, USA). Data was subjected to analysis of variance (ANOVA). Least significant difference values (LSD; P = 0.05) were calculated for mean separation (Landahl et al., 2009). The coefficient of variation (CV%), defined as the ratio of the standard deviation to the mean of the reference values was calculated and reported as a percentage (Alamar, 2007).

2.4.2. Vis/NIRS analysis, calibration and validation

Before analysis, the reflectance spectra in Indico format (Indico Pro 5.6 software, Analytical Spectral Devices Inc., USA) were transformed to absorbance (log (1/R)). Calculations of the average of 8 spectra obtained from each fruit, pre-processing and calibration methods were executed using the Unscrambler chemometric software (The Unscrambler Version 9.2, Camo Process, SA, Trondheim, Norway).

Several pre-processing methods including, smoothing using moving average and Savitzky-Golay methods, full multiple scatter correction (MSC), Savitzky-Golay first derivative and second derivative, minimum and maximum normalisation and vector normalisation were tested to correct light scatter and reduce the changes of light path length. The spectral pre-processing method which gave the highest R-value for validation and the lowest RMSEP was selected. After pre-processing trials, the optimal model performance was obtained using the Savitzky-Golay second derivative with the second order polynomial or MSC. Savitzky-Golay second derivative or MSC were used to correct light scattering properties, correct for additive, multiplicative effects of the spectra, and pathlength variations (Leonardi and Burns, 1999; Gómez et al., 2006; Nicolaï et al., 2007).

Principal component analysis (PCA) was perfomed to determine effective wavelength, discriminate fruit from four preharvest treatments and to detect spectral outliers. Outliers were removed before spectral preprocessing. Partial least squares regression (PLS) was applied to spectral data to develop prediction models for hue angle, CI, RBD index, water loss, dry matter, sucrose, glucose, fructose, and total carbohydrates. Outliers were determined from the residual sample variance plot after PCA and PLS. Samples that were located far from the zero line of the residual variance plot were identified as outliers and excluded (Kuang and Mouazen, 2011). A PLS variant known as partial least squares discriminant analysis (PLS-DA) was also used in order to classify fruit from different canopy positions based on the spectra. A method by Chen et al. (2011) was used in the application of PLS-DA. Briefly, fruit from each of the canopy positions in the calibration set was assigned a dummy variable as a reference value (outside = 1, outside bagged = 2, inside = 3 and inside bagged = 4). In addition, due to the discrete nature of RBD scores, samples were assigned a binary dummy variable as a reference value, which was an arbitrary number whether the sample belongs to a particular position or not. RBD affected fruit were set as reference data 1 while unaffected fruit were assigned to 0 (Teerachaichayut et al., 2011).

To develop PLS models, the dataset was randomly separated into two subsets, 48 fruit for calibration and 32 fruit for validation. An additional set of 40 fruit from the different part of the orchard was selected for external validation. The regression statistics of calibration models was described by the value of the root mean square error of calibration (RMSEC), root mean square error of validation or prediction (RMSEP), the correlation coefficients (R) between predicted and observed reference values, number of latent variables (LVs), and the residual predictive deviation (RPD), described by Williams and Sobering (1996) as the ratio of the standard deviation of the reference data for the validation set to the RMSEP. The ideal

model should have higher R and RPD values as well as lower RMSEC and RMSEP values. The optimal number of LVs was determined as the minimum number of LVs corresponding to the first lowest value of the RMSEC or RMSEP from the plot of the RMSEC or RMSEP for increasing number of LVs (Davey et al. 2009). The stability of the calibration model was tested by interchanging validation and calibration data sets and checking that the differences in the regression statistics obtained were small (Alvarez-Guerra et al., 2010).

3. Results and discussion

3.1. Rind breakdown disorder and biochemical profile of fruit from different canopy positions

Symptoms of rind breakdown disorder were visible on affected fruit after five weeks of continuous storage at 8°C. RBD index was significantly affected by preharvest manipulation of sunlight exposure (Table 1). Unbagged outside fruit had the lowest susceptibility to develop the disorder compared to other preharvest treatments. Fruit position within the canopy on its own did not show a significant difference on fruit susceptibility to RBD. However, exclusion of sunlight by bagging fruit resulted in increased fruit susceptibility, but only showed a significant difference on fruit located inside the canopy. These findings are consistent with those observed by Almela et al. (1992). In their study, these authors established that the sensitivity of fruit to development of rind spots related to RBD in 'Fortune' mandarins was influenced by different microclimates. Similar to observations reported by Cronje et al. (2011), fruit position within the canopy affected rind colour index (CI). Fruit borne on the outside of the tree canopy had the highest CI and hence were more orange while inside fruit had pale, yellow rinds.

Previous research suggested that rind water status is a factor prevailing in the susceptibility of citrus fruit to rind physiological disorders (Cohen et al., 1994; Alférez and Burns, 2004). In this study, fruit from the bagged treatments, both inside and outside of the canopy, were characterised by high postharvest mass loss, and this was essentially due to water loss by transpiration, as this can account for 90% of total water loss (Ben-Yehoshua, 1969). Water loss from the fruit results from a water pressure gradient prevailing between the fruit rind, which is close to saturation with water, and the less saturated ambient atmosphere (Ben-Yehoshua et al., 1994; Macnish et al., 1997).

Fruit position within the canopy also had a significant influence on rind biochemical properties. Results in Table 1 showed that unbagged fruit outside the canopy had higher dry matter content, glucose, and total phenolic acid concentrations compared to bagged fruit inside the canopy. The effect of preharvest canopy position and bagging treatments on fruit rind biochemical composition was documented by Cronje (2009) who reported that rind of fruit from the outside portion of the canopy had significantly higher sugar and carotenoid contents than shaded fruit borne inside canopy. In this study, unbagged fruit from outer portion of the canopy had significantly higher glucose concentration of 92.03 mg/g DM compared to inside fruit, which had glucose concentration of 66.92 mg/g DM. On the contrary, sucrose, and total carbohydrate of bagged fruit from inside the canopy were significantly higher in relation to other three positions. It was therefore noteworthy that these fruit also had highest susceptibility to RBD.

In addition to carbohydrate contents, phenolic acid concentration and antioxidant capacity were affected by fruit exposure to sunlight. Unbagged samples from the inside and outside position had relatively higher phenolic acid concentration of 39.29 and 37.65 mg/g DM when compared to outside bagged and inside bagged samples which had 33.48 and 34.68 mg/g DM, respectively (Table 1). Moreover, the antioxidant activity of inside bagged samples, i.e. fruit with the highest RBD, was much lower than those with low RBD disorder. It has reported previously that the presence of antioxidant species could be implicated to the development of various postharvest disorders including non-chilling peel pitting in 'Navelate' oranges (Cajuste and Lafuente, 2007). The results presented above are in accord with the notion that decreasing light levels around an individual fruit reduces rind condition and susceptibility to the disorder (Cronje et al., 2011). Pearson correlation and PCA analysis between physico-chemical properties and RBD development had very low correlation coefficients which serve to highlight the complexity of factors involved in the development of this disorder. Furthermore, the lack of a specific threshold values below or above which all fruit become affected and above or below which all fruit stay healthy suggests that several preharvest and postharvest factors also play a role.

3.2. Vis/NIR spectroscopy

3.2.1. Distribution of calibration and validation reference data

Table 2 shows the distributional statistics for reference datasets used in calibration and test validation. The reference measurements of all parameters in calibration and validation were normally distributed around the means. Although the selection method for calibration and validation was random, the validation data set was scrutinised to ensure that the validation data sets were confined within a boundary of the calibration set. The interpretation of calibration results depends greatly on the precision of the determined reference data and enough variation in both calibration and validation data (Lu et al., 2006). As is apparent in the min-max range and CV% values of the data presented, the calibration and validation values of the sample quality parameters cover a large range, which is helpful for developing calibration models for NIR spectroscopy (Clément et al., 2008). For instance, the mean concentration of sucrose values used for calibration and validation were 101.40 and 78.81 mg/g DM with standard deviation of 43.27 and 34.15 mg/g DM, respectively. The range of total carbohydrates in the calibration set was from 121.91 to 511.11 mg/g DM and the range of validation set was from 141.91 to 492.28 mg/g DM with corresponding CV % of 29.38 and 36.31%, respectively.

3.2.2. Spectral analysis and wavelength selection

The spectral variables which contribute the most to the model were determined from its regression coefficients curve. A wavelength with high regression coefficient value indicates that the variable is important to the model while the regression coefficient with a value close to zero does not contribute to the model. Fig. 2 portrays the regression coefficients of the total carbohydrates model developed using spectra obtained from intact 'Nules Clementine' mandarins subjected to different preharvest treatments. The pronounced peaks (high absolute correlation coefficients) in the NIR region of the curve are at 980, 1100, 1150, 1200, 1350, 1400, 1500, 1600 and 1650 nm were observed. The high correlation coefficients at 980 and 1100 nm corresponds with the second overtone of H-O-H stretching modes of water (Clément et al., 2008); 1200 nm is associated with the second overtone of C-H and C-H_2 stretching related to sugars; 1450 nm belongs to the first overtone of O-H stretching related to alcohol groups in sugars (Kawano et al., 1993; Golic et al., 2003). Based on the regression coefficients results, the informative wavelength bands for all carbohydrates, DM and water loss were between 900-1700 nm. These results are in agreement with the interpretation of these bands as combinations of first, second and third overtones of O-H and C-H stretching vibrations of water hydrogen bonds with sugar molecules (Golic et al., 2003; Tewari et al.,

2008). Colour parameters were best predicted in the visible region (450-750 nm) whilst RBD was predicted from 450–1000 nm. This was similar to results obtained by Zheng et al. (2010) for prediction of oleocellosis disorder. Considering that the regression coefficients determines how much the variable contribute to the model (Williams and Norris, 2001), this band may possibly be related to fruit sensitivity to rind physiological disorders such as oleocellosis and RBD.

3.2.3. Spectral pre-processing and wavelength selection

The spectra of solid samples such as fruit are influenced by physical properties such as shape, size, path length, etc. (Leonardi and Burns, 1999), which determine light scattering properties. As a common practice in NIRS, obtained spectra was subjected to several pre-processing methods and the suitable pre-processing methods were selected. Results presented in Table 3 show that Savitzky-Golay second derivative with the second order polynomial provided the best results for the PCA classification and PLS model for predicting RBD. MSC gave best results for PLS prediction of physico-chemical properties such as hue angle, colour index, water loss, dry matter content, and carbohydrate concentrations as well PLS-DA classification by preharvest treatments.

3.2.4. Vis/NIR- based PCA and PLS classification models

Individual spectra from 8 positions within the fruit and the average spectra were tested to develop PCA and PLS-DA classification models. Average spectra showed better models than individual spectra; and thus subsequent analyses were based on average spectra. PCA was performed on Vis/NIR spectra to compare spectral characteristics of fruit from different preharvest treatments. The PCA applied to the spectra using Savitzky Golay second derivative pre-processing revealed better grouping of the samples than other tested pre-processing methods. The data distribution in the PCA score plot presented in Fig. 3 displayed four clusters that could easily be identified. These clusters allowed distinction between fruit from different preharvest treatments. The first two principal components (PC) accounted for 68.0 % of the total variability, PC1 explains the 53.0 % of the variance and PC2 explained 15.0 % of the variance. The effective wavelength band for this classification was from 350 to 1200 nm with a strong absorption at 670 nm influenced by chlorophyll, two at 740 and 980 nm corresponding to water (O-H) functional groups and one at 1200 associated with C-H

stretching for carbohydrates. From this, it could be concluded that a combination of colour, carbohydrates and moisture content of the rind play an important role in discriminating different positions of the canopy. PCA models developed using spectra restricted to visible range only or to NIR range only were not able to show these clusters. This demonstrated the importance of the combination of visible and NIR range in the classification of fruit by their canopy position.

Spectral data was further subjected to discriminant analysis by assigning fruit from each canopy positions to a dummy variable (1, 2, 3 and 4 for outside, outside bagged, inside bagged and inside, respectively). Fig. 4 depicts performance of the PLS-DA model to classify fruit based on their origin within the tree canopy using full spectral range (350-2500 nm) and MSC spectral pre-processing. The prediction accuracy determined using 40 fruit from different trees and part of the orchard for external validation was high (R = 0.97, RMSEP = 0.30). The cut off value for PLS-DA discrimination was 0.50. Therefore, samples with predicted value of 0.50 or more were classified as having RBD, while samples with predicted values less than 0.50 were not affected by the disorder. The PLS-DA classification results in Fig. 5 show that the prediction model had low false positive (6.45%) and low false negatives 33%.

3.2.5. Vis/NIR- based PLS prediction models

Table 3 shows summary statistics for calibration and validation results for the prediction of different physico-chemical properties with PLS models. After testing different wavelength ranges based on observed peaks and information provided in the literature, wavelength ranges that gave the lowest RMSEC and RMSEP were selected to develop calibration models for each physico-chemical property. As would be expected, models for colour parameters (h and CI) were developed using visible range (350-700 nm) of the spectrum, whereas the RBD model was developed with the region between 350-1000 nm. This was in accordance with the range used by Zheng et al. (2010) to develop models for predicting susceptibility of citrus fruit to oleocellosis, another rind physiological disorder.

Prediction models for sucrose, fructose, glucose, total carbohydrates, TSS, dry matter and water loss were developed using wavelength range between 900 to 1800 nm. According to the absorption bands provided by Williams and Norris (2001), all these biochemical components have absorption bands in this spectral region. Results obtained in this study are similar to previous studies suggesting that the range from 350-1800 nm is suitable for

predicting colour parameters (Sun et al., 2009), dry matter (Guthrie et al., 2005a, b), sucrose, fructose, glucose (Tewari et al., 2008) and sugar concentration (Liu et al., 2010).

Models for predicting quality parameters such as colour index, hue angle, dry matter and water loss showed significantly high performance, with predictive R-values of 0.98, 0.97, 0.96 and 0.91, respectively and corresponding RMSEP of 0.43, 1.66, 0.92 % and 2.76 g (Fig. 6). NIR calibration and validation results demonstrated that sugars, dry matter, colour index and mass loss were predicted with significant accuracy. The prediction performance for sugars (sucrose, fructose, glucose, and total sugars) was high with R of 0.88, 0.94, 0.95, and 0.95 and corresponding RMSEP values of 24.36, 11.41, 11.58, 31.04 mg/g DM, respectively (Fig. 7). Although the accuracy is slightly lower, these results were comparable to those reported in the literature by Tewari et al. (2008) for the prediction of sucrose, fructose and glucose of grapefruit.

To test the effect of canopy position on model performance, calibration models were developed using fruit from each of the positions. The performance of calibration models developed using samples from a certain canopy were, in most cases relatively similar to those developed using a combined fruit from all positions. However, the prediction performance of fruit from separate canopy position in predicting parameters of another canopy position was lower than that of models developed using combined fruit. This might be attributed to the fact that the range of the cross validation data set from another canopy was not confined within a boundary of the calibration set.

As suggested by Davey et al. (2009) and Saeys et al. (2005), although the correlation between NIR predicted and reference values is high, it is also very critical to verify the accuracy of the model by referring to the RPD values. These authors stated that RPD values below 1.5 are considered unusable, those between 1.5 and 2.0 are suitable for rough prediction, those between 2.0 and 2.5 are suitable for quantitative predictions, while RPD values between 2.5 and above 3.0 are respectively considered good and excellent for prediction models. The low RPD values, 0.30 for RBD, and high RMSEP (0.30) clearly indicate poor accuracy of these models. Since this is a category variable ranging from 0 to 1, these errors are high. In Fig. 5, the model for predicting fruit susceptibility to RBD based on the binary data (1=affected and 0=unaffected) is presented. The poor accuracy of these models could be attributed to categorical nature of the data in both calibration and test set data and low incidence of RBD.

Furthermore, sucrose had lower RPD (2.41) for prediction compared to glucose, and fructose which had RPD values of 2.86 and 3.23 respectively. The low predictability of

sucrose could possibly results due to the difference in molecular weight of sucrose (MW=342.30 g/mol) compared to glucose and fructose (MW=180.16 g/mol) (Golic et al., 2003). This difference in molecular weight is such that there are 1.89 times fewer number of sucrose molecules than glucose and fructose in the same weight of sample. Therefore, the intensity of the bands associated with hydrogen bonding is smaller in sucrose than in glucose and fructose.

The complexity of biological factors involved in the development of RBD complicated the development of an acceptable model for predicting the disorder. However, the high ability of Vis/NIR to classify fruit based on their origin within the canopy using PLS-DA could have practical interest for online application. Another reason for the poor prediction model for RBD is that calibration and test set also contained a large proportion of samples in which RBD did not develop. In future, it will be necessary to increase sample size and use fruit from different localities in order to increase chances of the disorder. This is to ensure that the distribution of the disorder is wide, normal around the mean and evenly distributed along the entire range to avoid the Dunn effect (Williams and Norris, 2001; Davey et al., 2009).

4. Conclusion

In this study, the effects of fruit position within the canopy and bagging were significant in altering rind biochemical properties. PCA and PLS-DA models results displayed clusters that allowed distinction between fruit from different preharvest treatments. Vis/NIR calibration and validation results demonstrated that sugars, dry matter, colour index and water loss were predicted with significant accuracy. Further exploration of the statistics of the developed models revealed the high potential Vis/NIR spectroscopy to non-destructively detect rind biochemical profile.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterisation of rind breakdown of ‘Navelate’ sweet orange. Ann. Bot. 88, 415–422.

Alférez, F., Zacarìas, L., 2001. Postharvest pitting in navel oranges at non-chilling temperature: Influence of relative humidity. Acta Hort. 553, 307–308.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65 (Abstract).

Alvarez-Guerra, M., Ballabio, D., Amigo, J.M., Bro, R., Viguri, J.R., 2010. Development of models for predicting toxicity from sediment chemistry by partial least squares-discriminant analysis and counter-propagation artificial neural networks. Environ. Pollut. 158, 607–614.

Barry, G.H., Castle, M.S., Davies, F.S., 2000. Juice quality of 'Valencia' sweet orange among citrus-producing regions in Florida and between canopy positions. Proc. Int. Soc. Citricult. Congr. IX, 308–314.

Barry, G.H., Rabe, E., 2004. Clementine mandarin production in South Africa. Proc. Intl. Soc. Citricult. Congr. I, 360–363.

Ben-Yehoshua, S. 1969. Gas exchange, transpiration, and the commercial deterioration in storage of orange fruit. J. Am. Soc. Hort. Sci. 94, 524–528.

Ben-Yehoshua, S., Shapiro, B., Chen, Z.E., Lurie, S., 1983. Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress. Plant Physiol. 73, 87–93.

Blasco, J., Aleixos, N., Moltó, E., 2007. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. J. Food Eng. 81, 535–543.

Bobelyn, E., Serban, A., Nicu, M., Lammertyn, J., Nicolaï, B.M., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133–143.

Butz, P., Hofmann, C., Tauscher, B., 2005. Recent developments in non-invasive techniques for fresh fruit and vegetable internal quality analysis. Concise Rev. Food Sci. 70, 131–141.

Cajuste, J.F., Lafuente, T.M., 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. Postharvest Biol. Technol. 45, 193–203.

Chen, X., Wu, D., He, Y., Liu, S., 2011. Nondestructive differentiation of Panax species using visible and shortwave near-infrared spectroscopy. Food Bioprocess Tech. 4, 753–761.

Chikaizumi, S., 2000. Mechanism of rind-oil spot development in 'Encore' (*Citrus nobilis* Lour. X *C. Deliciosa* Ten.) fruit. J. Japan. Soc. Hort. Sci. 69, 149–155.

Clark, C.J., McGlone, V.A., DeSilva, H.N., Manning, M.A., Burdon, J., Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit (*Actanidia cinensis*) based on visible-NIR spectral characteristics at harvest. Postharvest Biol. Technol. 32, 147–158.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56, 9813–9818.

Cohen, E., Shapiro, B., Shalom, Y., Klein, J.D., 1994. Water loss: A non-destructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit. J. Am. Soc. Hort. Sci. 119, 983–986.

Crespo, P., Giné Bordonaba, J., Terry, L.A., Carlen, C., 2010. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. Food Chem. 122, 6–24.

Cronje, P.J.R., 2005. Peteca spot of lemons. S. Afr. Fruit J. Feb/March, 26–28.

Cronje, P.J.R., 2007. Postharvest rind disorders of citrus fruit. Citrus research international, Nelspruit, South Africa.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementines' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H, Huysamer, M., 2011. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Davey, M.W., Saeys., W., Hof, E., Ramon, H., Swennen, R.L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. J. Agric. Food Chem. 57, 1742–1751.

Duarte, A.M.M., Guardiola, J.L., 1995. Factors affecting rind pitting in the mandarin hybrids 'Fortune' and 'Nova'. The influence of exogenous growth regulators. Acta Hort. 379, 59–67.

Geeola, F., Geeola, F., Peiper, U.M., 1994. A spectrophotometric method for detecting surface bruises on 'Golden Delicious' apples. J. Agric. Eng. Res. 58, 47–51.

Giné Bordonaba, J., Terry, L.A. 2008. Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. J. Agric. Food Chem. 56, 7422–7430.

Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

Gómez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005a. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Aust. J. Agric. Res. 56, 405–416.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005b. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. Aust. J. Agric. Res. 56, 417–426.

Jimenez-Cuesta, M., Cuquerella, J., Martínez-Jávega, J.M., 1981. Determination of a colour index for citrus degreening. Proc. Int. Soc. Citricult. 2, 750–753.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Non-destructive determination of sugar content in 'Satsuma' mandarins using NIRS transmittance. J. Japan. Soc. Hort. Sci. 62, 465–470.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Kuang B., Mouazen, A.M., 2011. Calibration of visible and near infrared spectroscopy for soil analysis at the field scale on three European farms. Eur. J. Soil Sci. 62, 629–636.

Lafuente, M.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25, 49–57.

Lafuente, M.T., Marténez-Téllez, M.A. and Zacarías, L., 1997. Abscisic acid in the response of 'Fortune' mandarins to chilling, effects of maturity and high-temperature conditioning. J. Sci. Food Agric. 73, 494–502.

Landahl, S., Meyer, M.D., Terry, L.A., 2009. Spatial and temporal analysis of textural and biochemical changes of imported avocado cv. 'Hass' during fruit ripening. J. Agric. Food Chem. 57, 7039–7047.

Leonardi, L., Burns, D.H., 1999. Quantitative multiwavelength consistuent measurements using single-wavelength photo time-of-flight correction. Appl. Spectrosc, 53, 637–646.

Liu, Y., Sun, X., Ouyang, A., 2010. Non-destructive measurements of soluble solid content of navel orange fruit by visible-NIR spectrometric technique with PLSR and PCA-BPNN. LWT- Food Sci. Technol. 43, 602–607.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application of Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. 7, 794–799.

Macnish, A.J. Joyce, D. C., Hetherington, S.E., 1997. Packaging to reduce water loss can delay ripening of mango (*Mangifera indica* L. cv. 'Kensington Pride') fruit. Aust. J. Exp. Agric. 37, 463–467.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolaï, B., 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food Bioprocess Tech. 5, 425–444.

Miller, B.K., Delwiche, M.J., 1991. Spectral analysis of peach surface defects. Trans. Am. Soc. Agric. Eng. 34, 2509–2515.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., 1998. Nondestructive detection of selection drying, an internal disorder in tangerine. HortScience. 33, 310–312.

Saeys, W., Mouazen, A.M., Ramon, H., 2005. Potential onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst. Eng. 91, 393–402.

Sun, X., Zhang, H., Liu, Y., 2009. Nondestructive assessment of quality of 'Nanfeng' mandarin fruit by a portable near infrared spectroscopy. Int. J. Agric. Biol. Eng. 2, 65–71.

Teerachaichayut, S., Terdwongworakul, A., Thanapese, W., Kiji, K., 2011. Non-destructive prediction of hardening pericarp disorder in intact mangosteen by near infrared transmittance spectroscopy. J. Food Eng. 106, 206–211.

Terry, L.A., Chope, G.A., Giné Bordonaba, J., 2007. Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria x ananassa*) fruit quality. J. Agric. Food Chem. 55, 10812–10819.

Tewari, J.C., Dixit, V., Chi, B-K., Malik, K.A., 2008. Determination of origin and sugars of citrus fruit using genetic algorithm, correspondence analysis and partial least square combined with fiber optic NIR spectroscopy. Spectrosc. Acta Part A: Mol. Biomol. Spectrosc. 71, 1119–1127.

Wild, B.L., 1991. Postharvest factors governing the development of peteca spot on 'Meyer' lemons. HortScience. 26, 287–289.

Williams, P., Norris, K.H., 2001. Variables affecting near infrared spectroscopic analysis. In: Williams, P., Norris, K.H. (Eds). Near Infrared Technology in the Agriculture and Food Industries, 2nd ed. The American Association of Cereal Chemists, St. Paul, MNL, pp. 171–185.

Williams, P.C., Sobering, D.C., 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Davies A.M.C., Williams, P. (ed.). Near Infrared Spectroscopy: The future waves. Proc. 7th Interntl. Conf. Near Infra. Spectrosc. Montreal, Chichester, UK, pp. 185–188.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort. 125, 401–405.

Table 1: Physico-chemical profile of 'Nules Clementine'mandarin fruit from different preharvest canopy positions and bagging treatments. Values with different letters (mean and standard error of the mean) in the same row, demonstrate a significant difference. All values were measured after eight weeks in storage.

Quality Parameter	Canopy position			
	Outside	Outside bagged	Inside	Inside bagged
Rind hue angle	67.34±0.58ns	75.50±1.46ns	62.94±11.32ns	74.01±0.97ns
Rind colour index	6.12±0.19b	3.62±0.38a	3.65±0.57a	3.99±0.27a
Mass (g)	107.63±4.68b	104.89±4.28b	89.29±4.95a	107.61±6.38b
Rind breakdown index	0.05	0.45	0.20	0.75
Rind breakdown incidence (%)	5.00	20.00	10.00	45.00
Mass loss (g)	9.51±0.42ab	13.02±1.76bc	7.76±0.58a	13.62±1.67c
Total soluble solids (SSC)	13.71±0.24b	11.89±0.28a	12.11±0.27a	11.94±0.25a
Rind dry matter (%)	30.00±0.78b	26.98±0.51a	28.78±0.96ab	26.99±0.79a
Rind sucrose (mg/g DM)	95.31±6.07b	93.59±9.51b	63.30±8.65a	114.94±12.51b
Rind glucose (mg/g DM)	92.03±5.29b	85.44±8.94ab	66.92±6.97a	77.09±8.50ab
Rind fructose (mg/g DM)	126.48±5.19ab	130.21±9.76b	100.65±8.52a	122.26±9.71ab
Rind total sugars (mg/g DM)	313.82±11.58b	309.23±25.05b	230.88±22.82a	314.29±27.70b
Rind total phenolic acids (mg/g DM)	39.29±0.86b	33.48±1.10a	37.65±0.85b	34.68±1.25a
Rind AC (µmol trolox eq./g DM)	75.53±4.69c	52.72±3.52a	64.35±3.12b	53.47±2.10a

AC, Antioxidant capacity; NS, Non significant.

Table 2: Mean, standard deviation (SD), range and coefficient of variation (CV%) for calibration (n = 48), validation (n = 32) and external validation (n = 40) subsets of mandarin fruit.

Quality parameter	Calibration data set			External validation data set		
	Mean±SD	Range	CV%	Mean±SD	Range	CV%
Rind hue angle	73.14±5.11	63.53-89.27	7.05%	73.07±5.57	60.13-82.62	9.44%
Rind colour index	4.52±1.47	1.25-7.49	32.59%	4.24±1.66	1.70-7.04	39.20%
RBD index	0.49±0.80	0.00-0.75	205.8%	0.45±0.22	0.00-0.70	193.54%
Mass (g)	107.90±22.87	67.40-153.60	21.20%	101.50±21.74	68.10-147.70	19.93%
Mass loss (g)	11.33±5.21	4.51-28.53	45.99%	9.95±5.22	4.98-27.84	55.22%
Rind dry matter (%)	27.85±3.14	22.84-35.71	11.28%	28.41±3.09	23.16-34.71	11.52%
Rind sucrose (mg/g DM)	101.40±43.27	35.17-207.91	42.66%	89.39±42.23	36.42-206.61	44.41%
Rind glucose (mg/g DM)	83.87±30.11	22.19-152.1	35.91%	74.45±32.00	23.01-134.41	44.16%
Rind fructose (mg/g DM)	124.30±33.52	53.72-195.9	26.96%	112.81±33.27	54.38-180.63	33.80%
Rind total sugars (mg/g DM)	309.60±90.97	121.91-511.11	29.38%	276.64±94.23	131.03-489.45	38.36%

RBD, rind breakdown.

Table 3: An overview of statistics obtained during calibration (n = 48), validation (n = 32) and external validation (n = 40) of models for individual quality parameters.

Quality parameter			Calibration model			External validation model					Info.
	LV	Prepr	R	RMSEC	RPD	R	RMSEP	RPD	Slope	Bias	Region (nm)
Rind hue angle	3	MSC	0.97	1.21	4.22	0.96	1.39	4.01	0.98	0.04	350-700
Rind colour index	3	MSC	0.97	0.38	3.87	0.95	0.47	3.53	0.88	-0.07	350-700
RBD (binary scores)	5	2nd der.	0.77	0.27	0.67	0.69	0.30	0.33	0.52	-0.04	350-1000
Mass loss (g)	10	MSC	0.92	2.01	2.59	0.91	2.93	1.78	0.86	-1.99	900-1700
Rind dry matter (%)	8	MSC	0.98	0.68	4.62	0.94	1.01	3.06	0.91	0.17	900-1700
Rind sucrose (mg/g DM)	8	MSC	0.92	15.23	2.84	0.75	38.21	1.11	0.59	-26.87	900-1700
Rind glucose (mg/g DM)	10	MSC	0.95	9.18	3.28	0.95	11.88	2.53	0.9	7.23	900-1700
Rind fructose (mg/g DM)	14	MSC	0.98	6.33	5.30	0.93	13.97	2.38	0.97	1.22	900-1700
Rind total sugars (mg/g DM)	10	MSC	0.94	30.42	2.99	0.93	36.00	2.62	0.93	-9.66	900-1700

RBD, Rind breakdown; LV, Latent variable; Prepr., Preprocessing method; MSC, Multiple scatter correction; 2nd der., Second derivative; R, Correlation coefficient; RMSEC, Root mean square error of calibration; RPD, residual prediction deviation; RMSEP, Root mean square error of prediction.

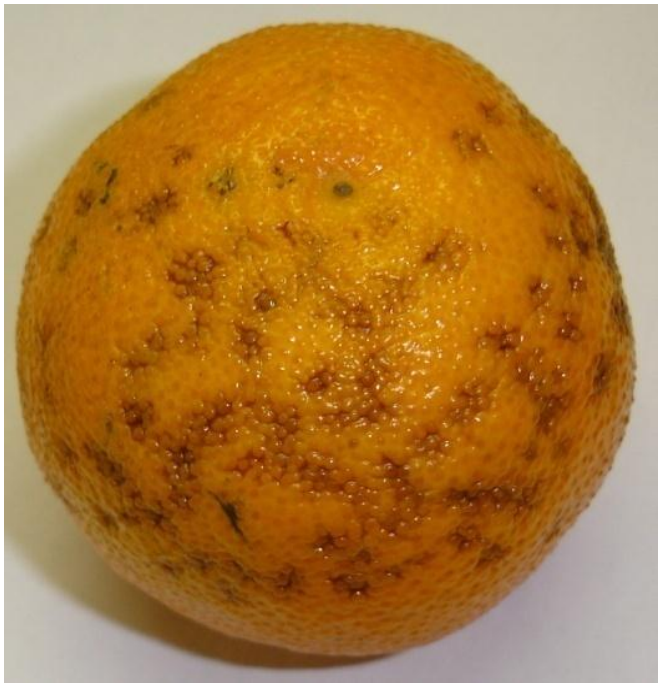

Fig. 1: Visual symptoms of non-chilling postharvest rind breakdown disorder of 'Nules Clementine' mandarin (Cronje et al., 2011).

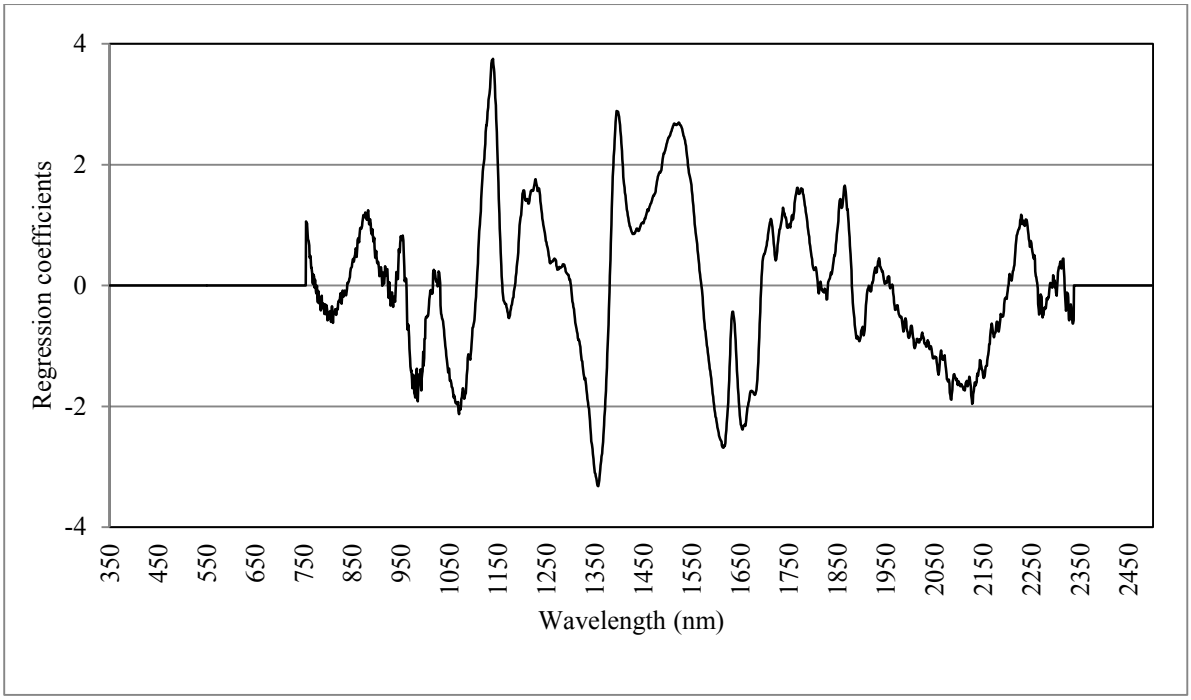

Fig. 2: Typical regression coefficients curve of the rind total sugars model of intact 'Nules Clementine' mandarin fruit with 10 PCs and NIR spectra range of 750-2350 nm.

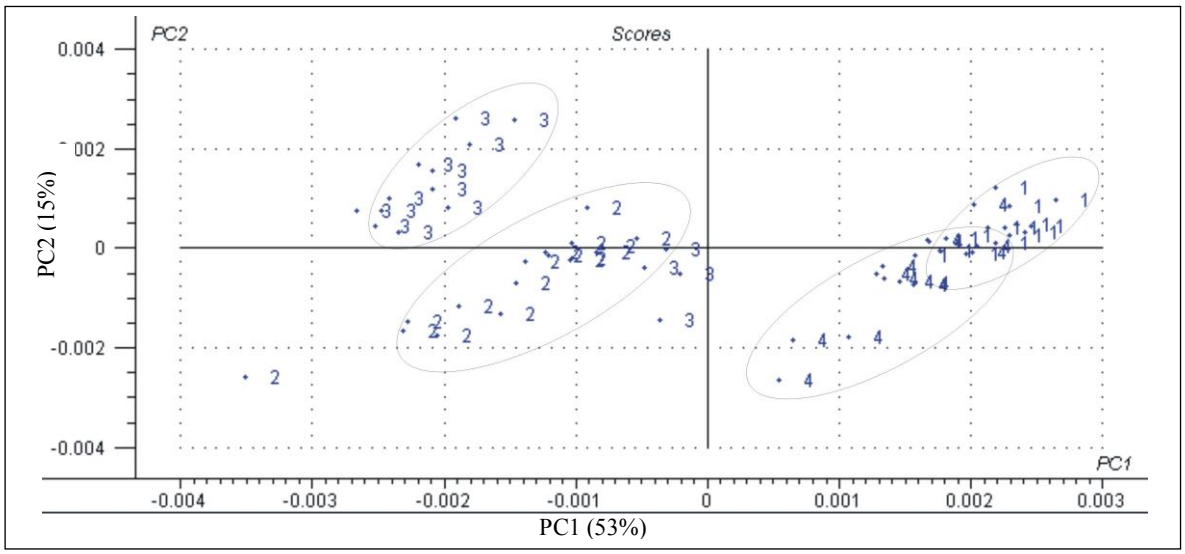

Fig. 3: PCA plot for the two PC factors showing spectral ability to sort based on their origin within the tree canopy. 1, 2, 3, and 4, respectively, represent fruit from outside the canopy, bagged fruit from outside the canopy, bagged fruit from inside the canopy, and fruit from inside the canopy.

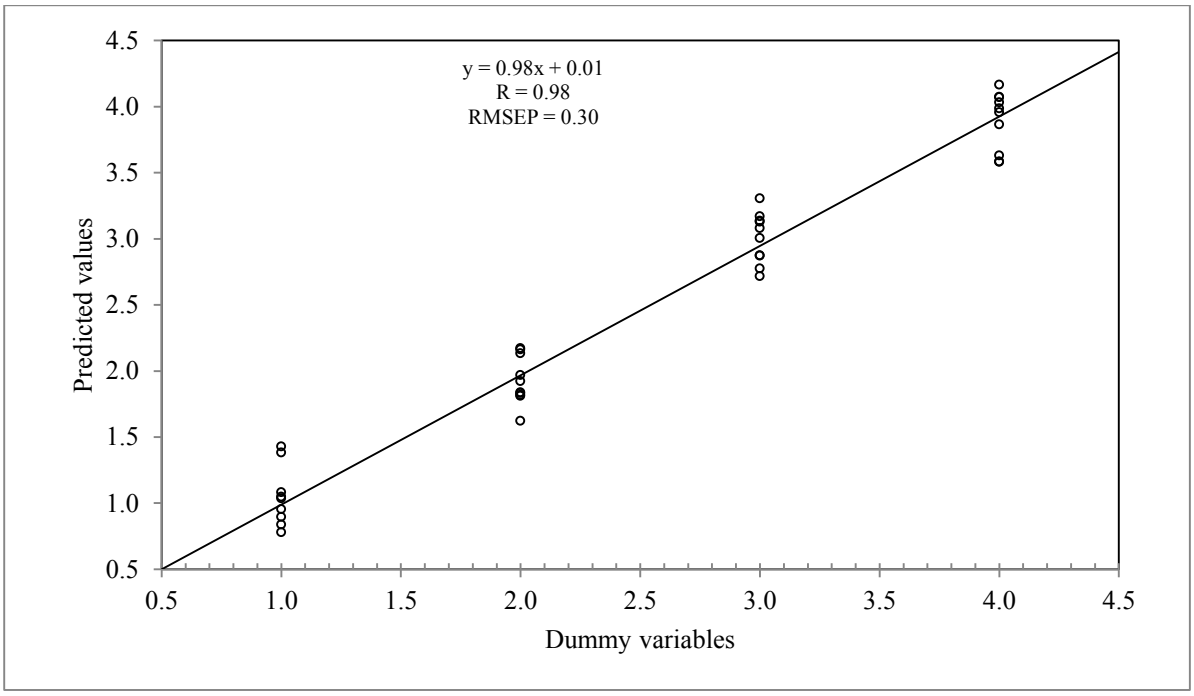

Fig. 4: PLS-DA models showing Vis/NIR spectral ability to predict fruit origin within the tree canopy. 1, 2, 3, and 4, represent fruit from outside the canopy, bagged fruit from outside the canopy, bagged fruit from inside the canopy, and fruit from inside the canopy, respectively.

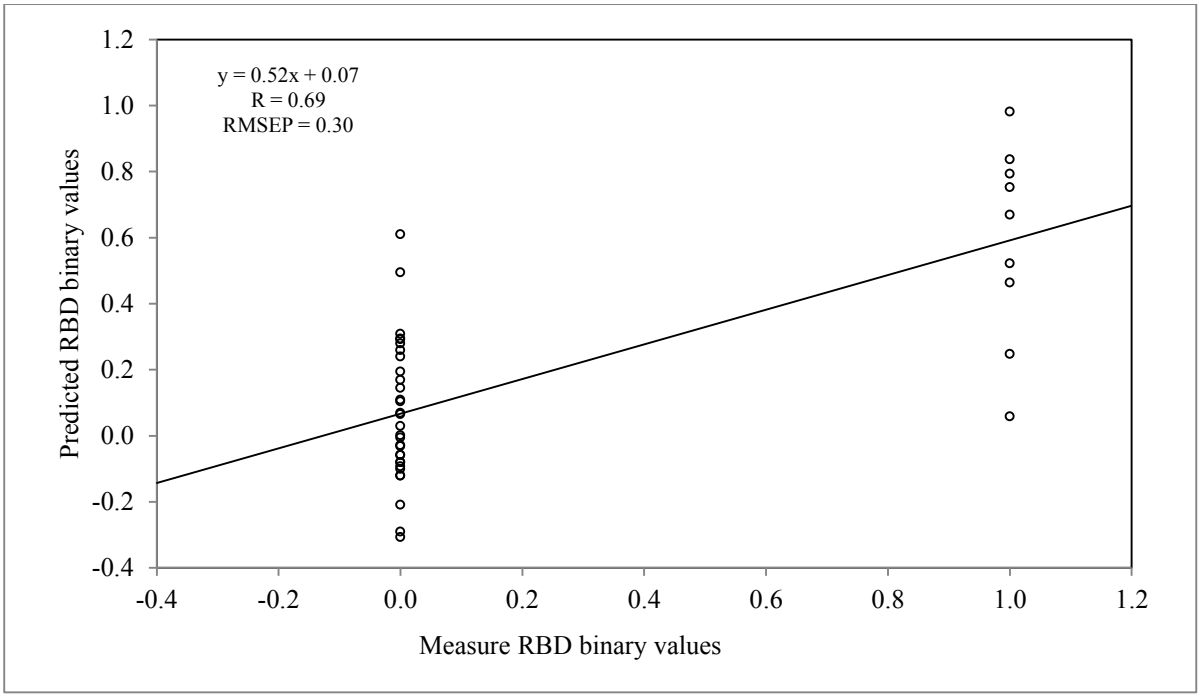

Fig. 5: PLS scatter plot showing Vis/NIR model performance to classify fruit based on the occurrence of RBD.

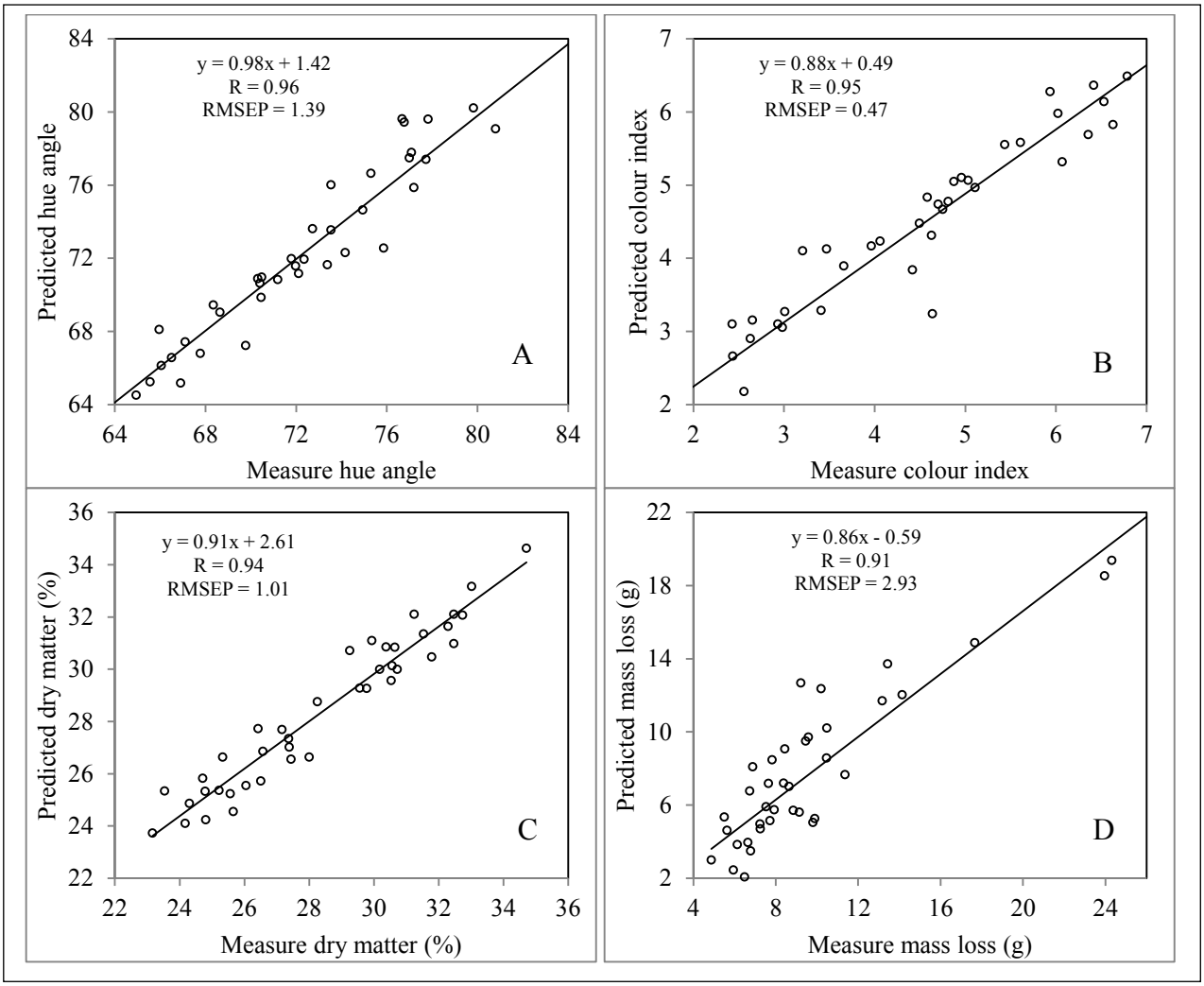

Fig. 6: Scatter plots of Vis/NIR predicted versus measure values of hue angle (A), colour index (B), dry matter (%) (C), and mass/water loss (g) (D). A and B predicted by visible wavelengths. C and D predicted by NIR wavelengths.

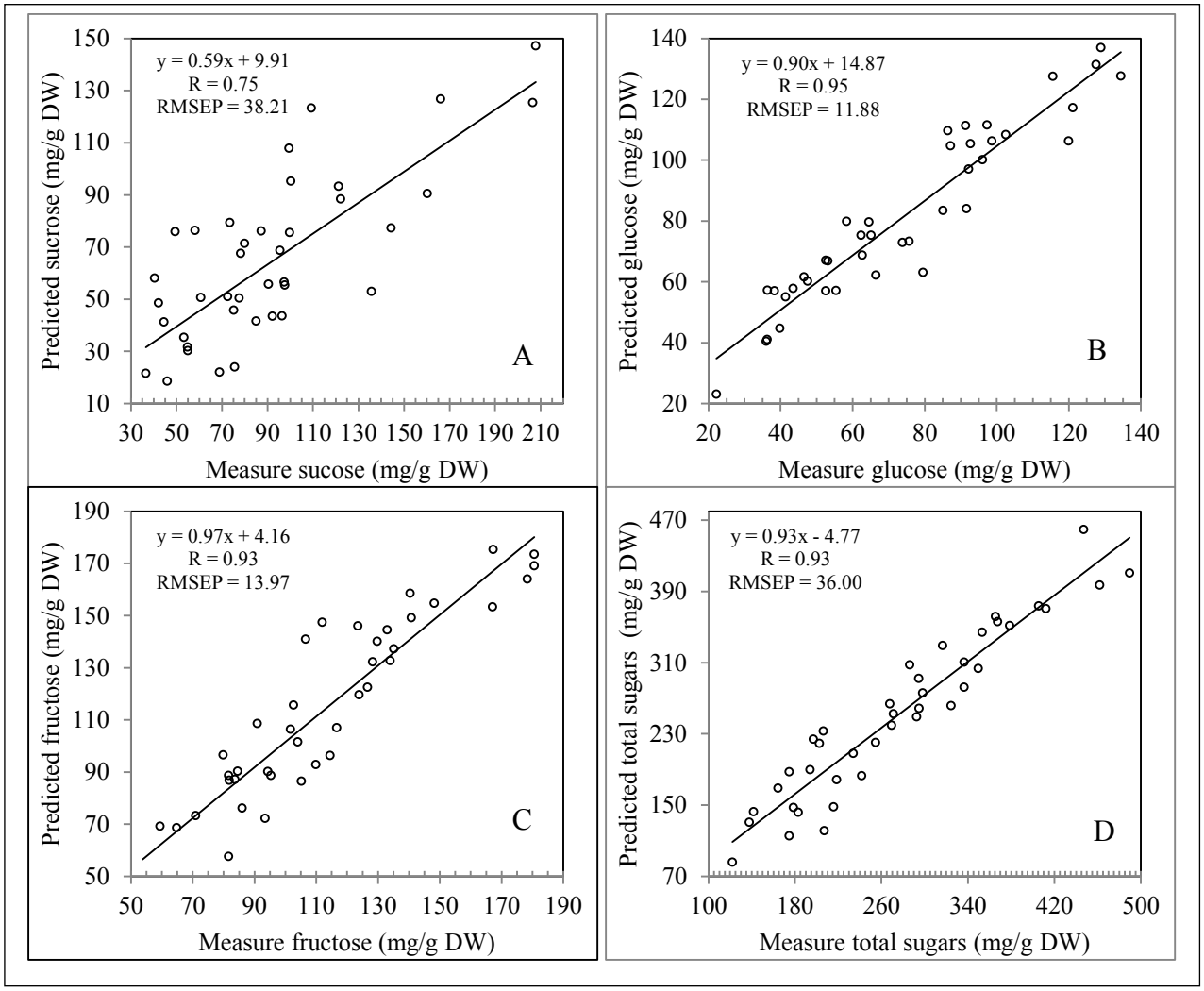

Fig. 7: Scatter plots for Vis/NIR predicted versus measured values of sucrose (A), glucose (B), fructose (C), and total carbohydrates (D). All results are expressed in mg/g DM.

CHAPTER 6

RESEARCH RESULTS

PAPER 5:

THE USE OF VIS/NIRS AND CHEMOMETRIC ANALYSIS TO PREDICT RIND BREADKDOWN AND POSTHARVEST BEHAVIOUR OF 'NULES CLEMENTINE' MANDARIN FRUIT

THE USE OF VIS/NIRS AND CHEMOMETRIC ANALYSIS TO PREDICT RIND BREAKDOWN AND POSTHARVEST BEHAVIOUR OF 'NULES CLEMENTINE' MANDARIN FRUIT

Abstract

The use of visible/near-infrared (Vis/NIR) spectroscopy to evaluate fruit physiological defects and predict postharvest quality attributes of fruit are topical in postharvest research. The use of chemometrics to analyse Vis/NIR spectra collected from intact 'Nules Clementine' mandarin fruit at harvest, in order to predict the rind biochemical profile after eight weeks of storage was explored. The study further aimed to predict fruit susceptibility to physiological rind breakdown (RBD). Vis/NIRS signals of 150 fruit were obtained immediately after harvest. Reference data on rind physico-chemical properties were obtained after eight weeks of cold storage at 8±0.5 °C, including RBD, colour index (CI), rind dry matter (DM), and concentration of non-structural carbohydrates (sucrose, glucose, fructose, total carbohydrates). Partial least squares (PLS) regression was applied to spectral data to develop prediction models of fruit properties. Principal component analysis (PCA) followed by PLS-discriminant analysis (PLS-DA) were also used to classify fruit according to canopy position and susceptibility to RBD. Optimal PLS model performances for DM, sucrose, glucose and fructose were obtained using models based on multiple scatter correction, with respective residual predictive deviation (RPD) of 3.39, 1.75, 2.19 and 3.08. The PLS models for DM and carbohydrates were developed using 900-1700 nm wavelength band, while models for CI were developed and validated using 450-750 nm. Clear clusters of fruit distribution in the PCA and PLS-DA models based on canopy position and susceptibility to RBD were obtained. The results demonstrated the potential applications of Vis/NIR spectroscopy and chemometrics in predicting postharvest behaviour of mandarin fruit.

Keywords: Rind breakdown · Citrus · Non-destructive · Spectral pre-processing · Rind physiological disorder.

1. Introduction

External appearance is the primary parameter used to evaluate quality of citrus fruit and the presence of skin defects is one of the most influential factors in determining the price of fresh fruit. External citrus fruit quality classification is currently based on the evaluation of fruit surface colour, size, shape and freedom from defects. These quality parameters are usually evaluated by humans or by machine vision systems (Xudong et al., 2009). Under most grading systems, fruit with slight external defects are graded and marketed with sound fruit, thereby reducing the quality of the batch. Alternatively, fruit with slight defects are graded out and removed together with seriously damaged fruit, thus causing economic loses (Blasco et al., 2007). The challenge is significant regarding citrus rind disorders that do not manifest at harvest or during grading, but develop about 1-5 weeks after harvest; such as rind breakdown disorder (RBD) of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) (Cronje et al., 2011; Magwaza et al., 2012a, b), RBD of 'Navel' orange [*C. sinensis* L. (Osb.)] (Alférez et al., 2003) and non-chilling postharvest rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Alférez and Burns, 2004). The challenge is therefore to develop a non-destructive method that will determine rind quality on the packing line and assist in pre-symptomatic sorting and segregation of fruit into different quality tiers.

Non-destructive optical methods based on visible/near-infrared (Vis/NIR) spectroscopy (Vis/NIRS) have been developed and evaluated for non-destructive internal quality assessment of fruit and vegetables, including citrus (Butz et al., 2005; Antonucci et al., 2011). Very limited research work has been conducted to develop a technology that can assess, predict and monitor fruit rind physiological disorders. This is because until recently, most non-destructive quality measurement technologies were developed to assess fruit according to their internal quality attributes rather than for external quality. However, the success of Vis/NIRS to predict surface bruising in apple (Geeola et al., 1994), surface defects in peach (Miller and Delwiche, 1991), storage disorder in kiwifruit (Clark et al., 2004) and internal drying in tangerine (Peiris et al., 1998) suggests there is potential for this technology in determining the sensitivity or propensity of specific citrus fruit to develop non-chilling associated physiological rind disorders. The trend is constantly shifting towards developing more reliable and cost effective technologies to non-destructively screen fruit physiological disorders. One of the recent successes of Vis/NIRS application was demonstrated by Zheng et al. (2010) who used it to predict susceptibility of citrus fruit to oleocellosis.

Vis/NIRS depends on chemometrics which involves multivariate analysis for interpreting large data sets (Wetzel, 1983; Wang and Paliwal, 2007). Calibration methods include partial least squares regression (PLS), multivariate linear regression (MLR), principal component analysis (PCA) and support vector machine and artificial neural networks (ANN) (Nicolaï et al., 2007a; Yang et al., 2011; Lorente et al., 2013). Currently, PLS is probably the most widely applied regression method in chemometrics, and the approach has been used to evaluate the potential of Vis/NIRS in measuring the quality characteristics of 'Satsuma' mandarin fruit (Gómez et al., 2006). Besides resulting in better prediction models compared to MLR, PLS analysis results in models which always have the lowest number of latent variables (LVs) since PLS models exclude LVs that are not important to describe the variance of the quality parameter (De Jong, 1993).

The spectra of solid and scattering samples such as intact fruit are influenced by physical properties such as shape and size (Leonardi and Burns, 1999). This creates spectral noise when analysing quality parameters for which such physical characteristics are not important (Blanco and Villarroya, 2002). In order to remove this noise, facilitate handling and to develop more simple and robust models, the complex spectral data are often pre-treated by different statistical procedures (Nicolaï et al., 2007b; Magwaza et al., 2012c). Thus, the selection of suitable pre-processing or pre-treatment methods is an important step in the process of spectral analysis. Some of the more frequently used pre-processing methods for Vis/NIRS signal include normalisation, derivatives (usually first and second), the multiplicative signal correction also known as multiplicative scatter correction (MSC), the standard vector normalisation (SNV), smoothing using moving average, and de-trending (Blanco and Villarroya, 2002). Derivative spectroscopy is a method which has demonstrated success in correcting light scattering contributions provided that the spectrum describes a large wavelength range (Leonardi and Burns, 1999). To reduce the effect of fruit size on citrus spectra, Kawano et al. (1993) normalised the second derivative spectra by dividing it by the second derivative value at 844 nm, which had a high correlation to the diameter of the fruit. However, derivative spectroscopy does not correct the pathlength variations. MSC is suggested to correct for pathlength variations.

In this study, the application of chemometric analysis of spectra of intact 'Nules Clementine' mandarin fruit at harvest to predict future rind biochemical profile and to predict susceptibility of fruit rind to develop RBD was investigated.

2. Materials and methods

2.1. Fruit samples

The study was conducted in 2012 using 'Nules Clementine' mandarin fruit harvested from a commercial orchard located in Paarl area of the Western Cape Province, South Africa (33°43'27.44''S; 18°57'21.28''E). A total of 100 fruit (50 from the inside position and 50 from the outside position of the canopy) from 10 trees were selected for non-destructive and destructive measurements. Of the 100 fruit harvested, 60 were used for calibration and the remaining 40 were used for validation during model development. An independent population of 50 fruit (for validation) was harvested from a commercial orchard in Citrusdal, Western Cape Province, South Africa (32° 35' 18.26" S, 19° 01' 14.69" E) using the selection procedure described above (from inside and outside canopy positions). Fruit were harvested at optimum maturity according to industry practice based on maturity index, which is the ratio of total soluble solids (TSS) to titratable acidity. These fruit then received all commercial postharvest practices, including drenching (Thiabenzole, 500 mg/L; Imazalil, 500 mg/L and 2,4-dichlorophenoxyacetic acid, 125 mg/L) and waxing (polyethylene citrus wax, Citrashine®, Johannesburg, South Africa). After phytosanitary inspection and export certification, fruit from different canopy positions were packed in separate carton boxes, sent at ambient temperature via a courier service to Cranfield University (CU) in the United Kingdom, where experiments were conducted. Fruit arrived at CU after 48 hours and were stored for 24 hours at 20 °C and 80% relative humidity to equilibrate, prior to taking NIR measurements.

2.2. Spectral acquisition

Upon arrival at CU, spectra of intact fruit samples were acquired using a method described by Magwaza et al. (2012a, b). Briefly, spectra were collected using a mobile fibre-optic spectrophotometer (350-2500 nm) (LabSpec2500® Near Infrared Analyser, Analytical Spectral Devices Inc., CO, USA) in diffuse reflectance mode, equipped with one Si array (350-1000 nm) and two Peltier cooled InGaAs detectors (1000-1800 nm and 1800-2500 nm). Reflectance spectra were acquired from 8 positions on the fruit; 4 from equatorial spots, 2 from the stem-end and 2 from the stylar-end of the fruit. The first set of spectra was acquired

before storage and the second set was acquired from the same fruit (and the same spots within the fruit) after eight weeks of cold storage.

2.3. Storage and destructive (reference) measurements

After the first round of spectral acquisition fruit were stored for eight weeks in a cold room with delivery air temperature of 8±0.5°C which is known to cause the highest incidence of RBD (Khumalo, 2006). Destructive data on physico-chemical properties including colour, RBD, DM and non-structural carbohydrates (sucrose, glucose and fructose) of fruit were obtained after 8 weeks of storage. Rind colour components were measured in L*a*b* colour space using a Minolta CR-400 colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203). From the L*, a* and b* colour parameters, the colour index (CI) was calculated according to equation 1 (Jimenez-Cuesta et al., 1981; Pathare et al., 2012):

$$CI = \frac{1000 \times a}{L \times b} \quad (1)$$

During cold storage, fruit were evaluated weekly for the incidence of RBD, over eight weeks. RBD incidence was scored on a subjective scale from 0 = no breakdown to 3 = severe breakdown (Fig. 1). RBD was then expressed as RBD index as described by Alférez and Burns (2004) using equation 2.

$$RBDI = \frac{\sum \{RBD(0-3) \times \text{No.of fruit in each class}\}}{\text{Total number of fruit}} \quad (2)$$

After storage, rind was peeled by hand from the rest of fruit, snap frozen in liquid nitrogen and stored at -40°C until further analysis. Frozen samples were then freeze-dried in a Labogene ScanVac CoolSafe Freeze Dryer System (CS55-4, Denmark) for 7 days at 0.015 kPA and -55°C. Lyophilised samples were weighed and water content was calculated from freeze dried samples and expressed as a percentage of fresh mass, after which samples were ground using a pestle and mortar into fine powder. Non-structural carbohydrates (sucrose, glucose and fructose) were extracted and quantified using a method described previously (Magwaza et al., 2012b).

2.4. Data analysis

Statistical analysis of destructive measurements was carried out using SPSS 20.0 for Windows (SPSS Inc. Chicago, IL, USA). Data were subjected to analysis of variance (ANOVA). Least significant difference values (LSD; $p \leq 0.05$) were calculated for the comparison of means.

2.5. Chemometrics

The reflectance spectra in Indico format (Indico Pro 5.6 software, Analytical Spectral Devices Inc., CO, USA) were transformed to absorbance (log (1/R)). Individual spectra from 8 positions within the fruit and the average spectra of 8 spectra from each fruit were tested to develop PLS, PCA and PLS-DA models. Average spectra showed better models than individual spectra (data not shown); and thus results reported herein are based on average spectra. Calculations of the average of 8 spectra obtained from each fruit, pre-processing and calibration methods were executed using The Unscrambler® chemometric software (Version 9.2, Camo Process, SA., Norway).

Different pre-processing methods including MSC, SNV, Savitzky Golay first derivative and second derivative (2^{nd} order polynomial), were applied to normalise and smooth spectral data prior to regression to correct for light scatter and reduce the changes of light path length.

Average Vis/NIRS spectra were subjected to PCA to determinate effective wavelength, detect outliers and to discriminate fruit from different canopy positions. PLS 1 regression/prediction models were developed using spectral data for each quality attribute. A PLS variant known as partial least squares discriminant analysis (PLS-DA) was also used in order to classify fruit from different canopy positions according to the spectra. A method by Chen et al. (2011), with slight modifications, was used in the application of PLS-DA. Fruit from each of the canopy positions in the calibration set was assigned a dummy variable as a reference value (outside = 1 and inside = 2). In addition, due to the discrete nature of RBD scores, samples were assigned a binary dummy variable as a reference value, which was an arbitrary number whether the sample belongs to a particular position or not. RBD affected fruit were set as reference data one (1), while unaffected fruit were assigned to 0 (Teerachaichayut et al., 2011).

During model development, the dataset was subjected to test set validation where fruit were randomly separated into two subsets, 60% for calibration and 40% for validation.

Although the sample selection method for calibration and validation was random (using the random function of the Unscrambler software), validation data sets were scrutinised to ensure that the validation data sets were confined within a range of values of the calibration set. Models was described by the value of the root mean square error of calibration (RMSEC), root mean square error of validation or prediction (RMSEP), the correlation coefficient (R), which represents the proportion of explained variance of the response variable in the calibration (R_c) or validation dataset (R_v). The number of latent variables (LVs), and the residual predictive deviation (RPD), described by Williams and Sobering (1996) as the ratio of the standard deviation of the destructive data for the validation set to the RMSEP. The ideal model should have high R and RPD values as well as low RMSEC and RMSEP values. The optimal number of LVs was determined as the minimum number of LVs corresponding to the first lowest value of the RMSEC or RMSEP from the plot of the RMSEC or RMSEP for increasing number of LVs (Davey et al., 2009).

The spectral variables which contributed the most to the model were determined from the regression coefficients curve. Wavelength bands with high regression coefficient values are important to the mode. Outliers were evaluated using the score plots, X-residuals and leverage plots on the PLS and PCA models (Shiroma and Rodriguez-Saona, 2009). Samples that were located far from the zero line of the residual variance plot were identified as outliers and excluded (Kuang and Mouazen, 2011).

The stability of the calibration model was tested by interchanging validation and calibration data sets and checking that the differences in the regression statistics obtained were small (Alvarez-Guerra et al., 2010). Prediction model robustness was tested by external validation with spectra of fruit harvested from a farm in Citrusdal, an orchard located about 100 km from the orchard of fruit used during model development.

3. Results and discussion

3.1. Description spectra

The reflectance spectra presented in Fig. 2 portray the typical spectra obtained from intact ‘Nules Clementine’ mandarins harvested from different canopy positions. Each line represents the average spectra acquired from 50 fruit in each canopy position before and after storage, respectively. Spectral features such as reflectance peaks were similar to those obtained by Gómez et al. (2006). The edge at the beginning (350 to 450 nm) of each

spectrum was characterised by noise and was removed before calibration. Strong absorption bands around 670, 740, 960, 1200, 1450, 1780 and 1930 nm were observed. Absorption at 670 nm is due to red absorbing pigments, particularly chlorophyll (Clément et al., 2008); 740 nm corresponds to third overtone of O-H stretching; 980 nm is associated with second overtone of H-O-H stretching modes of water; 1200 is the combination of second overtones of C-H and C-H_2 stretching; 1450 belongs to the first overtone of O-H stretching; 1780 nm is a combination of first overtone of C-H and CH_2 stretching; and 1930 nm is the combination of O-H, C-H and C-H_2 deformations associated with sugar solution (Kawano et al., 1993; Golic et al., 2003; Tewari et al., 2008). It should be noted that before storage, the average spectra of outside fruit had a distinctly stronger absorbance in the waveband between 590 and 900 nm compared to inside fruit. After 8 weeks of storage, the difference between spectral data acquired from outside and inside fruit was less pronounced due to loss of chlorophyll (green pigment) during storage. Results observed in this study are similar to those obtained by Zheng et al. (2010) for prediction of oleocellosis disorder, where large variations in absorbance spectra were observed among fruit with different sensitivities in the same waveband between 590 and 900 nm. In this study, fruit harvested from the inside position of the canopy were more susceptible to RBD than outside fruit (Fig. 3), a trend that is similar to previous work by Cronje et al. (2011) and in Chapter 5. Since the intensities of reflectance vary with concentrations of biochemical constituents of the sample (Williams and Norris, 2001) the band may possibly be related to fruit sensitivity to rind physiological disorders such as oleocellosis and RBD.

3.2. Description of destructive data for NIR calibrations

The distributional statistics of destructive data used in calibration and independent validation are summarised in Table 1. The reference measurements for calibration and validation data sets were normally distributed round the means, covered a wide range and had enough variation, represented by the coefficient of variation (CV%). High variation of the reference data is helpful in developing reliable prediction models for Vis/NIRS (Lu et al., 2006; Clément et al., 2008). The mean values of the CI for calibration and validation populations were 4.38 and 4.42, respectively, with the corresponding CV% values of 37.81 and 35.75%. The mean concentration of sucrose values used for calibration and validation were 88.19 and 92.68 mg/g DM with standard deviation of 41.63 and 42.73 mg/g DM, respectively. The concentration of total non-structural carbohydrates in the rind tissue ranged

between 122.03 and 501.12 mg/g DM in the calibration population and the range of validation set was from 137.66 to 512.01 mg/g DM with corresponding CV% of 34.25 and 32.30, respectively.

3.3. Spectral pre-processing and wavelength selection

Similar to our previous study (Magwaza et al., 2012b), the models constructed using individual spectra from eight positions within the fruit were unacceptably poor (data not presented). In all cases, average spectra showed better model performance than individual spectra from each position. Hence, in the current study, the average spectra were used to develop PLS, PCA and PLS-DA models. Poor performance of individual spectra could be the result of spatial distribution and level of attributes within the fruit. In citrus and other fruit types, chemical composition has been reported to vary from stem to blossom end, from sun to shade sides, and from different canopy positions of the fruit (Miyamoto and Kitano, 1995; Guthrie and Walsh, 1997; Peiris et al., 1999; Guthrie et al., 2005). This suggests that Vis/NIR spectra acquisition needs to be repeated at several positions around the fruit in order to minimise the effect of variation within fruit. However, this might not be practically compatible with a typical speed of commercial sorting lines, which may be as high as 10 fruit per second per lane (Nicolaï et al., 2007b). Parallel installed lines with turning fruit and a number of spectrophotometers might overcome this problem.

Spectral data of solid and scattering samples such as intact mandarin fruit are complex as they are influenced by physical properties such as shape, size, etc. (Leonardi and Burns, 1999). As such, spectral pre-processing facilitates handling and development of simpler and more robust models (Butz et al., 2005; Magwaza et al., 2012c). After several pre-processing methods tests performed in the current study, prediction models that gave the higher R, lower RMSEC and RMSEP, a small difference between RMSEC and RMSEP and high RPD was selected to predict the quality parameter of interest (Lammertyn et al., 2000).

Results in Table 2 show that CI was best predicted using raw spectra without spectral pre-processing in the visible region (450 to 750 nm). Since colour parameters are likely to be directly detected in the visible range, it is logical that model performance for CI did not need spectral pre-processing. The CI results demonstrated a classic example of the risk associated with removing useful information from the data by spectral pre-processing. The best stable model for predicting rind DM (RPD = 3.39) was achieved using models developed by MSC pre-processing method. In the case of DM, the model based on Savitzky-Golay first

derivative (second order polynomial) pre-processing also had acceptable prediction potential (RPD = 3.47), but MSC had the smallest difference between R_c and R_v, and was thus more reliable. Although DM was best predicted based on spectra treated by MSC, the results obtained using Savitzky Golay first derivative spectral pre-processing were marginally lower, suggesting that both methods could be used for DM prediction of 'Nule Clementine' mandarin rinds.

MSC also gave best results for PLS prediction of rind carbohydrates concentration such as sucrose, glucose fructose, and total carbohydrates with RPD values of 1.75, 2.19, 3.08, and 3.06, respectively. Savitzky-Golay second derivative with the second order polynomial provided the better results for the PCA classification of fruit from different canopy positions (Fig. 4 and Fig. 5) and PLS model for predicting RBD (Fig. 6). Previous research showed that Savitzky-Golay first and second derivative corrected light scattering properties while MSC corrected for additive, multiplicative effects of the spectra, and pathlength variations (Leonardi and Burns, 1999; Gómez et al., 2006). It is important to note that the DM, fructose, glucose, sucrose and total sugars models developed in the succeeding sections are based on Vis/NIRS after MSC pre-processing while CI models were developed without pre-processing.

The optimal number of LVs was determined as the minimum number corresponding to the first lowest value of the residual y-variance, from the plot of residual y-variance against number of LVs and the difference between RMSEC and RMSEP (Fig. 7A) (Davey et al., 2009). In the DM PLS model, the optimum number of LVs was observed as eight. The residual variance did not change after LV 8; hence adding another LV explained very small variance and would have resulted in a complex model and overfitting from including noise. With regard to fructose, the number of LVs (14) used to construct the models was high and violated the statistical rule of thumb, which states that the ratio of the number of samples for calibration (in this case 100) to the number of LVs should be equal to or larger than 10 (Lammertyn et al., 2000). Despite this large number of LVs used to construct the model, the model seemed to be accurate and stable during the validating exercise.

The contributions of spectral variables to the model were determined from its regression coefficients curve. Wavelength bands with high absolute regression coefficient values are important for the model while regression coefficients with a value close to zero do not contribute to the model (Williams and Norris, 2001). A typical regression coefficient curve obtained during prediction model development for dry matter content in 'Nules Clementine' mandarin rinds is portrayed in Fig. 7B. High absolute regression coefficients were observed

in the NIR region of the curve at 950, 1200, 1320 and 1700 nm. As mentioned above, these high absolute regression coefficients correspond with the second overtone of H-O-H stretching modes of water, the second overtone of C-H and C-H_2 stretching related to sugars molecules as well as second and third overtones of OH and CH stretching vibrations of water hydrogen bonds with sugar molecules related to vibration of water hydrogen bonds with sugars molecules (Kawano et al., 1993; Golic et al., 2003; Clément et al., 2008; Tewari et al., 2008). From the regression coefficients results, the informative wavelength bands for all carbohydrates and DM were between 900-1700 nm, while CI and RBD were best predicted at 450-750 and 450-1000 nm, respectively.

3.4. Vis/NIR-based PCA and PLS models

PCA was performed on Vis/NIRS spectra to compare the characteristics of fruit from different positions on the tree. The PCA was applied to the spectra collected before storage and eight weeks postharvest. The distribution in the PCA score plot of fruit spectra acquired before storage showed two distinctive clusters corresponding to two canopy positions. This grouping was only possible on spectra transformed using Savitzky Golay second derivative pre-processing method (Fig. 4). These clusters allowed distinction between fruit from different canopy positions with accuracy of 84%, i.e. only eight fruit could not be identified as 1 or 2. The first two PCs accounted for 86.0 % of the total variability (Fig. 4), PC1 explained the 71.0 % of the variance and PC2 explained 15.0 % of the variance. The effective wavelength band for this classification was from 450 to 1200 nm with a strong absorption at 670 nm influenced by chlorophyll, two bands at 740 and 980 nm corresponding to water (O-H) functional groups and one at 1200 nm associated with C-H stretching for carbohydrates. This confirms that a combination of colour, carbohydrates and moisture content of the rind play an important role in discriminating between fruit from different positions of the canopy. As previously reported by Magwaza et al. (2012a, b), PCA models developed using spectra restricted to either visible range or to NIR range did not show these clusters demonstrating the importance of the combination of visible and NIR range in the classification of fruit by their canopy position. Spectral data collected after eight weeks of postharvest was also subjected to a similar analysis, but clusters were not easily identifiable (Fig. 5). In this case, the first two PCs accounted for 57.0 % of the total variability, PC1 explained the 42.0 % of the variance and PC2 explained 15.0 % of the variance. Improved rind colour during storage

is one of the possible reasons explaining poor classifications and misidentification observed after eight weeks of storage.

To further test the potential of Vis/NIR spectral information to discriminate fruit from different canopy positions, spectral data obtained before storage was subjected to discriminant analysis (PLS-DA) by assigning fruit from each canopy positions to a dummy variable (1, and 2 for outside and inside, respectively). Performance of the PLS-DA model to classify fruit based on their origin within the tree canopy using spectral range between 450 and 2400 nm and MSC spectral pre-processing is shown in Fig. 8. The results in this study showed that fruit from inside the canopy had higher susceptibility to the disorder than outside fruit. In practical terms, the ability of Vis/NIRS to non-destructively classify fruit based on their origin within the canopy, suggests the potential of this technology to classify individual fruit in a packing line, for either local (inside fruit) or export market and long term storage (outside fruit).

Due to discrete nature of RBD scores, correlating it with complicated NIR data was difficult and model statistics were poor ($R < 0.10$). As a result, samples were assigned a binary dummy variable (0 and 1), which indicated absence or presence of RBD, respectively (Teerachaichayut et al., 2011). After this analysis, the model statistical parameters for external prediction were improved to $R = 0.61$, RMSEP = 0.34 and RPD = 0.45. Considering that RBD was a category binary variable ranging from 0 to 1, the RMSEP of 0.34 was too high (Table 3 and Fig. 6). In addition, the small y-variance explained by this model (18%) also proved this model to be poor and not able to accurately predict RBD. The upper limit for PLS-DA discrimination of fruit without RBD was 0.5, and samples with predicted value of 0.5 or higher were classified as having RBD, while samples with predicted values higher than 0.5 were not affected by the disorder (Fig. 6). The RBD PLS-DA prediction model had low false positive (11.5%) and relatively high false negatives (57.0%) resulting in the overall accuracy of 72.5%.

The prediction performance of the models developed and validated using Vis/NIRS signals acquired from fruit before storage and eight weeks after storage are summarised in Table 3. PLS models based on spectra acquired before storage was better for predicting CI ($R = 0.94$, RMSEP = 0.38 and RPD = 4.12) than models based on spectra acquired after 8 weeks of storage ($R = 0.89$, RMSEP = 0.65 and RPD = 2.43). For RBD, a similar trend was observed, where prediction was better when using spectra acquired before storage compared to spectra after storage. In 'Nules Clementine' mandarins, rind colour of fruit harvested from inside and outside the canopy has been previously reported to improve during storage (Cronje

et al., 2011). The loss of quantifying ability reported in the current study on rind colour could be attributed to the inclusion of the visible waveband in these models. The prediction performance of fructose, glucose, total sugars and DM was slightly higher for the models based on spectra acquired after storage compared to those acquired from fruit before storage. An example of this improved trend of model performance with time is shown on DM, where the RPD value of the model with spectra obtained before storage was 3.39 while that for spectra acquired after 8 weeks of storage was 3.84. NIRS is expected to perform well to predict sugar concentration as reported before (Magwaza et al., 2012b).

4. Conclusion

The results of this study demonstrated the potential of using Vis/NIRS to predict rind biochemical composition during storage. Rind CI, carbohydrates and DM were all predicted with high accuracy. However, scanning fruit more than once to get a mean would be impracticable on an on-line sorting system. This limits the implementation of the Vis/NIRS for real-time sorting. The study further showed that the technology is highly dependent on the chemometric procedures employed during construction of the prediction models. For instance, a comparison of different pre-processing methods showed that spectral pre-processing influences the performance of the model. As it would be expected with Vis/NIRS, colour parameters were best predicted by models without spectral pre-processing, demonstrating that spectral pre-processing is not always required.

In terms of predicting RBD, calibration and validation statistics were very low with RPD of ± 0.45. There are standards for RPD values where values below 1.5 are pracically unusable. Therefore, the low RPD values clearly indicate poor accuracy of these models. Previous studies have shown that RBD-susceptible fruit located inside the canopy have lower rind carbohydrates concentration (Cronje et al., 2011). However, the complexity of biological factors involved in the development of RBD may be part of the reasons why it was difficult to develop a reliable prediction model for RBD. Regardless of poor prediction of RBD, the study showed some interesting information about rind physicochemical properties such as rind colour, rind non-structural carbohydrates and dry matter content, which could be used for potential on-line application of Vis/NIRS.

PCA and PLS-DA models based on spectra acquired before harvest were able to discriminate fruit based on their position within the canopy. The accuracy of the two regression methods was very high demonstrating that both methods could be used,

individually or in combination, for screening purposes. In previous studies (Cronje et al., 2011; Magwaza et al., 2012a, b), we established that fruit located inside the canopy were more susceptible to RBD than inside fruit. The ability of Vis/NIRS to discriminate between inside and outside fruit suggests the potential of this technology to discriminate fruit based on their susceptibility to RBD. This information could be used as an on-line deciding tool, during packing, to decide on fruit destined for long distance export market (outside) and those destined for short distance or local market (inside fruit).

Furthermore, the results from this study showed the potential of Vis/NIRS to predict postharvest rind physico-chemical properties, particularly CI, DM and carbohydrates for up to eight weeks post-storage. Taking this into account, the ability of Vis/NIRS to predict carbohydrates shows the potential of this technology to predict postharvest behaviour of rind biochemical properties. In addition, the upper or lower limits of carbohydrates concentration in which the disorder may occur are still not known. Hence, further research still needs to be conducted to explain whether Vis/NIRS-predicted carbohydrates concentration is useful for determining fruit susceptibility to postharvest RBD.

References

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alvarez-Guerra, M., Ballabio, D., Amigo, J.M., Bro, R., Viguri, J.R., 2010. Development of models for predicting toxicity from sediment chemistry by partial least squares-discriminant analysis and counter-propagation artificial neural networks. Environ. Pollut. 58, 607–614.

Antonucci, F., Pallottino, F., Paglia, G., Palma, A., D'Aquino, S., Menesatti, P., 2011. Non-destructive estimation of mandarin maturity status through portable VIS-NIR spectrophotometer. Food Bioprocess Tech. 4, 809–813.

Blanco, M., Villarroya, I., 2002. NIR spectroscopy: a rapid-response analytical tool. Trends Anal. Chem. 21, 240–250.

Blasco, J., Aleixos, N., Moltó, E., 2007. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. J. Food Eng. 81, 535–543.

Butz, P., Hofmann, C., Tauscher, B., 2005. Recent developments in non-invasive techniques for fresh fruit and vegetable internal quality analysis. J. Food Sci. 70, 131–141.

Chen, X., Wu, D., He, Y., Liu, S., 2011. Nondestructive differentiation of *Panax* species using visible and shortwave near-infrared spectroscopy. Food Bioprocess Tech. 4, 753–761.

Clark, C.J., McGlone, V.A., DeSilva, H.N., Manning, M.A., Burdon, J., Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit (*Actanidia cinensis*) based on visible-NIR spectral characteristics at harvest. Postharvest Biol. Technol. 32, 147–158.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56, 9813–9818.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Davey, M.W., Saeys, W., Hof, E. Ramon, H., Swennen, R.L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. J. Agric. Food Chem. 57, 1742–1751.

De Jong, S., 1993. PLS fits closer than PCR. J. Chemomet. 7, 551–557.

Geeola, F., Geeola, F., Peiper, U.M., 1994. A spectrophotometric method for detecting surface bruises on 'Golden Delicious' apples. J. Agric. Eng. Res. 58, 47–51.

Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

Gómez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Guthrie, J., Walsh, K., 1997. Non-invasive assessment of pineapple and mango fruit quality using near infrared spectroscopy. Austr. J. Exp. Agric. 37, 253–263.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Austr. J. Agric. Res. 56, 405–416.

Jimenez-Cuesta, M., Cuquerella, J., Martinez-Javega, J.M., 1981. Determination of a colour index for citrus degreening. Proc. Int. Soc. Citricul. 2, 750–753.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Non-destructive determination of sugar content in 'Satsuma' mandarins using NIRS transmittance. J. Japan Soc. Hort. Sci. 62, 465–470.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Kuang B., Mouazen, A.M., 2011. Calibration of visible and near infrared spectroscopy for soil analysis at the field scale on three European farms. Eur. J. Soil Sci. 62, 629–636.

Lammertyn, J., Peirs, J., De Baerdemaeker, J., Nicolaï, B.M., 2000. Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121–132.

Leonardi, L., Burns, D.H., 1999. Quantitative multiwavelength consistuent measurements using single-wavelength photo time-of-flight correction. Appl. Spectrosc. 53, 637–646.

Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., Blasco, J., 2013. Selection of optimal wavelength features for decay detection in citrus fruit using the ROC curve and neural networks. Food Bioprocess Tech. 6, 530–541.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. 7, 794–799.

Magwaza, L.S., Opara, U.L. Cronje, P.J.R. Nieuwoudt, H.H. Landahl, S., Terry, L. A., 2012a. Quantifying the effects of fruit position in the canopy on physical and biochemical properties and predicting susceptibility to Rind Breakdown Disorder of 'Nules Clementine' Mandarin (*Citrus reticulata* Blanco) Using Vis/NIR Spectroscopy. Acta Hort. 1007, 89–91.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H., Mouazen, A.M., Saeys, W., Nicolai, B.M., 2012b. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest Biol. Technol. 74, 1–10.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolaï, B., 2012c. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food Bioprocess Tech. 5, 425–444.

Miller, B.K., Delwiche, M.J., 1991. Spectral analysis of peach surface defects. Trans. Am. Soc. Agric. Eng. 34, 2509–2515.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3**,** 227–237.

Nicolaï, B.M., Theron, K.I., Lammertyn, J., 2007a. Kernel PLS regression on wavelet transformed NIR spectra for prediction of sugar content of apple. Chemomet. Intell. Lab. Syst. 85, 243–252.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I. K., Lammertyn, J., 2007b. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Pathare, P.B., Opara, U.L., Al-Said, F.A., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Tech. 6, 36–60.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., 1998. Non-destructive detection of selection drying, an internal disorder in tangerine. HortScience 33, 310–312.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. HortScience 34, 114–118.

Shiroma, C., Rodriguez-Saona, S., 2009. Application of NIR and MIR spectroscopy in quality control of potato chips. J. Food Comp. Anal. 22, 596–605.

Teerachaichayut, S., Terdwongworakul, A., Thanapese, W., Kiji, K., 2011. Non-destructive prediction of hardening pericarp disorder in intact mangosteen by near infrared transmittance spectroscopy. J. Food Eng. 106, 206–211.

Tewari, J.C., Dixit, V., Chi, B.-K., Malik, K.A., 2008. Determination of origin and sugars of citrus fruit using genetic algorithm, correspondence analysis and partial least square combined with fiber optic NIR spectroscopy. Spectrochim. Acta Mol. Biomol. Spectrosc. 71, 1119–1127.

Wang, W., Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. Sensory and Instrumental Food Chemistry 1, 193–207.

Wetzel, D.L., 1983. Near-infrared reflectance analysis. Anal. Chem. 55, 1165–1176.

Williams, P., Norris, K.H., 2001. Variables affecting near infrared spectroscopic analysis. In Near Infrared Technology in the Agriculture and Food Industries pp. 171–185. P. Williams and K. H. Norris Eds. 2nd ed. The American Association of Cereal Chemists, St. Paul, MNL.

Williams, P. C., Sobering, D. C., 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In Proceedings of the 7th International Conference of Near Infrared Spectroscopy: Near Infrared Spectroscopy: The future waves, pp. 185–188. A. M. C. Davies and P. Williams eds. Montreal, Chichester, UK.

Xudong, S., Hailiang, Z., Yande, L., 2009. Nondestructive assessment of quality of 'Nanfeng' mandarin fruit by a portable near infrared spectroscopy. International J. Agric. Biol. Eng. 2, 65–71.

Yang, H., Kuang, B., Mouazen, A.M., 2011. In situ determination of growing stages and harvest time of tomato (Lycopersicon esculentum) fruits using fibre optic vis-NIR spectroscopy. Appl. Spectrosc. 35, 931–938.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort. 125, 401–405.

Table 1: Mean, standard deviation (SD), range and coefficient of variation (CV%) for calibration (n = 100) and external validation (n = 50) subsets of mandarin fruit physico-chemical properties.

Quality parameter	Calibration data set			Validation data set		
	Mean±SD	Range	CV%	Mean±SD	Range	CV%
Colour index	4.38±1.66	0.07-7.49	37.81	4.42±1.58	0.07-7.43	35.75
Rind dry matter (%)	28.42±3.07	22.16-35.41	10.82	28.12±3.19	22.84-35.46	11.34
Rind fructose (mg/g DM)	120.12±34.70	53.72-195.88	28.88	120.58±35.09	64.826-195.88	29.10
Rind glucose (mg/g DM)	81.20±30.59	22.19-152.15	37.68	80.70±31.09	36.01-152.16	38.53
Rind sucrose (mg/g DM)	88.19±41.63	35.85-207.95	47.32	92.68±42.73	35.90-206.30	44.47
Rind total carbohydrates (mg/g DM)	289.50±99.14	122.03-501.12	34.25	293.95±94.95	137.66-512.01	32.30

Table 2: Results for calibration and prediction of the partial least squares (PLS) models developed and validated using different spectral data pre-processing methods. These values are based on spectral data acquired at harvest.

Quality parameter	Pre.Pr[b]	LV[h]	Calibration			Validation				Info. Region[n] (nm)
			R_c[i]	RMSEC[j]	Slope	R_v[k]	RMSEP[l]	RPD[m]	Slope	
Colour index	None[c]	2	0.95	0.37	0.95	0.94	0.38	4.12	0.94	450-750
	SVN[d]	2	0.94	0.39	0.94	0.94	0.39	4.02	0.93	450-750
	1st der[e]	2	0.90	0.49	0.90	0.88	0.62	2.56	0.90	450-750
	2nd der[f]	2	0.90	0.49	0.90	0.88	0.62	2.56	0.88	450-750
	MSC[g]	2	0.95	0.37	0.94	0.94	0.44	3.60	0.93	450-750
RBD (binary scores)	None[c]	5	0.44	0.35	0.19	0.38	0.36	0.17	0.38	450-2400
	SVN[d]	5	0.33	0.41	0.11	-0.27	0.42	-0.08	0.21	450-2400
	1st der[e]	5	0.63	0.32	0.39	0.64	0.30	0.40	0.76	450-2400
	2nd der[f]	5	0.79	0.27	0.67	0.70	0.34	0.45	0.45	450-2400
	MSC[g]	5	0.67	0.28	0.45	0.44	0.35	0.25	0.39	450-2400
Rind DM[a] (%)	None	8	0.95	0.73	0.95	0.91	1.24	2.56	0.96	900-1700
	SVN	8	0.91	0.93	0.91	0.91	0.99	3.23	0.91	900-1700
	1st der	8	0.96	0.64	0.96	0.92	0.92	3.47	0.93	900-1700
	2nd der	8	0.84	1.28	0.83	0.83	1.35	2.37	0.83	900-1700
	MSC	8	0.93	0.68	0.95	0.94	0.94	3.39	0.92	900-1700
Rind fructose (mg/g DM)	None	14	0.87	13.21	0.87	0.85	13.60	2.58	0.85	900-1700
	SVN	14	0.88	11.96	0.88	0.82	18.20	1.93	0.79	900-1700
	1st der	14	0.99	3.99	0.99	0.89	18.43	1.90	0.92	900-1700
	2nd der	14	0.84	14.14	0.84	0.73	23.34	1.50	0.77	900-1700
	MSC	14	0.91	9.18	0.90	0.90	11.41	3.08	0.88	900-1700
Rind glucose (mg/g DM)	None	10	0.84	12.53	0.84	0.81	14.90	2.09	0.81	900-1700
	SVN	10	0.54	20.45	0.54	0.62	16.98	1.83	0.58	900-1700
	1st der	10	0.97	5.02	0.98	0.48	20.57	1.51	0.57	900-1700
	2nd der	10	0.69	16.73	0.69	0.59	24.31	1.28	0.62	900-1700
	MSC	10	0.89	10.32	0.88	0.88	14.19	2.19	0.86	900-1700
Rind sucrose (mg/g DM)	None	10	0.57	27.46	0.57	0.45	36.81	1.12	0.59	900-1700
	SVN	10	0.49	29.36	0.49	0.42	34.18	1.21	0.43	900-1700
	1st der	10	0.79	18.22	0.79	0.77	25.38	1.62	0.77	900-1700
	2nd der	10	0.72	24.11	0.72	0.63	28.62	1.44	0.71	900-1700
	MSC	10	0.92	12.37	0.92	0.83	24.36	1.75	0.90	900-1700
Rind total carbohydrates (mg/g DM)	None	10	0.93	26.06	0.93	0.83	60.03	1.58	0.95	900-1700
	SVN	10	0.62	60.42	0.62	0.65	48.70	1.95	0.62	900-1700
	1st der	10	0.79	42.71	0.79	0.78	47.29	2.01	0.78	900-1700
	2nd der	10	0.93	26.83	0.93	0.86	47.64	1.99	0.88	900-1700
	MSC	10	0.89	30.42	0.89	0.90	31.04	3.06	0.91	900-1700

[a]*DM*, rind dry matter, [b]*Pre.Pr* pre-processing method, [c]*None* no spectral pre-processing, [d]*SVN* standard vector normalisation, [e]*1st der* first derivative, [f]*2nd der* second derivative, [g]*MSC* multiple scatter correction, [h]*LV* latent variables, [i]R_c correlation coefficient for calibration, [j]*RMSEC* root mean square error of calibration, [k]R_v correlation coefficient for validation, [l]*RMSEP* root mean square error of prediction, [m]*RPD* residual predictive deviation, [n]*Info region*, informative region of the spectrum.

Table 3: Model performance using spectra acquired before storage and after 8 weeks of storage.

Quality parameter	Time (weeks)	Pre.Pr[b]	LV[f]	Calibration			Validation			
				R_c[g]	RMSEC[h]	Slope	R_v[i]	RMSEP[j]	RPD[k]	Slope
Colour index	0	None[c]	2	0.95	0.37	0.95	0.94	0.38	4.12	0.94
	8		2	0.91	0.63	0.93	0.89	0.65	2.43	0.90
RBD (binary scores)	0	2nd der[d]	5	0.79	0.27	0.67	0.70	0.34	0.45	0.45
	8		5	0.77	0.29	0.59	0.61	0.36	0.42	0.45
Rind DM[a] (%)	0	MSC[e]	8	0.93	0.68	0.95	0.94	0.94	3.39	0.92
	8		8	0.96	0.64	0.96	0.96	0.83	3.84	0.93
Rind fructose (mg/g DM)	0	MSC	14	0.91	9.18	0.90	0.90	11.41	3.08	0.88
	8		14	0.96	6.33	0.97	0.94	10.01	3.51	0.97
Rind glucose (mg/g DM)	0	MSC	10	0.89	10.32	0.88	0.88	14.19	2.19	0.86
	8		10	0.91	9.18	0.90	0.90	11.41	2.72	0.88
Rind sucrose (mg/g DM)	0	MSC	8	0.92	12.37	0.92	0.83	24.36	1.75	0.90
	8		8	0.89	13.44	0.91	0.75	38.21	1.39	0.59
Rind total sugars (mg/g DM)	0	MSC	10	0.89	30.42	0.89	0.90	31.04	3.06	0.91
	8		10	0.93	25.35	0.91	0.88	31.35	3.03	0.87

[a]*DM* rind dry matter, [b]*Pre.Pr* pre-processing method, [c]*None* no spectral pre-processing,[d]*2nd der* second derivative, [e]*MSC* multiple scatter correction, [f]*LV* latent variables, [g]R_c correlation coefficient for calibration, [h]*RMSEC* root mean square error of calibration, [i]R_v correlation coefficient for validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Fig. 1: Different levels of Rind breakdown (RBD) of 'Nules Clementine' mandarin fruit, left to right showing increasing RBD intensity; from no (0, 0% affected), little (1, 25% area affected), moderate (2, 50% affected), and severe RBD (3, 75 to 100% affected).

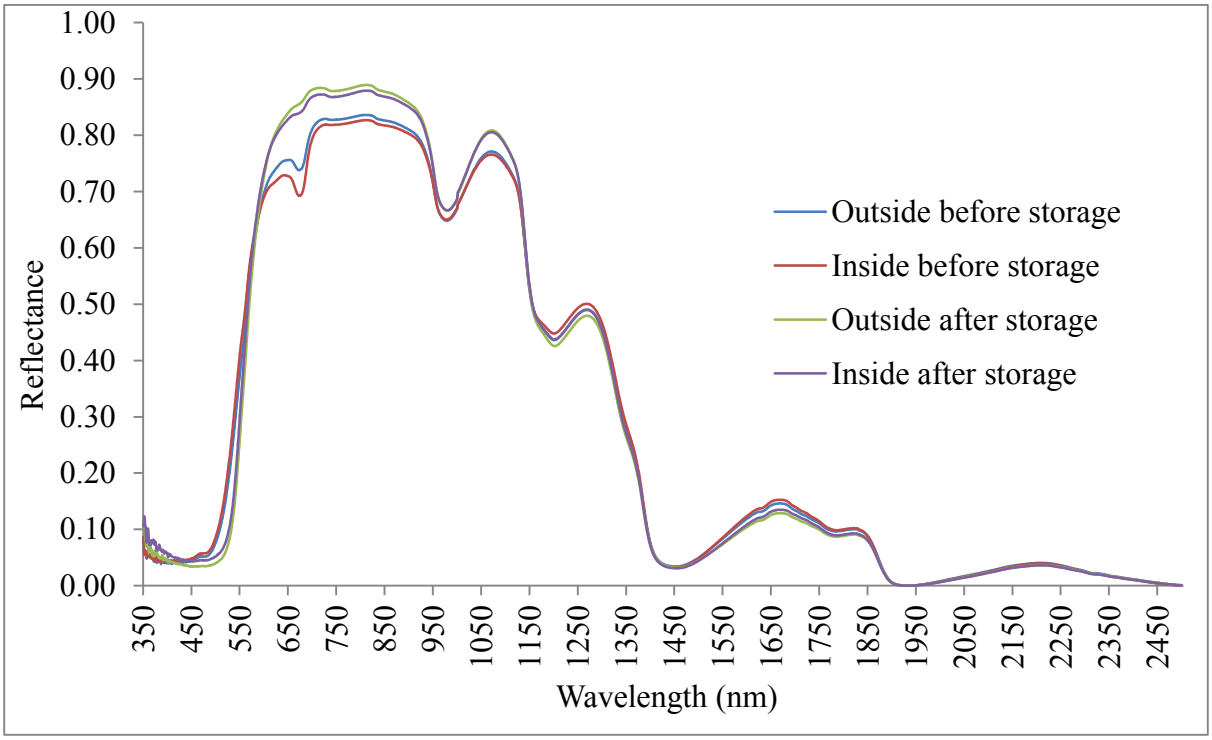

Fig. 2: Typical average Vis/NIRS reflectance spectra (350-2500 nm) of intact 'Nules Clementine' mandarin fruit harvested from different positions of the canopy, after baseline correction. The first set of spectra was acquired before storage from outside fruit (*blue line*) and inside fruit (*red line*). The second set of spectra was acquired after eight weeks of storage from the same fruit, harvested from outside (*green line*) and inside (*purple line*) canopy positions.

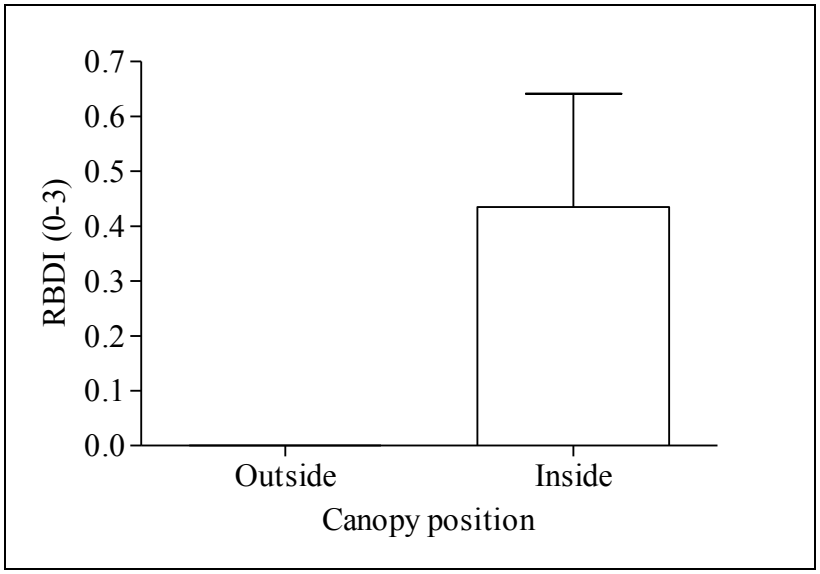

Fig. 3: Effect of fruit position within the canopy on the incidence of rind breakdown disorder of 'Nules Clementine' mandarin.

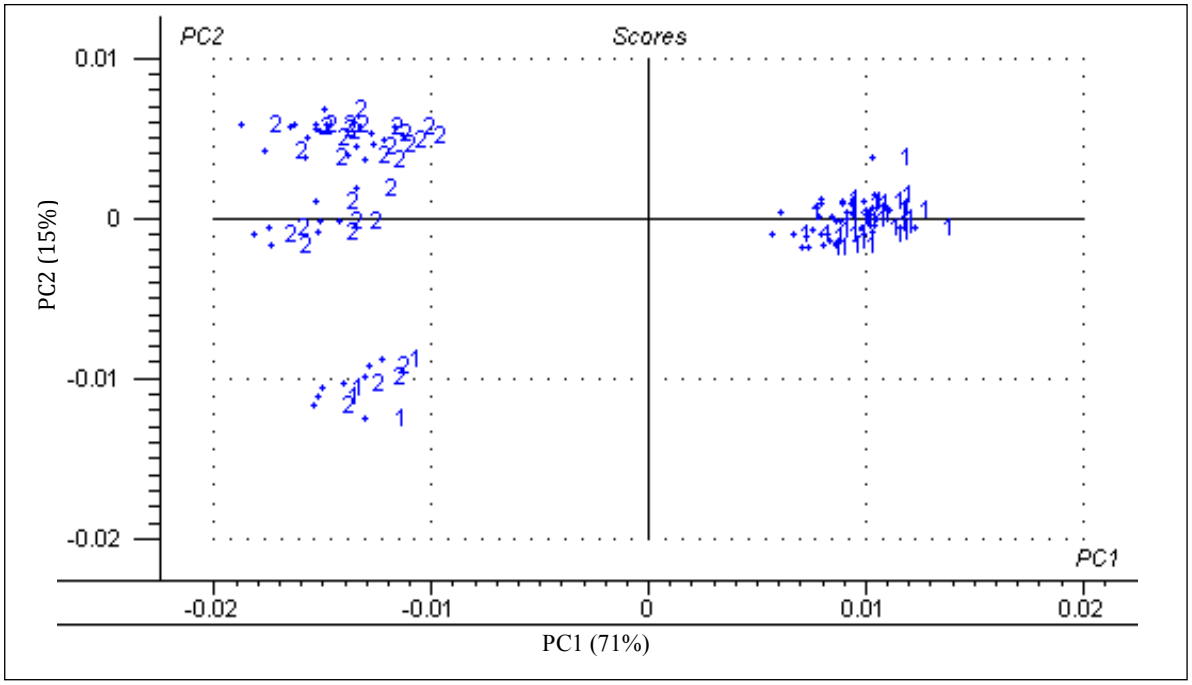

Fig. 4: Principal component (PC) analysis (PCA) score plot showing the ability of spectra acquired before storage, to sort fruit based on their origin within the tree canopy. 1 and 2 represent fruit from outside and inside the canopy, respectively.

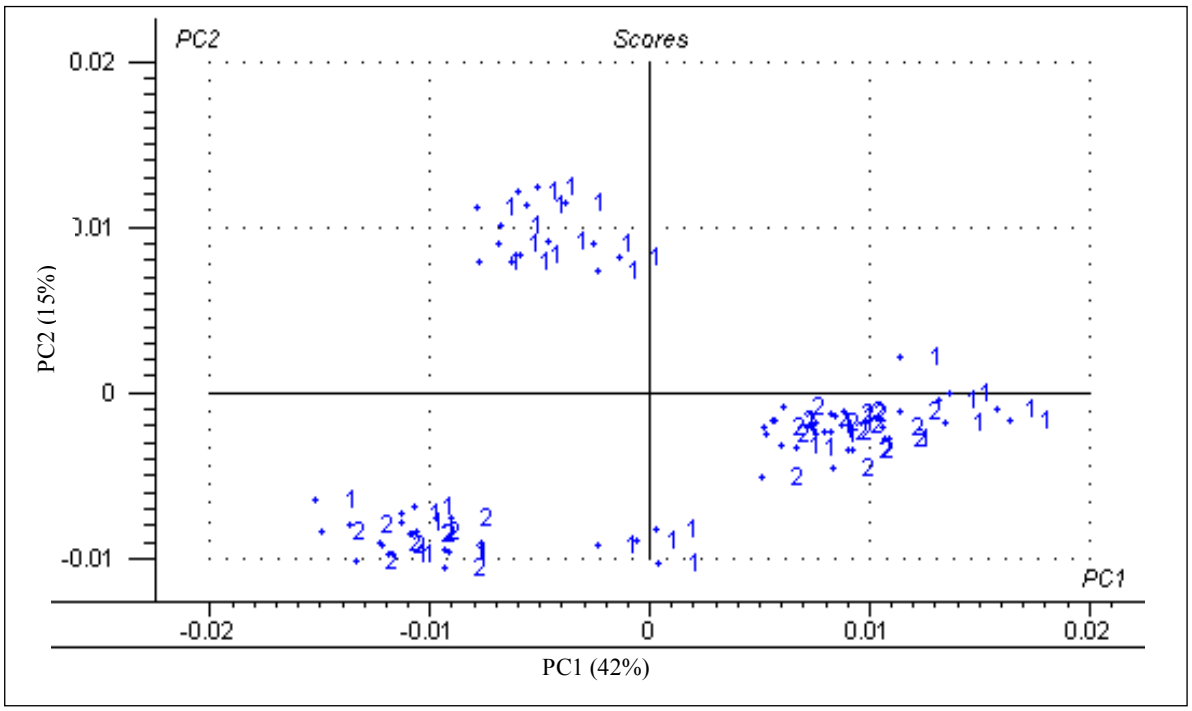

Fig. 5: Principal component (PC) analysis (PCA) score plot showing the ability of spectra acquired after eight weeks of storage, to sort fruit based on their origin within the tree canopy. 1 and 2 represent fruit from outside and inside the canopy, respectively.

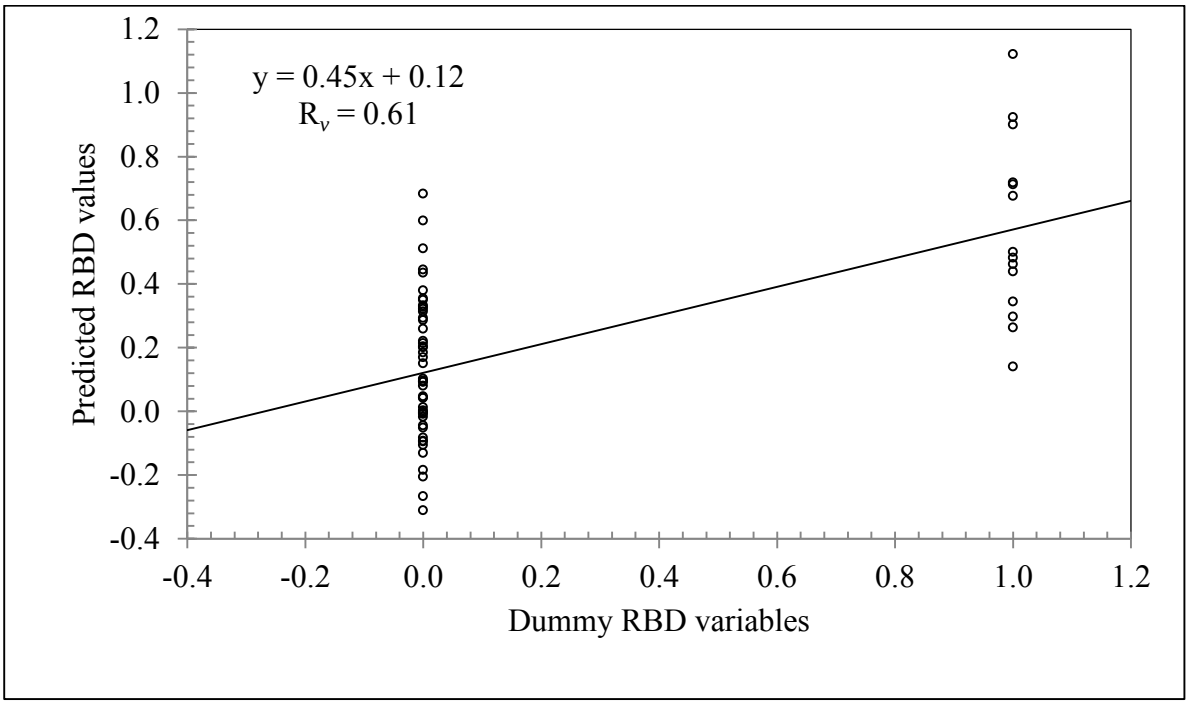

Fig. 6: Partial least squares (PLS) scatter plot showing visible and near infrared (Vis/NIR) model performance to classify fruit based on the occurrence of rind breakdown (RBD). 0 and 1 are fruit with and without RBD, respectively.

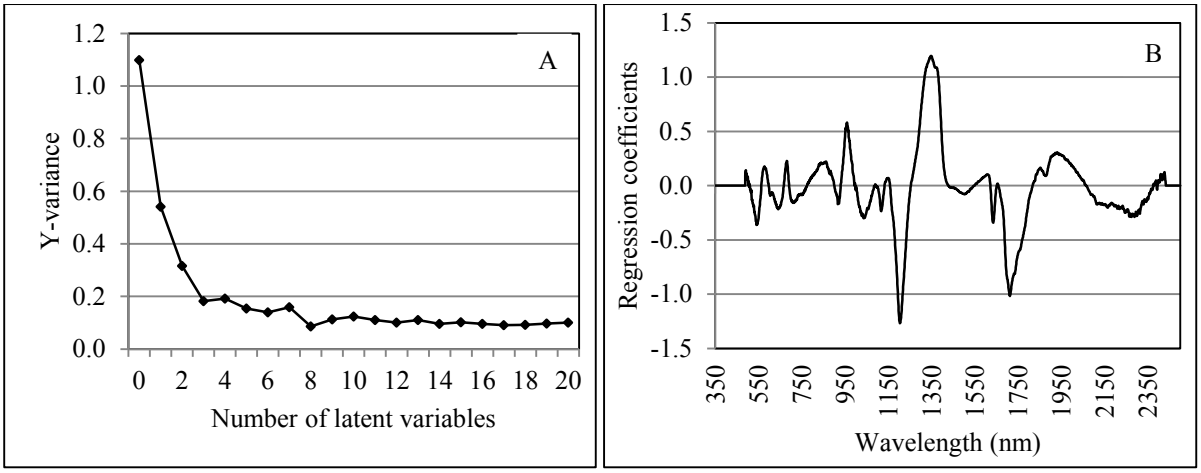

Fig. 7: Residual y-variance as a function of the number of latent variables (LVs) in the dry matter (DM) model (A), and regression coefficients curve of the DM model of intact 'Nules Clementine' mandarin fruit with eight LVs and NIR spectral range of 450–2450 nm (B) based on multiple scatter correction spectral pre-processing.

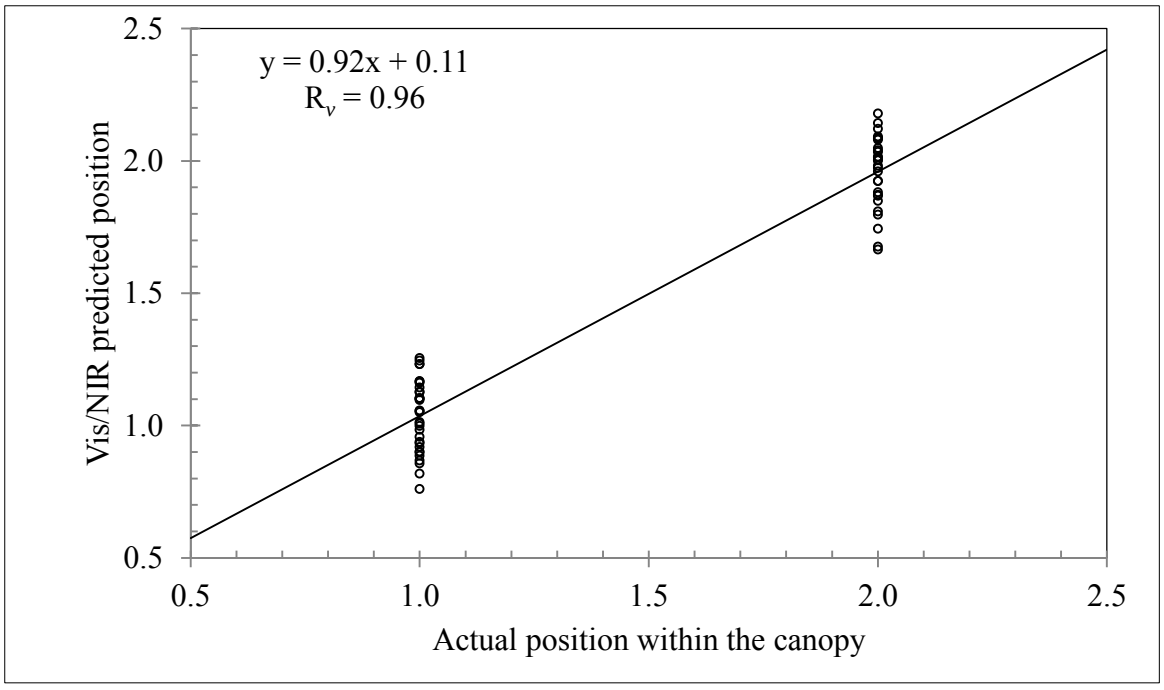

Fig. 8: PLS-DA models showing Vis/NIR spectral ability to predict fruit origin within the tree canopy. Spectra for this model were acquired before storage. 1 and 2 are fruit with and without RBD, respectively.

CHAPTER 7

RESEARCH RESULTS

PAPER 6:

ROBUST VIS/NIRS MODELS FOR THE PREDICTION OF RIND QUALITY IN 'NULES CLEMENTINE' MANDARIN FRUIT DURING POSTHARVEST STORAGE*

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ROBUST VIS/NIRS MODELS FOR THE PREDICTION OF RIND QUALITY IN 'NULES CLEMENTINE' MANDARIN FRUIT DURING POSTHARVEST STORAGE

Abstract

The robustness of visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS) models is a crucial requirement for assessment of fruit quality parameters. This research was conducted to examine the relationships between fruit canopy position, limited light by fruit bagging, rind biochemical composition and susceptibility of 'Nules Clementine' mandarin to rind breakdown disorder (RBD) during storage. The second aim was to validate Vis/NIRS models used for the assessment of rind physico-chemical properties of intact 'Nules Clementine' mandarins based on independent validation approach. The thrid aim of this study was investigate the robustness of partial least squares (PLS) calibration models developed with data from individual orchard locations with those developed from combined orchard locations and two seasons in predicting postharvest rind physico-chemical properties related to RBD. Vis/NIRS signals of fruit from four orchards in the Western Cape Province in South Africa (Citrusdal, Paarl, Porterville and Stellenbosch) were acquired on freshly harvested fruit using a LabSpec® spectrometer. Reference physico-chemical properties were measured after 8 weeks of storage at 8±0.5°C, including RBD, rind hue angle ($h°$), rind dry matter (DM), and the concentration non-structural carbohydrates (sucrose, glucose, fructose, and total carbohydrates). In Porterville, Paarl, and Stellenbosch, bagged fruit inside the canopy had significantly ($p<0.001$) higher incidence of RBD compared to sun-exposed outside fruit. Concomitant to this, bagged fruit from inside the canopy in Porterville, Citrusdal, and Stellenbosch had higher percentage of mass loss. In all four orchards, bagged inside the canopy had lower dry matter (DM) content in the rind tissue, suggesting involvement of the parameters in fruit susceptibility to RBD. Inside bagged fruit harvested from Porterville had higher concentration of non-structural carbohydrates (fructose, sucrose, and total sugars) than outside fruit. Similarly, in Stellenbosch, fruit from the outside position had lower rind sucrose (95.3 mg/g DM) compared to inside bagged fruit (114.9 mg/g DM) but higher glucose concentration (92.0 mg/g DM) compared to inside fruit (66.9 mg/g DM). Principal component analysis (PCA) of Vis/NIRS signals was executed to separate fruit according to canopy position. The projection of the samples in the PCA score plots displayed clusters that allowed the distinction between fruit from different preharvest treatments. Clear PCA

clustering based on canopy position of fruit was obtained. PLS regression with leave-one-out full cross validation was used to develop calibration models of studied parameters. The models were externally validated using data from a different location or season not included in the calibration. Prediction performance of PLS models of a single orchard location validated with data of an independent location was low but encouraging, with residual predictive deviation (RPD) values ranging from 0.95 to 1.58 for fructose models. The fructose calibration models developed using two combined orchard locations had higher prediction accuracy (RPD ranging between 1.32 and 1.97) than models of one orchard location. The performance of models developed from three orchard location in 2012 to predict parameters of 2011 was better (RPD for fructose model = 2.50) than models developed from individual orchards and two combined orchards. The results from this study demonstrated that Vis/NIRS models offer a considerable robustness for non-invasive prediction of rind quality attributes which might predispose 'Nules Clementine' mandarin fruit to RBD. The ability of PCA to discriminate between inside and outside fruit using Vis/NIRS signal suggests the potential of this technology to discriminate fruit based on their susceptibility to RBD.

Keywords: Nonstructural carbohydrates · Postharvest physiological disorder · Rind breakdown · Citrus.

1. Introduction

The marketability of citrus fruit, for fresh consumption, is highly dependent on external appearance and rind quality (Khalid et al., 2012). Physiological rind disorders or defects are important factors affecting external quality of citrus fruit and are the major causes for economic losses to the industry worldwide (Alférez et al., 2003; Porat et al., 2004; Cajuste and Lafuente, 2007). A postharvest physiological rind disorder of 'Nules Clementine' mandarin, commonly referred to as rind breakdown (RBD) is among several commercially important defects affecting the citrus industry (Cronje et al., 2007).

In earlier studies conducted in South Africa, it has been demonstrated that the sensitivity of fruit to development of RBD was influenced by different microclimates (Cronje et al., 2011a, b; Magwaza et al., 2012a, b). Fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affected the concentrations of carbohydrates and mineral elements in the rind during fruit development. Cronje et al. (2011b) concluded that the reduction of transpiration potential by lower temperatures and higher humidity inside the canopy could be responsible for the reduced accumulation of carbohydrates. It was also hypothesised that limited photosynthetically active radiation on shaded portions of the canopy reduced fruit photosynthesis rate and osmotic potential, which in turn reduce rind condition and increase susceptibility to RBD (Cronje et al., 2011a, b; Magwaza et al., 2012b; 2013a). Although higher incidence of RBD on inside fruit compared with the outside fruit was consistent for four consecutive seasons, it should be noted that all these studies were conducted in one citrus growing region of South Africa. Therefore, the proposed hypothesis for the mechanism underlying the development of RBD cannot be generalised for all growing regions. This has prompted an extension of previous investigations to include other regions, as part of testing the hypothesis describing the effect high or low light on the sensitivity of 'Nules Clementine' mandarin fruit to RBD.

One of the main difficulties related to RBD is that the disorder, characteristically, does not manifest during harvest or fruit grading in the packing house, but develops about three to five weeks after harvest (van Rensburg and Bruwer, 2000; van Rensburg et al., 2004; Cronje et al., 2011a, b; Magwaza et al., 2012b; 2013a, 2013b). This delayed phenomenon is in essence the difficulty that faces the industry. The challenge is therefore to develop technology to evaluate rind quality on the packing line, in order to classify individual fruit for either local or export market, as losses in the postharvest chain can result in a significant reduction of income.

Visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS) has become one of the mostly used methods for a non-destructive evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Miyamoto and Kitano, 1995; Nicolaï et al., 2007; Cozzolino et al., 2010; Magwaza et al., 2012c; Wedding et al., 2013). The feasibility of Vis/NIRS to examine physico-chemical properties in relation to quality of citrus fruit was discussed in a review by Magwaza et al. (2012a, Chapter 3). Vis/NIRS depends on chemometrics which involves multivariate analysis for interpreting large data sets (Wetzel, 1983; Wang and Paliwal, 2007). Multivariate statistical techniques such as partial least squares (PLS) regression and principal component (PC) analysis (PCA) are applied to extract the required information about quality attributes from the Vis/NIR spectrum (Cozzolino et al., 2011). The foregoing literature evidence demonstrates that PLS is probably the most widely applied method in chemometrics today (Gómez et al., 2006; Lorente et al., 2013). However, very few studies on Vis/NIRS involved testing citrus PLS calibration models of fruits from independent populations such as, different harvest dates, seasons, localities, temperatures, and cultivars. In most studies, full cross validation was considered to be adequate to demonstrate that Vis/NIRS can be used to non-destructively evaluate quality aspects of interest. However, in practical and commercial in-line application, the actual accuracy of the model must be estimated with an appropriate test set or independent validation data set (Miyamoto and Kitano, 1995; Ou et al., 1997; Guthrie et al., 2005; Cozzolino et al., 2011). The stability of the model when tested using unknown samples and external factors can demonstrate its robustness.

Robustness of calibration models has become a critical issue in Vis/NIRS and an active area of research (Nicolaï et al., 2007; Bobelyn et al., 2010). The application of Vis/NIRS in horticulture requires an assessment of the robustness of the developed calibration model across populations of fruit grown under differing conditions (Guthrie et al., 2005). Therefore, the validity of the calibration models for future predictions depends on how well the calibration set represents the composition of future samples (Peirs et al., 2003; Wedding et al., 2013). Validation of a model on a population independent of that used in calibration effectively tests for over-fitting of the model and makes Vis/NIRS models suitable for in-line application (Guthrie et al., 1998; Guthrie et al., 2005). For a Vis/NIRS model to be regarded as valid and robust, its performance should be accurate, stable across seasons, for a given variety, and relatively insensitive towards unknown changes in external factors (Miyamoto and Kitano, 1995; Peiris et al., 1999; Nicolaï et al., 2007; Cozzolino et al., 2011).

Differing growing conditions such as within tree, orchard, geographical and seasonal variability may result in differences in physical and chemical properties of the fruit (Barry et al., 2003; Cronje et al., 2011a, b; Khalid et al., 2012). This change in fruit physico-chemical properties result in altered fruit optical characteristics and spectral band assignment (Golic et al., 2003; Guthrie et al., 2005). As a result of these variations in horticultural products, region and seasonal variation has been considered important to include in Vis/NIRS experimental design because different growing conditions in different places and seasons affect fruit analyte levels with concomitant effects on model predictions (McGlone et al., 2002).

Recent studies demonstrated the feasibility of Vis/NIRS to non-destructively predict rind biochemical profile of 'Nules Clementine' mandarins after eight weeks of postharvest storage (Magwaza et al., 2012b, c). However, the PLS models developed from these studies were validated using fruit from the same orchard. According to Louw and Theron (2010), the best way to develop robust Vis/NIRS models for biological products is to acquire calibration data over a sufficient period of time to obtain an appropriate range of variation in the population due to environmental conditions across seasons. The ability of Vis/NIRS to evaluate rind quality prompted the necessity for further analysis of these parameters on samples from other orchard locations, to evaluate the influence of between- and within-orchard variability on PLS model performance.

Therefore, the aim of this study was threefold, first, to examine the relationships between fruit canopy position, bagging treatments and growing locations on rind biochemical composition and susceptibility of 'Nules Clementine' mandarin to RBD during storage. The second aim was to validate Vis/NIRS models used for the assessment of rind physico-chemical properties of intact 'Nules Clementine' mandarins based on independent validation approach. The third aim was to demonstrate robustness of calibration models across two seasons and four orchard locations in order to predict postharvest changes of parameters related to rind quality as well as fruit susceptibility to RBD.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Carbohydrates standards (sucrose, D-glucose and D-fructose) were purchased from Sigma Aldrich (Dorset, UK). Extraction solvent was methanol of high performance liquid chromatography (HPLC) grade purchased from Fisher

Scientific Chemicals (Leics., UK). Solutions and solvents were prepared with Milli-Q water (Milipore Inc.; $\sigma = 18$ M Ω cm^{-1}).

2.2. Site, fruit sampling and postharvest handling

During 2011, 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) were harvested from an orchard at Stellenbosch University experimental farm (33°53'4.56"S, 18°37'36.84"E) in order to develop initial calibration models to evaluate the potential of Vis/NIRS for the assessment of rind physico-chemical properties (Magwaza et al., 2012b). To add seasonal and orchard variation into the PLS models, fruit of the same cultivar were sampled in 2012 season from three commercial orchards in different growing locations of the Western Cape Province, South Africa. These experimental sites were located in Paarl (33°43'27.44"S; 18°57'21.28"E), Porterville (33°01'00"S, 18°58'59"E) and Citrusdal (32°35'18.26"S, 19°1'14.69"E). The chronological experimental design and distribution of samples during model development and validation is illustrated in Fig. 1. In all orchards over the two seasons, 15 uniform trees were identified and marked for sampling. On each tree, six fruit of uniform size from the sun-exposed (outside) and six from shaded canopy position (inside), were randomly selected and tagged in a zone between 1 to 2 m from the ground, four months before commercial maturity. Half of the selected fruit (3 fruit) from each of the canopy positions was covered with brown paper bags without removing or covering subtending leaves (Cronje, 2009), about four months before harvest, as previously discussed by Magwaza et al. (2012b). The study therefore consisted of four preharvest treatments, i.e. outside unbagged, outside bagged, inside unbagged, and inside bagged.

In both seasons, fruit were harvested at commercial maturity (16-18 May) and sorted at the Postharvest Research Laboratory at Stellenbosch University to remove any defective fruit. These fruit were weighed and received postharvest treatments according to industry practices (except degreening) as previously described (Cronje et al., 2011b; Magwaza et al., 2012b). In 2011, 120 blemish free fruit (30 fruit from each treatment) were selected for non-destructive evaluation and these same samples were analysed by destructive methods. During the 2012 season, a sample of 100 tagged fruit (25 from each preharvest treatment) without physical defects was harvested from each of the three experimental sites, following the same postharvest handling procedure used in 2011. In both seasons, fruit were separately packed in boxes after phytosanitary inspection and certification, marked, and sent at ambient temperature by air freight to Cranfield University (CU) in the United Kingdom, where

measurements and analysis of fruit were conducted. Fruit arrived at CU after 48 hours and were stored for 24 hours in an air-conditioned laboratory at 20°C to equilibrate, prior to taking the first Vis/NIRS measurements. Therefore, Vis/NIRS spectra were acquired within less than several days after harvest.

After Vis/NIRS scanning, fruits were stored for 8 weeks at 8±0.5°C, a temperature which is known to cause the highest degree of RBD incidence (Khumalo, 2006). During eight weeks in storage, fruit were scored weekly, for the incidence of RBD. RBD was scored by visual inspection on a subjective scale from no (0), little (1), moderate (2), and severe (3) rind breakdown.

2.3. Spectral acquisition

Vis/NIRS signals of intact mandarin fruit were acquired based on a method described by Magwaza et al. (2012a, b), using a mobile fibre-optic Vis/NIRS spectrophotometer (350-2500 nm, LabSpec2500® Near Infrared Analyser, Analytical Spectral Devices Inc., USA) in diffuse reflectance mode equipped with one Si array (350-1000 nm) and two Peltier cooled InGaAs detectors (1000-1800 nm and 1800-2500 nm). The sampling interval of the instrument was 1 nm and the spectral resolution was 3 nm at 700 nm and 10 nm at 1400 nm and 2100 nm. The integration time was less than 500 ms per spectrum collected. A high intensity probe with an in-built light source (quartz-halogen bulb of 3000 K) and detection fibre at 35° angle to the light source was used (Kuang and Mouazen, 2011). The fruit were placed in direct contact with the high intensity probe.

The Vis/NIRS system was calibrated with a 100% reflection white tile to provide background reference spectrum prior to scanning the fruit and periodically at 30 min intervals. A total of 10 scans were obtained from exactly the same position on the fruit and were automatically averaged to obtain a single spectrum. Reflectance spectra were acquired from eight positions on the fruit; four from equatorial spots, two from the stem-end and two from the stylar-end of the fruit. The first set of Vis/NIRS signals was acquired before storage and the second set was acquired from the same fruit (and the same positions on the fruit) after eight weeks of cold storage (Magwaza et al., 2013). In the current study, we are reporting models developed using Vis/NIRS spectra acquired before storage.

2.4. Physico-chemical measurements

To fulfil the aim of predicting postharvest behaviour, reference measurements such as RBD, colour parameters such as lightness (L*), chroma (C*) and hue angle (*h*°), dry matter content and non-structural carbohydrates (sucrose, glucose and fructose) of the rind were obtained after 8 weeks of postharvest storage at 8±0.5°C using methods described below.

2.4.1. Mass and rind colour

Individual fruit mass (g) was measured using a calibrated and sensitive balance (±0.5 g, Mettler Toledo, ML3002E / 01, Switzerland). Rind colour components of individual fruit were measured objectively in three values, the CIE L*, C* and hue angle (*h*°) space using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203) (Terry et al., 2007; Pathare et al., 2013). An average of three readings at three equidistant points around the equatorial axis of the fruits was automatically calculated.

2.4.2. Sample preparation

After 8 weeks of storage, the rinds (flavedo and albedo) were peeled, snap frozen in liquid nitrogen and stored in a -40°C freezer. Frozen samples were freeze-dried in a Labogene ScanVac CoolSafe Freeze Dryer System (CS55-4, Lynge, Denmark) for 7 days at 0.015 kPa and -55°C. Dried samples were weighed and dry matter (DM) content was calculated from freeze dried samples and expressed as a percentage of fresh mass. These samples were ground into fine powder using a pestle and mortar and returned into the freezer until extraction for further analysis of non-structural carbohydrates.

2.4.3. Extraction and HPLC quantification of non-structural carbohydrates

Extraction and determination of non-structural carbohydrates were executed using a method described elsewhere (Crespo et al, 2010; Magwaza et al., 2012a, b). Briefly, non-structural carbohydrates were extracted from 150 ± 0.5 mg of dried rind powder using 62.5% (v/v) aqueous methanol, stirred for 30 min, filtered through a 0.2 µm syringe filter and placed in a vial for HPLC analysis. Concentrations of fructose, glucose and sucrose were determined using a HPLC binary pump system (Agilent Technologies, UK), equipped with a refractive index detector (RID, G1362A), based on that previously described (Terry et al., 2007).

Sample extracts were diluted in HPLC grade water (1:10), and injected into a Rezex RCM monosaccharide Ca^{+} (8%) column of 7.8 mm diameter x 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} guard column of 3 mm x 4mm x (Phenomenex). The column temperature was set at 80°C using a thermostated column compartment (G1316A, Agilent). The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min. The presence and concentration of the selected non-structural carbohydrates were calculated by comparing peak area of samples against peak area of known standard concentrations (0.05-1.25 mg/mL; average $R^2 = 0.991$).

2.5. Data analysis

2.5.1. Statistical analysis

Statistical analyses of fruit physico-chemical properties were carried out using statistical software (STATISTICA, Vers. 11.0, StatSoft Inc. USA). Data was subjected to analysis of variance (ANOVA) and means were separated by least significant difference (LSD; $p = 0.01$) using Duncan's multiple-range tests. Two-way ANOVA was perfomed to determine significant differences in all measured quality parameters. The statistical analysis was used to identify the main effects and interactions between the preharvest canopy position treatments and growing location. Pareto analysis at 95% confidence interval was carried out to assess the effects of canopy position and growing location (each at four levels), and the interaction between the two factors on RBD susceptibility (Mahajan et al., 2008; Caleb et al., 2012).

2.5.2. Chemometrics, calibration and validation procedures

From previous studies (Magwaza et al., 2012b, c; 2013), it was established that for all measured variables, performance of PLS models based on individual spectra from any one of the eight positions within the fruit were poor compared to the results obtained average spectrum of eight spectra from each fruit. Therefore, the eight-fruit reflectance spectra in Indico format (Indico Pro 5.6 software, Analytical Spectral Devices Inc., CO, USA) were averaged in one spectrum prior to PCA and PLSR using Unscrambler 9.8 (Camo Softaware, AS, Norway). Due to technical performance associated with the LabSpec2500® Near Infrared Analyser, considerable noise observed from 350 to 450 nm and 2401 to 2500 nm were removed from the spectra before the calibration exercise. Thus, all Vis/NIRS analysis

was based on 451 – 2400 nm. PCA was performed to identify the combination of latent variables (LVs) that contributed most to the model, detect spectral outliers, and to discriminate between fruit based on biological variations due to canopy position, orchard location, production season, and how these characteristics change during storage. Before the calibration, the spectra were subjected to several pre-processing methods including Savitzky–Golay first and second derivatives (second order polynomial) and multiple scatter correction (MSC). Each pre-processing method was first applied individually and later tested in combination with others. After spectral pre-treatment, the best model performances for all measured rind attributes were obtained using MSC except for colour models which performed better without spectral pre-processing.

Calibrations were developed using PLS regression and full leave-one-out cross validation (internal validation). The criteria for testing performance of developed models was based on the root mean square error of cross validation (RMSECV), root mean square error of validation or prediction (RMSEP), the correlation coefficient (R), which represents the proportion of explained variance of the response variable in the calibration (R_{cv}) or validation dataset (R_p), the number of LVs, and the residual predictive deviation (RPD) (Cayuela and Weiland, 2010; Bobelyn et al., 2010; Jamshidi et al., 2012). RPD is described by Williams and Sobering (1996) as the ratio of the standard deviation of the reference data for the validation set to the RMSEP. The ideal model should have high R and RPD values, as well as low RMSECV and RMSEP values and a small difference between the last two statistical parameters. A large difference between RMSEC and RMSEP indicates the possibility that too many LVs were used in the model (overfitting) and noise is modelled (Gómez et al., 2006). The optimal number of LVs was determined as the minimum number of LVs corresponding to the first lowest value from the RMSEC or RMSEP plot for increasing number of LVs (Davey et al., 2009).

The robustness of the models was investigated by looking at the two major sources of variation generally accepted in horticultural produce which are seasonality and orchard location. The sequence of model development and calibration procedures is graphically illustrated in Fig. 1. To test the effect of orchard location, calibration models were developed using data sets from one orchard and validating them externally using the data from another orchard locations, individually. The second step in the investigation of orchard location effect on model performance, PLS calibration models were using two combined locations and validated with an independent orchard. In order to evaluate the effect of the season on model performance, the models developed with combined data set encompassing all three locations

in 2012 season were used to predict parameters from 2011 season. Due to the reason of fruit availability, these models were not based on a full factorial design and therefore there is an overlap between season and origin (i.e. 2011 and Stellenbosch refer to the same data set). To develop robust models for all attributes, 60% of fruit from all locations and seasons were assigned to calibration set and the remaining 40% were used in the validation sets.

In an attempt to develop an RBD susceptibility classification model, samples were assigned to binary dummy variable, which indicated whether the sample belongs to particular RBD group or not. RBD affected fruit were set as reference data 1 while unaffected fruit were assigned to 0 (Teerachaichayut et al., 2011). These values were subjected to PLS variant known as partial least squares discriminant analysis (PLS-DA) in order to evaluate the ability of obtained spectra to classify fruit with different susceptibility to RBD.

3. Results and discussion

3.1. Canopy Position and Bagging Effect on Fruit Physico-chemical Properties

3.1.1. Rind breakdown disorder

Symptoms of the disorder were visible on affected fruit after two weeks of postharvest storage. The effects of preharvest canopy positions and orchard locations, as well as their interaction, on the development of RBD are summarised in a surface plot (Fig. 2) and Pareto chart (Fig. 3). Both figures showed that the interaction between orchard location and preharvest canopy position treatments as well the main effect of orchard location were not significant, indicating that, regardless of orchard location, preharvest canopy position had a significant influence on RBD (p<0.001). Inside fruit were more susceptible to the disorder compared to other preharvest treatments, while bagging of inside fruit resulted in the highest RBD incidence. Compared to disorders such as rind staining in 'Fortune' mandarins (Almela et al. 1992; Duarte and Guardiola 1995) and rind breakdown in 'Navel' oranges (Agustí et al. 2001) where peel disorders were associated with sun/heat exposure, RBD in 'Nules Clementine' occurred on inside fruit because the disorder is associated with low mineral and carbohydrate allocation and therefore premature senescence. These results are similar to those reported by Cronje et al. (2011a) and Magwaza et al. (2012b), corroborating the hypothesis that canopy microclimate affect rind development via rind photosynthesis and mineral

nutrient accumulation (Cronje et al., 2011b) and could contribute significantly to determine the rind condition.

3.1.2. Physical profile of fruit from different canopy positions

An overview of physical properties of fruit from different orchard locations, canopy positions and bagging treatments is presented in Table 1. Variance analysis carried out for rind hue angle, dry matter content and mass loss showed significant interactions between canopy position and orchard location. However, it should also be noted that the effects of individual main factors were also significant for all three parameters. Therefore, on interpreting variable response due to individual main effects, it is important to consider the effects of interaction. Similar colour associated features were observed by Cronje et al. (2011b), Khalid et al. (2012) and Magwaza et al. (2012b), whereby fruit located in the inside position of the canopy were yellower in appearance with higher hue angle values ranging from 74.4 –90.2° compared to more orange (67.2 to 82.8°) colour of outside fruit. The yellower rind colour of inside fruit at harvest indicates a reduced concentration of carotenoids (Cronje et al., 2011a). In 'Satsuma' mandarins, it was also observed that bagging fruit with brown paper bags inhibited chlorophyll degradation and reduced carotenoid formation in the rind resulting in pale yellow fruit at harvest (Hiratsuka et al., 2012).

In the current study, for both canopy position and orchard location, bagged fruit had high postharvest mass loss, which could be due to reduced natural wax formation resulting in high water loss by transpiration, as this accounts for 90% of total mass loss (Ben-Yehoshua et al., 1983; 2001). Changes in the water status of the flavedo and albedo has been shown to be one of the primary postharvest factors contributing to the susceptibility of citrus to rind non-chilling physiological disorders (Alférez et al., 2003; 2010). In this study, bagged fruit from inside the canopy, with higher percentage of mass loss were more susceptible to RBD. Fruit from outside the canopy had significantly (p<0.05) higher dry matter (30 to 35%) than inside fruit (27 to 30%) (Table 1), demonstrating a close relationship between sunlight intensity within the tree canopy and the levels of accumulated structural and non-structural carbohydrates. Fruit bagging has been reported to raise relative humidity (RH) and temperature surrounding the fruit (Cronje et al., 2011b). Higher RH inside the bag could have weakened fruit sink strength as a result of reduced transpiration in (Hiratsuka et al., 2012). Furthermore, increased RH possibly increased water content in the rind of bagged fruit explaining reduced dry matter. A similar phenomenon has been observed in kiwi fruit, where

radiation interception reduced carbohydrates and dry matter accumulation (Tombesi et al., 1993). In 'Valencia' oranges Bean and Todd (1960) and Yen and Koch (1990) demonstrated that more than 98% of CO_2 fixed by fruit photosynthesis is distributed into rind tissues and less than 2% into the pulp. From the results presented in the current study, it could be argued that radiation interception by the fruit itself may also have played an important role in rind condition, since shading is known to inhibit fruit photosynthetic ability and weaken sink strength of mandarin fruit (Yen and Koch, 1990; Cronje et al., 2011b). The results from this study confirm a previously formulated hypothesis stating that reduced light intensity around an individual fruit reduces rind condition, which increases the susceptibility to RBD (Cronje et al., 2011b).

3.1.3. Non-structural carbohydrates profile of fruit from different canopy positions

The three main non-structural carbohydrate evaluated were sucrose, glucose and fructose. In the rinds of 'Nules Clementine' mandarin fruit, fructose was the most abundant carbohydrate, ranging from 78.5 to 130.2 mg/g DM. Sucrose concentration ranged from 30.8 to 144.9 mg/g DM, while glucose ranged between 52.6 and 92.0 mg/g DM (Table 2). The concentrations of these carbohydrates were close to those previously reported in the rinds of 'Marsh' grapefruit, 'Valencia' and oranges (Ting and Deszyck, 1961; Rosales and Burns, 2011). In this study, the interaction between preharvest canopy position and orchard location, significantly influenced the concentration of non-structural carbohydrates, but variability due to main effects was also noted. Reduced radiation by bagging has previously been reported to negatively affect non-structural carbohydrates concentration of citrus fruit (Hiratsuka et al., 2012).

Results in Table 2 showed the opposite trend in that shaded and bagged fruit harvested from Porterville had higher concentration of fructose, sucrose, and total sugars than sun-exposed fruit. Sucrose of bagged inside fruit from Stellenbosch followed a similar pattern, showing the higher (114.9 mg/g DM) concentration compared to outside fruit (95.3 mg/g DM). The concentration of non-structural carbohydrates in the rind has been reported to be implicated in the development of RBD in 'Nules Clementine' mandarins (Cronje et al., 2011a; Magwaza et al., 2012b). Concomitant to this, results of the current study also indicated that in these three orchard locations, shaded fruit containing higher non-structural carbohydrates, and more susceptible to RBD during storage, suggesting that the carbohydrate content was not a limiting factor in the susceptibility of 'Nules Clementine' mandarins to

RBD in these orchards. Another possibility worth considering is that stressed fruit from inside the canopy could have produced carbohydrates in the rind during storage. Previously reported results on 'Valencia' oranges have demonstrated that synthesis of carbohydrates from organic acids is possible (Echeverria and Valich, 1989). Based on this evidence and the assumption that bagged fruit from inside the canopy had 50% higher citric acid concentration (as observed in our previous study, unpublished), production of carbohydrates from acids may be a possible mechanism involved in the higher levels of non-structural carbohydrates in shaded inside fruit.

Lower osmotic potential due to higher rind sucrose, an osmoregulatory compound in plant cells (Yakushiji et al., 1996; Huang et al., 2000), has previously been reported, demonstrating a possible link between fruit position, rind carbohydrates content and ultimately development of RBD (Cronje et al. 2011a). The high non-structural carbohydrates concentrations in the rind of inside fruit is possibly a stress response, triggered by low light levels, to maintain osmotic potential in the shaded rind tissue. This imbalance could possibly lead to a reduction in rind condition, which manifests as RBD development. The lower concentration of total sugars in the rind of sun-exposed fruit in Porterville and Paarl might also indicate that most of the carbohydrates were converted into higher dry matter of these fruit, shown in Table 1. Different carbohydrates responses to reduced sunlight indicated that carbohydrates might not be a universal biochemical marker or the primary cause for RBD, since it is highly dependent on climatic conditions and other environmental factors during growth and development as well as during postharvest storage.

In fruit harvested from the Citrusdal orchard, results similar to those reported by Cronje et al. (2011a) and Hiratsuka et al. (2012) were observed, lower non-structural carbohydrates concentrations in rind tissue of shaded fruit compared to sun-exposed fruit. Considering that the increase in carbohydrates has been reported to play a protective role in plants against stress conditions (Purvis and Grieson, 1982), postharvest stress conditions during senescence may have induced an accumulation of non-structural carbohydrates in the rind of 'Nules Clementine' mandarin. These results are not unique to citrus fruit. Similar observations were recorded in apples where sun-exposed outside fruit had higher soluble carbohydrates concentration than inside fruit (Nilsson and Gustavsson, 2007), although rind architecture is different. Previous reports have shown that sucrose does not change in the flavedo of fruit attached to the plant during extreme stress conditions but fructose and glucose increased (Holland et al., 1999). Furthermore, sucrose in citrus does not interchange between fruit different tissues of citrus fruit, but is translocated from other parts of the plant (Purvis and

Yelenosky, 1983). Therefore, the decreased total sugar concentration in mandarin rinds from outside positions in locations such as Porterville and Paarl, could be explained by the consumption of carbohydrates reserves for maintenance respiration during growth and conversion into structural dry matter (Holland et al., 1999). The rind of outside fruit, which were exposed to the sun, utilises more carbohydrates for respiration as the fruit is subjected to preharvest stresses.

3.1.4. Laboratory reference based PCA output

In order to gain a better insight of how measured physico-chemical properties correlate with each other and with RBD, laboratory measurements data were subjected to PCA. The total variability was explained by the first two principal components (PCs), with PC1 and PC2 accounting for 44% and 14% of the variation in the data, respectively (Fig. 4). PCA showed that the ratio of non-reducing (sucrose) to reducing (glucose + fructose) carbohydrates (Suc:Fru+Glu), mass loss and canopy position were positively correlated with RBD, confirming significant contribution to the development of the disorder. Supporting this, Suc:Fru+Glu ratios in Table 2 were lower in fruit exposed to sunlight and higher on inside fruit, except for bagged fruit borne inside the canopy of the Porterville orchard where it was lower. The direction of the vector of DM was opposite from that of RBD indicating a negative relationship, as was also shown in Table 1. Although all non-structural carbohydrates were located in the same plane as RBD, the correlation was not very strong. This observation and those shown in Table 2 suggest that rind non-structural carbohydrates may not be a primary indicator of fruit susceptibility to the disorder as DM, water loss and canopy position would. However, non-structural carbohydrates and the ratio of sucrose to the sum of glucose and fructose can still be used as a secondary biochemical marker of fruit sensitivity to the disorder. Previous studies have demonstrated that Vis/NIRS has significantly greater accuracy for determining sugars than other quality parameters in citrus fruit (Gòmez et al., 2006). The ability of Vis/NIRS to predict rind non-structural carbohydrates as a possible biochemical indicator of fruit susceptibility to RBD was therefore explored.

3.2. Vis/NIR spectroscopy

3.2.1. Vis/NIRS based PCA

PCA scores and loadings for the first two PCs (Fig. 5) display sample projection using spectra acquired before storage and after eight weeks postharvest. Clear separation was observed demonstrating the effect of storage on spectral and physico-chemical properties. The first two PCs explained 78% (PC1 = 59%, PC2 = 18%) of the total variance. Separation in Fig. 5A clearly showed that the storage effect on fruit spectral and physico-chemical properties was mostly along the first PC, which contributed most to the sample distribution. Interestingly, samples were stretched along PC1 before storage and clustered in a small area after postharvest storage. The loss of variability on samples after storage could be attributed to rind biochemical changes associated with fruit senescence, such as decrease in carotenoids content, decrease in chlorophyll (Cronje et al., 2011a) and increase in carbohydrates concentration (Magwaza et al., 2013c).

Examining the PCA loadings plot in Fig. 5B revealed a significant wave band contributing the visible range at 670 nm, which is attributed to strong chlorophyll absorption. Two main absorption peaks in the near infrared range at 980 and 1200 nm, correspond to water (O-H) functional groups and C-H stretching for carbohydrates, respectively (Golic et al., 2003; Tewari et al., 2008). Strong absorption in these bands suggests that the combination of colour, carbohydrates and moisture content of the rind played an important role in discriminating fruit from different positions of the canopy. As previously reported by Magwaza et al. (2012b), PCA models developed using spectra restricted to either the visible range or to the NIR range did not show these clusters, demonstrating the importance of the combination of visible and NIR ranges in the classification of fruit by their canopy position. In ‘Nules Clementine’ mandarins, rind colour of harvested fruit has been previously reported to improve during storage (Cronje et al., 2011a). The loss of sample variation observed after postharvest storage could be attributed to colour change and other biochemical changes associated with senescence.

PCA was also performed on Vis/NIRS spectra to compare spectral characteristics of fruit from different canopy positions of the tree for individual orchards. The PC similarity map of the PCA of spectra acquired from Porterville fruit showed four distinctive clusters of samples from four preharvest canopy positions (Fig. 6). These clusters allowed distinction between fruit from different preharvest canopy positions with accuracy of 95%, as only five fruit from inside canopy were misidentified as to belong to the outside cluster. The distribution of the clusters clearly showed that the canopy position effect was directed along the first PC and fruit bagging was related to the second PC. Similar clusters were also observed in the PCA

similarity map developed using Vis/NIRS signal of fruit harvested the Stellenbosch orchard, although the accuracy was lower, showing some overlap between clusters (Fig. 7). In Porterville and Stellenbosch, the first two PCs accounted for 65 % (Fig. 6) and 76 % (Fig. 7) of the total variation, respectively. In the Stellenbosch orchard, PC1 and PC2 explained the 70 % and 6 % of the variance, respectively. In Paarl and Citrusdal, the PCA score plots for the first two principal components were not able to clearly distinguish between fruit from different preharvest canopy positions and bagging treatments. Adding more PCs (PC4) to the projection score plot improved the clustering, although there was still an overlap between samples from different canopy positions (Fig. 8). In Citrusdal, addition of more PCs to the PCA did not improve the sample separation; hence sample distribution was based on the first two PCs (Fig. 9).

Spectral data of all four locations were pooled in one matrix and subjected to a PCA, which resulted in a similar map showing Stellenbosch population harvested in 2011 to be clearly separated from other orchard locations of the 2012 season (Fig. 10). However, there was no clear separation among the three locations harvested in 2012, indicating that season effect was bigger than the orchard effect. Consistent with the RBD surface plot (Fig. 2) and Pareto analysis (Fig. 3) showing the effect of fruit position on RBD, clustering of samples by canopy position was possible in all locations although the level of accuracy was not the same. In the current study it was demonstrated that fruit located inside the canopy were more susceptible to RBD than outside fruit. Therefore, the ability of PCA models of Vis/NIRS to discriminate between inside and outside fruit suggests the potential of this technology to discriminate fruit based on their susceptibility to RBD. This information could be used as an on-line decision tool during packing, to decide on fruit destined for long distance export market (outside) and those destined for short distance or local market (inside fruit).

3.2.2. Distribution of calibration and validation reference data

Descriptive statistics showed that data of most measured quality parameters were normally distributed around the mean as indicated by the skewness in Table 3. A normally distributed data set should have a skewness value ranging from -1 to +1 with the absolute value of this statistical parameter as close to zero as possible (Puchert et al., 2010). RBD, mass loss, and sucrose were exceptions to this description, with absolute skewness values higher than 1. The skewness has recently been shown to affect interpretation of PLS model statistical parameters such as RPD (Bellon-Maurel et al., 2010). These researchers stated that

in highly skewed samples, the use of RPD, which was developed for biological data sets showing normal distribution, may be inappropriate. This is because, the standard deviation (a numerator in the RPD ratio) of highly skewed data with many low values samples, such as the distribution of RBD (Fig. 11), may not be statistically correct.

The statistics (mean, standard deviation and CV %) for populations of 'Nules Clementine' mandarins used in calibrations and independent validation stages for each parameter analysed are presented in Tables 4 to 10. Lu et al. (2006) noted that the validity and prediction accuracy of calibration models largely depend on the precision of the measured physical and biochemical data and the presence of enough variation in both calibration and validation data sets. Ideally, sample sets for calibration and validation of the study parameters should show a large sample variation to be better predicted by Vis/NIRS (Pérez-Marín et al., 2005; Clément et al., 2008; Sánchez et al., 2013). With the exception of colour parameters, such as L*, C*, and *h*°, all measured parameters in this study had high CV % values of up to 43.4 % for sucrose demonstrating that both calibration and validation data sets covered a wide range of values.

3.2.3. PLS prediction models

3.2.3.1. Performance of individual orchard models

Tables 4 to 6 display statistics for PLS calibration models developed using fruit from one orchard and validated with fruit from two other orchards separately. The three measured colour parameters showed the best prediction in terms of R-value, RMSEP and RPD. Correlation coefficients between predicted and measured L*, C* and *h*° for models calibrated with fruit from Porterville and validated with fruit from Paarl and Citrusdal ranged from 0.91 to 0.95 and from 0.89 to 0.97, respectively (Table 4). A similar trend was observed with models calibrated with fruit from Paarl (Table 5) and Citrusdal (Table 6). Colour parameters models such as those for *h*° and L* were also characterised by lower LVs (3 and 2, respectively), RMSEP (1.40 to 2.98) and higher RPD values (2.12 to 3.85). The lower latent variables and high prediction accuracy of colour parameters could be attributed to the significant waveband for these parameters occurring in the visible range (from 451 to 750 nm). In all lightness, chroma and hue angle prediction models, the absolute values of bias were rather small (0.06 to 1.96), indicating stability of these models with regard to location. As previously stated by Clément et al. (2008), the inclusion of the visible range in the

prediction of colour from Vis/NIRS signal is equivalent to matching two sets of spectrophotometric data. However, Vis/NIRS provide the possibility of integrating quantitative assessment of external colour parameters and rind biochemical quality attributes in one system.

From the previous study (Magwaza et al., 2012b) and regression coefficients results obtained in this study (data not shown), the informative wavelength bands for all non-structural carbohydrates and DM were between 900-1700 nm. For all three orchards, the prediction statistics for fructose, glucose, sucrose and total carbohydrates were encouraging but less accurate compared to the statistics for colour parameters. Carbohydrates models had high RMSEP ranging between 6.76 and 16.84 mg/g DM, for sucrose and from 30.2 to 54.8 mg/g DM for total carbohydrates. The validation RPD values were 1.58 for fructose model calibrated with Paarl to predict Porterville (Table 5) and 1.27 for glucose models developed and validated with data from Porterville and Paarl, respectively (Table 4). For fruit and vegetables models, Saeys et al. (2005) and Davey et al. (2009) suggested that that RPD values below 1.5 are considered unusable, from 1.5 to 2.0 are suitable for rough prediction, those between 2.0 and 2.5 are suitable for quantitative predictions, while RPD values from 2.5 to 3.0 and above are respectively considered good and excellent prediction models. In the this study, the RPD values were higher than 1.5 but lower than 2.0 for most carbohydrates models and the correlation between Vis/NIRS-predicted and measured values for most carbohydrates models were biologically acceptable ($R>0.70$). This demonstrates the importance of using the combination of statistical parameters to evaluate calibration model performance.

A significant absolute bias of up to 10.28 (Table 4) was also observed from prediction models for rind glucose using calibration models based on a single orchard population or one location. The higher bias of carbohydrates models demonstrated that the models were not robust and therefore sensitive to the external factors such as orchard location. The lower prediction accuracy for carbohydrates models could have resulted from the fact that the range of the measured validation data set from another orchard was not confined within the boundary of the calibration set. In general most calibration models developed from one orchard population violated a rule of thumb which states that samples used to build a calibration model should be selected from samples similar to those that will be measured during validation or analysed in the future (Wang et al., 1991; Cozzolino et al., 2011). An example of this phenomenon is demonstrated in the scatter plot for rind DM model calibrated with data from Citrusdal and validated with data of Paarl fruit (Fig. 12A). A spiking method

described by Guerrero et al. (2010) and Kuang and Mouazen (2013) was used to solve this problem. Spiking of calibration models with a few (1-5) samples from the target prediction orchard location followed by recalibration improved model performance (RMSEP = 2.4 %) (Fig. 12B) compared to RMSEP of 3.4 % observed in a model without spiking in Fig. 12A.

3.2.3.2. Performance two combined orchards locations

In citrus fruit, the largest source of variation has been reported to be caused by the differences among trees within an orchard, while variation due to canopy position, and growing location also contribute significantly to the total variance (Barry et al., 2003). In this study the tree-to-tree and within canopy position variance were included to ensure sufficient variation in the sample population from individual orchards. To illustrate the robustness of the calibration models with respect to orchard variance, the performance of calibration models developed using combined data set from two locations were evaluated by predicting quality parameters of the third orchard and prediction statistics reported in Tables 7 to 9. An improvement of the accuracy was evident when the calibration data of both orchards were combined. For instance, the RMSEP for DM model developed from a combination of Porterville and Paarl and validated with Citrusdal was lower (1.96 %) (Table 9) compared to individual models for Porterville and Paarl in predicting Citrusdal, with RMSEP of 2.10 % (Table 4) and 1.93 % (Table 5), respectively. This was also evident on the glucose models where combined samples from Citrusdal and Paarl used to predict Porterville (Table 7) resulted in relatively higher prediction statistics (R = 0.91, RMSEP = 14.50 mg/g DM, RPD = 1.66) compared to, for instance, the Citrusdal calibration model to predict Paarl (Table 6) (R = 0.65, RMSEP = 16.62 mg/g DM, RPD = 1.12).

Similarly, the total soluble sugars prediction model which combined Citrusdal and Porterville fruit to predict Paarl (Table 8) was better (RMSEP = 28.68 mg/g DM) than those for individual models for Citrusdal and Porterville to predict Paarl fruit which had RMSEP values of 42.41 mg/g DM (Table 6) and 37.40 mg/g DM (Table 4), respectively. This effect was also observed for other quality parameters on different combinations of two orchards for calibration, but could not be generalised. As a result of the large impact of orchard on variation, it appears that combining data sets of different orchards for calibration resulted in improved model accuracy. Higher variation in combined orchards than in individual orchards is responsible for this high prediction power as previously demonstrated by Clément et al. (2008) that a parameter showing a large sample variation is likely to be better predicted by

Vis/NIRS. However, the advantage of combined orchards was not realised on colour parameters which showed reduced accuracy. RMSEP for *h*° was reduced from 2.5-3.85 range on individual orchard models to between 2.4 and 3.4 on combined orchards models. Higher RMSEP values for colour on combined orchard models support the notion by Kuang and Mouazen (2011), who stated that larger sample variation increases model robustness but may also reduce accuracy. Therefore, a statistical compromise between robustness and accuracy should found when developing Vis/NIRS calibration models.

3.2.3.3. Performance one season models in predicting another season

To test the effect of seasonal variation on model performance, calibration models were developed using fruit from 2012 and validated with data from 2011 (Table 10). The performances of L*, C* and DM calibration models for 2012 were relatively poor in predicting 2011 data, with respective RPD-values of 0.68, 0.95 and 0.70. This low prediction accuracy demonstrated the variation effects across seasons and location. These findings support the notion stating that seasonal and orchard variation significantly affects calibration models for horticultural products (Peirs et al., 2003). Spectral differences due to the biological variability of samples from different orchards and season may indicate that future samples cannot be predicted with high accuracy (Peirs et al., 2003; Wedding et al., 2013). Several studies on citrus fruit rind quality have suggested that variations in regional climate, soil type, hours of sunlight, fruit age, and microclimatic conditions during the growing season and within an orchard may influence biochemical profile and roughness of the rind (Golic and Walsh, 2006; Khalid et al., 2012). The biochemical profile will affect absorption bands, whereas roughness will affect light scattering. While the second may be reduced by proper spectra preprocessing, the absorption of biochemical components are finger prints that can be quantified by accounting for variation in the data set used for establishing calibration models. It is therefore important to note that reflection based system like the one used in this study are also more likely to be sensitive to changes in sample surface coarseness compared to transmittance systems (Golic and Walsh, 2006). The variations in these properties affect the Vis/NIRS model performance in predicting quality parameters of interest. Models developed in 2012, across three orchards or locations performed better in predicting *h*° (RMSEP = 1.91; RPD = 2.91), fructose (RMSEP = 14.03 mg/g DM; RPD = 2.50), and glucose (RMSEP = 15.24 mg/g DM; RPD = 2.04) of fruit from Stellenbosch in 2011.

3.2.3.4. A robust models to cover two seasons and four orchard locations

To develop robust models for measured quality parameters, models which cover all orchard locations and seasons were developed by assigning 60% of fruit to the calibration 40% to the validation set (Table 11). The models that were developed by incorporating all orchards and seasons gave better model statistics for all quality parameters compared to models developed using a single orchard or single season. For instance, the DM model developed from a wide range of rind colour gave a correlation coefficient of 0.89, RMSEP of 1.99 % Dm and an RPD of 2.28. It is noteworthy that the RPD values for all models except for sucrose were above 2.0. Poor prediction statistics for sucrose could possibly resulted from high skewness in data distribution (Table 3, Fig. 13E) and/or the difference in molecular weight of sucrose (MW=342.30 g/mol) compared to glucose and fructose (MW=180.16 g/mol) (Golic et al., 2003). The higher molecular weight of sucrose results in 1.89 times less sucrose molecules than glucose and fructose molecules in the same weight of sample. Therefore, the intensity of the bands associated with hydrogen bonding is smaller for sucrose compared to glucose and fructose (Golic et al., 2003).

In Fig. 13, scatter plots for predicted against measured values for *h*°, rind dry matter, fructose, glucose, sucrose, and total sugars using a model including all fruit combination are presented. The better models developed across a population with larger range of the attribute in question demonstrates the importance of enough variability in the reference samples as stated by Davey et al. (2009). These results are also in agreement with previous findings showing that incorporating data from multiple orchards and seasons in the calibration model improves the predictive performance, in comparison to those developed using individual season and/or orchard (Peiris et al., 1998; Peirs et al., 2003; Guthrie et al., 2005; Wedding et al., 2013). This is supported by Melfsen et al. (2012) and Peirs et al. (2003) who stated that the accuracy of validation results from very diverse and completely randomised data sets is over-optimistic with regard to future samples.

3.2.3.5. Prediction of RBD

In previous studies (Magwaza et al., 2012b, c; 2013), it was established that models for predicting RBD (on a scale 0-3) are poor ($R < 0.10$). Similarly, in this study, reliable results for the prediction of RBD were difficult to obtain with Vis/NIRS (data not shown). The hypothesis behind poor prediction of RBD models was based on the discrete nature of RBD

scores, and trying to correlate such simple data with complicated Vis/NIRS data is difficult, and resulted in poor model statistics. Therefore, data was subjected to PLS-DA to develop RBD-susceptibility classification model. Prediction performance results presented in Table 12 show that PLS-DA model statistics for RBD-binary data were still poor (RPD for validation < 1.50) regardless of sample origin and season. These results concur with the observations reported by Magwaza et al. (2012b, c; 2013), where RBD was predicted better using PLS-DA compared to PLS.

4. Conclusion

The rind of fruit in outside tree canopy, which developed under high sunlight conditions, were better coloured (orange) compared to fruit located inside the canopy (yellow). In all four sampled orchards, bagged fruit inside the canopy had significantly higher postharvest mass loss and lower DM in the rind tissue. Associated with higher mass loss and lower DM, bagged fruit inside the canopy had significantly higher incidence of RBD compared to sun-exposed outside fruit, indicating a close correlation of the parameters with fruit susceptibility to RBD. The results showed that in all four orchard locations, the concentrations of rind non-structural carbohydrates were significantly affected by fruit position and bagging treatments. Inside and bagged fruit harvested from Porterville, Paarl and Stellenbosch had higher concentrations of non-structural carbohydrates (fructose, glucose, and sucrose) than outside fruit. However, Citrusdal was an exception to this trend, with higher non-structural carbohydrates in rinds of fruit harvested from outside position of the canopy compared to shaded fruit. Although carbohydrates have been previously identified to play a protective role in plants against stress conditions, the differences in carbohydrates responses to reduced sunlight indicated that their levels in the rind might not be a universal biochemical marker or the primary cause for RBD, since it is highly dependent on climatic conditions and other environmental factors during growth and development as well as during postharvest storage. PCA models based on Vis/NIR signals were developed in order to discriminate between fruit according to canopy position, orchard location and postharvest storage based on their NIR spectrum features. The ability of Vis/NIRS to discriminate between inside and outside canopy fruit suggests the potential of this technology to discriminate between fruit based on their susceptibility to RBD. This information could be used as an in-line sorting tool, during packing, to decide on fruit destined for long distance export market (outside) and those destined for short distance or local market (inside fruit).

Reliable results for the prediction of RBD were difficult to obtain with Vis/NIRS. Nevertheless, PLS results showed the potential of using Vis/NIRS to predict rind physico-chemical attributes after storage. Rind carbohydrates and DM which are associated with RBD were predicted with high accuracy except for sucrose which had lower prediction statistics. Vis/NIRS results obtained in the current study also demonstrated the importance of model robustness in predicting for new populations. Calibration models developed using fruit from two orchards (growing locations), in most cases gave improved prediction accuracy compared to models developed based on data from a single orchard. Similarly, when variability in orchard location and season was added to the calibration models, significantly lower RMSEP values were obtained. In this study, one of the factors which may be responsible for sample variability and reduced validation was the inclusion of the samples that belong to different orchard location in the validation model. As a result the analyte of interest as well as the concentrations of other compositional factors deviated from the calibration set. Spiking calibration models with a few samples from the target prediction orchard improved model performance. While the ultimate goal of any technique is to be universal, it is important to stress that special attention should be paid on model robustness in predicting new populations from different orchard location and production or marketing seasons. Considering the observed variation between growing locations and seasons, it is advisable to continuously upgrade calibration models based on successive seasons and new orchard locations.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterization of rind breakdown of ‘Navelate’ sweet orange. Ann. Bot. 88, 415–422.

Alamar, M.C., 2007. Contributions to the estimation of soluble solids content and texture of apple fruit. PhD thesis, Universidad Politécnica de Valencia, Spain.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in ‘Navel’ oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Alquezar, B., Burns, J.K., Zacarìas, L., 2010. Variation in water, osmotic and turgor potential in peel of ‘Marsh’ grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65.

Barry, G.H., Castle, W.S., Davies, F.S., Litten, R.C., 2003. Variability in juice quality of 'Valencia' sweet orange and sample size estimation for juice quality experiments. J. Am. Soc. Hortic. Sci. 128, 803–808.

Bean, R.C., Todd, G.W., 1960. Photosynthesis and respiration in developing fruits. I. $^{14}CO2$ uptake by young oranges in the light and in the dark. Plant Physiol. 35, 425–429.

Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J-M., McBratney, A., 2010. Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. Trends Anal. Chem. 29, 1073–1081.

Ben-Yehoshua, S., Shapiro, B., Chen, Z.E., Lurie, S, 1983. Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress. Plant Physiol. 73, 87–93.

Ben-Yehoshua, S., J. Peretz, R. Moran, B. Lavie, and J.J. Kim. 2001. Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*Citrus sinensis*, Osbeck). Postharvest Biol. Technol. 22:19–27.

Bobelyn, E., Serban, A., Nicu, M., Lammertyn, J., Nicolaï, B.M., Saeys, W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. 55, 133–143.

Cajuste, J.F., Lafuente, T.M., 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. Postharvest Biol. Technol. 45, 193–203.

Caleb, O.J., Mahajan, P.V., Opara, U.L., Witthuhn, C.R., 2012. Modelling the respiration rates of pomegranate fruit and arils. Postharvest Biol. Technol. 64, 49–54.

Cayuela, J.A., Weiland, C., 2010. Intact orange quality prediction with two portable NIR spectrometers. Postharvest Biol. Technol. 58, 113–120.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56, 9813–9818.

Cozzolino, D., Cyankar, W.U., Shah, N., Smith, P., 2011. Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. Food Res. Int. 44, 1888–1896.

Crespo, P., Giné Bordonaba, J., Terry, L.A., Carlen, C., 2010. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. Food Chem. 122, 6–24**.**

Cronje, P.J.R., 2007. Postharvest rind disorders of citrus fruit. Citrus Research International, Nelspruit, South Africa.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementines' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H, Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Scientia Hort. 130, 829–837.

Davey, M.W., Saeys., W., Hof, E., Ramon, H., Swennen, R.L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. J. Agric. Food Chem. 57, 1742–1751.

Duarte, A.M.M., Guardiola, J.L., 1995. Factors affecting rind pitting in the mandarin hybrids 'Fortune' and 'Nova'. The influence of exogenous growth regulators. Acta Hort. 379, 59–67.

Echeverria, E., Valich, J., 1989. Enzymes of sugar and acid metabolism in stored Valencia oranges. J. Am. Soc. Hort. Sci. 114, 445–449.

Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

Golic, M., Walsh, K.B., 2006. Robustness of calibration models based on near infrared spectroscopy for the in-line grading of stone fruit for total soluble solids content. Anal. Chim. Acta 555, 286–291.

Gómez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Guerrero, C., Zornoza, R., Gómez, I., Mataix-Beneyto, J., 2010. Spiking of NIR regional models using samples from target sites: effect of model size on prediction accuracy. Geoderma 158, 66–77.

Guthrie, J., Wedding, B., Walsh, K., 1998. Robustness of NIR calibrations for soluble solids in intact melons and pineapple. J. Near Infrared Spectrosc. 6, 259–265.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. Aust. J. Agric. Res. 56, 417–426.

Hiratsuka, S., Yokoyama, Y., Nishimura, H., Miyazaki, T., Nada, K., 2012. Fruit photosynthesis and phosphoenolpyruvate carboxylase activity as affected by lightproof fruit bagging in Satsuma mandarin. J. Am. Soc. Hort. Sci. 137, 215–220.

Holland, N., Sala, J.M., Menezes, H.C., Lafuente, M.T., 1999. Carbohydrate content and metabolism as related to maturity and chilling sensitivity, of cv. Fortune mandarins. J.Agric. Food Chem. 47, 2513–2518.

Huang, X-M., Huang, H-B., Gao, F-F., 2000. The growth potential generated in citrus fruit under water stress and its relevant mechanisms. Sci. Hort. 83, 227–240.

Jamshidi, B., Minaei, S., Mohajerani, E., Ghassemian, H., 2012. Reflectance Vis/NIR spectroscopy for nondestructive taste characterization of Valencia oranges. Comput. Electron. Agric. 85, 64–69.

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M, 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of 'Kinnow' mandarin (*Citrus nobilis* Lour x *Citrus deliciosa*Tenora). Scientia Hort. 135, 137–144.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Kuang B., Mouazen, A.M., 2011. Calibration of visible and near infrared spectroscopy for soil analysis at the field scale on three European farms. Eur. J. Soil Sci. 62, 629–636.

Kuang, B., Mouazen, A.M., 2013. Effect of spiking strategy and ratio on calibration of on-line visible and near infrared soil sensor for measurement in European farms. Soil Till. Res. 128, 125–136.

Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., Blasco, J., 2013. Selection of optimal wavelength features for decay detection in citrus fruit using the ROC curve and neural networks. Food Bioprocess Technol. 6, 530–541.

Louw, E.D., Theron, K.I., 2010. Robust prediction models for quality parameters in Japanese plums (*Prunus salicina* L.) using NIR spectroscopy. Postharvest Biol. Technol. 58, 176–184.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application of Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. B 7, 794–799.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P., Saeys, W., Nicolaï, B., 2012a. NIR spectroscopy applications for internal and external quality analysis of citrus fruit – a review. Food Bioprocess Technol. 5, 425–444.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H., Mouazen, A.M., Saeys, W., Nicolaï, B.M., 2012b. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest Biol. Technol. 74, 1–10.

Magwaza, L.S., Opara, U.L. Cronje, P.J.R. Nieuwoudt, H.H. Landahl, S., Terry, L. A., 2012c. Quantifying the effects of fruit position in the canopy on physical and biochemical properties and predicting susceptibility to rind breakdown disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) using Vis/NIR spectroscopy. Acta Hort. 1007, 83–91.

Magwaza, L.S., Opara, U.L., Cronje, P.J.R., Landahl, S., Nicolaï, B.M., Terry, L.A., 2013a. Non-chilling physiological rind disorders in citrus fruit. Hort. Rev. 41, (In Press).

Magwaza, L.S., Ford, H.D., Cronje, P.J.R., Opara, U.L., Landahl, S., Tatam, R.P., Terry, L.A., 2013b. Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins. Postharvest Biol. Technol. (In Press): http://dx.doi.org/10.1016/j.postharvbio.2013.03.019.

Magwaza, L.S., Opara, U.L., Landahl, L., Cronje, P.J.R., Nieuwoudt, H.H., Mouazen, A.M., Terry, L.A., 2013. The use of Vis/NIRS and chemometrics to predict fruit defects and postharvest behaviour of 'Nules Clementine' mandarin fruit. Biosyst. Eng. (Submitted).

Mahajan, P.V., Oliveira, F.A.R., Macedo, I., 2008. Effect of temperature and humidity on the transpiration rate of the whole mushrooms. J. Food Eng. 84, 281–288.

McGlone, V.A., Jordan, R.B., Martinsen, P.J., 2002. Vis-NIR estimation at harvest of pre- and post-storage quality indices for 'Royal Gala' apple. Postharvest Biol. Technol. 25, 135–144.

Melfsen, A., Hartung, E., Haeussermann, A., 2013. Robustness of near-infrared calibration models for the prediction of milk constituents during the milking process. J. Dairy Res. 80, 103–112.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3, 227–237.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I. K., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Nicolaï, B.M., Bulens, I., De Baerdemaker, J., De Ketelaere, B., Hertog, M.L.A.T.M., Verboven, P., Lemmertyn, J., 2009. Non-destructive evaluation: detection of external and internal attributes frequently associated with quality and damage. In: Florkowiski et al. (eds). Postharvest Handling: A Systems Approach, pp 421-442. Academic Press, Elsevier, Amsterdam.

Nilsson, T., Gustavsson, K.-E., 2007. Postharvest physiology of 'Aroma' apples in relation to position on the tree. Postharvest Biol. Technol. 43, 36–46.

Ou, A.S., Lin, S., Lin, T., Wu, S., Tiarn, M., 1997. Studies on the determination of quality-related constituents in 'Ponkan' Mandarin by near infrared spectroscopy. J. Chin. Agric. Chem. Soc. 35, 462–474.

Pathare, P.B., Opara, U.L., Al-Said, F.A., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Technol. 6, 36–60.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1998. Near-infrared spectroscopic method for nondestructive determination of soluble solids content of peaches. J. Amer. Soc. Hort. Sci. 123, 898–905.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. HortScience. 34, 114–118.

Peirs, A., Tirry, J., Verlinden, B., Darius, P., Nicolaï, B.M., 2003. Effect of biological variability on the robustness of NIR models for soluble solids content of apples. Postharvest Biol. Technol. 28, 269–280.

Pérez-Marín, D., Garrido-Varo, A., Guerrero, J.E., 2005. Implementation of LOCAL algorithm with near-infrared spectroscopy for compliance assurance in compound feeding stuffs. Appl. Spectrosc. 59, 69–77.

Porat. R., Weiss, B., Cohen, L., Daus, A., Aharoni., N., 2004. Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biol. Technol. 33, 35–43.

Puchert, T., Lochmann, D., Menezes, J.C., Reich, G., 2010. Near-infrared chemical imaging (NIR-CI) for counterfeit drug identification—A four-stage concept with a novel approach of data processing (Linear Image Signature). J. Pharmaceut. Biomed. Anal. 51, 138–145.

Purvis, A.C., Grierson, W., 1982. Accumulation of reducing sugars and resistance of grapefruit peel to chilling injury as related to winter temperature. J. Am. Soc. Hort. Sci. 107, 139–142.

Purvis, A.C., Yelenosky, G., 1983. Translocation of carbohydrates and proline in young grapefruit trees at low temperatures. Plant Physiol. 73, 877–880.

Rosales, R., Burns, J.K., 2011. Phytohormone changes and carbohydrate status in sweet orange fruit from huanglongbing-infected trees. J. Plant Growth Regul. 30, 312–321.

Saeys, W., Mouazen, A.M., Ramon, H., 2005. Potential onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst. Eng. 91, 393–402.

Sánchez, M-T., De la Haba, M-J., Serrano, I., Pérez-Marín, D., 2013. Application of NIRS for non-destructive measurements of quality parameters in intact oranges during on-tree ripening and at harvest. Food Anal. Method. 6, 826–837.

Shiroma, C., Rodriguez-Saona, S., 2009. Application of NIR and MIR spectroscopy in quality control of potato chips. J. Food Comp. Anal. 22, 596–605.

Sun, X., Zhang, H., Liu, Y., 2009. Nondestructive assessment of quality of 'Nanfeng' mandarin fruit by a portable near infrared spectroscopy. Int. J. Agric. Biol. Eng. 2, 65–71.

Teerachaichayut, S., Terdwongworakul, A., Thanapese, W., Kiji, K., 2011. Non-destructive prediction of hardening pericarp disorder in intact mangosteen by near infrared transmittance spectroscopy. J. Food Eng. 106, 206–211.

Terry, L.A., Chope, G.A., Giné Bordonaba, J., 2007. Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria x ananassa*) fruit quality. J. Agric. Food Chem. 55, 10812–10819.

Tewari, J.C., Dixit, V., Chi, B-K., Malik, K.A., 2008. Determination of origin and sugars of citrus fruit using genetic algorithm, correspondence analysis and partial least square

combined with fiber optic NIR spectroscopy. Spectroc. Acta Part A: Mol. Biomol. Spectrosc. 71, 1119–1127.

Ting, S.V., Deszyck, 1961. The Carbohydrates in the Peel of Oranges and Grapefruit. J. Food Sci. 26, 146–152.

Tombesi, A., Antognozzi, E., Palliotti, A., 1993. Influence of light exposure on characteristics and storage life of Kiwifruit. New Zeal. J. Crop Hort. Sci. 21, 87–92.

van Rensburg, P.J.J., Bruwer, M., 2000. Factors influencing rind breakdown of 'Clementine' mandarin fruit. Proc. Int. Soc. Citricul. 3, 1173.

van Rensburg, P.J.J., Cronje, P.J.R., Joubert, J., Gambetta, G., Bruwer, M., 2004. Factors influencing rind breakdown in citrus fruit. Proc. Int. Soc. Citricul. 2, 1051–1061.

Wang, W., Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. Sens. Instr. Food Chem. 1, 193–207.

Wang, Y., Veltkamp, D.J., Kowalski, B.R., 1991. Multivariate instrument standardization. Anal. Chem. 63, 2750–2756.

Wedding, B.B., Wright, C., Grau, S., White, R.D., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biol. Technol. 75, 9–16.

Wetzel, D.L., 1983. Near infrared reflectance analysis: Sleeper among Spectroscopic Techniques. Anal. Chem. 55, 1165–1176.

Williams, P.C., Sobering, D.C., 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Davies A.M.C., Williams, P. (ed.). Near Infrared Spectroscopy: The future waves. Proc. 7th Interntl. Conf. Near Infra. Spectrosc. Montreal, Chichester, UK, pp. 185–188.

Yakushiji, H., Nonami, H., Fukuyama, T., Takagi, N., Hashimoto, Y., 1996. Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. J. Am. Soc. Hort. Sci. 121, 466–472.

Yang, H., Kuang, B., Mouazen, A.M., 2011. In situ determination of growing stages and harvest time of tomato (Lycopersicon esculentum) fruits using fibre optic vis-NIR spectroscopy. Appl. Spectrosc. 35, 931–938.

Yen, C-R., Koch, K.E., 1990. Developmental changes in translocation and localization of ^{14}C-labbeled assimilates in grapefruit: light and dark CO_2 fixation by leaves and fruit. J. Am. Soc. Hort. Sci. 115, 815–819.

Table 1: Physico-chemical properties of fruit from different canopy positions and bagging treatments after 8 weeks in storage. Different letters next to values (mean and standard error of the mean) for the same quality parameter are significantly different from each other based on a 2-factorial analysis of variance.

Quality parameter	Growing region	Canopy position			
		Outside	Outside bagged	Inside	Inside bagged
Rind hue angle	Porterville	82.65±1.85 b	81.14±2.06 bc	77.02±0.92 cd	75.06±1.51 d
LSD = 1.057	Paarl	82.77±1.71 b	83.21±1.91 b	81.10±1.02 bc	82.39±1.54 b
L *P*<0.001					
CP *p*<0.001	Citrusdal	75.32±1.38 d	83.47±1.59 b	82.12±1.15 b	90.17±1.80 a
L*CP *P*<0.001	Stellenbosch	67.21±0.45 e	76.22±1.31 d	73.44±1.09 d	74.39±1.00 d
Fruit mass loss	Porterville	14.27±0.52 bcdef	15.83±1.29 bcde	16.28±0.78 bc	22.50±1.77 a
(%)	Paarl	13.02±0.28 cdef	12.06±0.47 f	11.80±0.57 fg	12.92±0.67 cdef
LSD = 0.607					
L *P*<0.001540	Citrusdal	13.27±0.30 cde	17.26±0.84 b	16.28±0.85 bcd	24.26±0.57 a
CP *p*<0.001					
L*CP *P*<0.001	Stellenbosch	8.88±0.27 g	12.73±1.82 ef	8.75±0.53 g	12.76±1.50 ef
Rind dry matter	Porterville	35.02±0.83 b	33.58±0.50 bc	33.41±0.66 bcd	28.93±0.74 fgh
(%)	Paarl	38.44±0.49 a	37.42±0.87 a	35.02±0.83 b	31.31±1.12 de
LSD = 0.517					
L *P*<0.001	Citrusdal	31.84±0.55 cde	30.79±0.55 efg	31.15±0.49 ef	30.17±0.42 efg
CP *p*<0.001					
L*CP *P*<0.001	Stellenbosch	30.00±0.78 efg	26.98±0.51 h	28.78±0.96 hg	26.99±0.79 h

LSD, least significant difference; *L*, orchard location; *CP*, preharvest canopy position treatments; *L*CP*, interaction between orchard location and canopy position.

Table 2: Positional and bagging effects on carbohydrates composition in rinds of 'Nules Clementine' mandarins harvested from different citrus growing regions. Means of the same quality parameter with different letters are significantly different ($P \leq 0.05$).

Quality parameter	Growing region	Canopy position			
		Outside	Outside bagged	Inside	Inside bagged
Rind fructose	Porterville	78.46±3.87 h	93.76±4.52 efgh	91.79±4.70 fgh	124.35±9.35 abc
(mg/g DM)	Paarl	83.33±3.09 h	86.46±2.32 gh	81.07±3.83 h	95.57±6.93 efgh
LSD = 3.771 L *P*<0.001 CP *P*=0.026 L*CP *P*<0.001	Citrusdal	109.74±2.63 bcde	107.66±4.25 cdef	114.39±4.75 abcd	89.07±4.39 gh
	Stellenbosch	126.48±5.19 ab	130.21±9.76 a	100.65±8.52 defg	122.26±9.71 abc
Rind glucose	Porterville	52.64±3.28 h	61.15±3.92 fgh	59.38±4.12 gh	87.82±7.70 ab
(mg/g DM)	Paarl	61.58±3.42 efgh	67.13±2.59defgh	56.47±4.15 gh	65.81±5.71 defgh
LSD = 3.388 L *P*<0.001 CP *P*=0.289 L*CP *P*<0.001	Citrusdal	75.96±2.40 bcdef	71.37±3.94 cefg	79.06±3.97 abcd	52.74±4.35 h
	Stellenbosch	92.03±5.29 a	85.44±8.94 abc	66.92±6.97 dfgh	77.09±8.50 abcde
Rind sucrose	Porterville	31.37±1.02 f	33.13±1.86 ef	45.62±4.06 ed	53.57±5.81 cd
(mg/g DM)	Paarl	31.68±0.79 f	29.58±0.75 f	41.43±3.74 def	41.46±2.53 def
LSD = 3.014 L *P*<0.001 CP *P*<0.001 L*CP *P*<0.001	Citrusdal	32.68±0.95 ef	31.46±1.09 f	36.02±2.69 ef	30.79±1.91 f
	Stellenbosch	95.31±6.07 b	93.59±9.51 b	63.30±8.65 c	114.94±12.51 a
Rind total sugars	Porterville	162.46±7.58 f	188.05±9.67 def	196.79±12.05 cdef	265.74±22.32 b
(mg/g DM)	Paarl	176.58±6.66 de	183.16±5.18 def	178.96±9.96 def	202.84±14.14 cdef
LSD = 9.104 L *P*<0.001 CP *P*=0.016 L*CP *P*<0.001	Citrusdal	218.39±5.29 cd	210.48±8.84 cde	229.48±10.86 bc	172.60±10.31 ef
	Stellenbosch	313.82±11.58 a	309.23±25.05 a	230.88±22.82 bc	314.29±27.70 a
Su:Glu+Fru	Porterville	0.26±0.02 defg	0.22±0.01 fgh	0.30±0.02 de	0.25±0.01 defgh
LSD = 0.017 L *P*<0.001 CP *P*<0.0016 L*CP *P*<0.001	Paarl	0.23±0.01 efgh	0.20±0.01 gh	0.31±0.02 cd	0.29±0.04 def
	Citrusdal	0.18±0.01 h	0.18±0.01 gh	0.19±0.01 gh	0.22±0.01 fgh
	Stellenbosch	0.45±0.04 b	0.46±0.05 b	0.37±0.03 c	0.61±0.07 a

Suc:Fru+Glu non-reducing (sucrose) to reducing (glucose + fructose) ratio; *LSD* least significant difference; *L* orchard location; *CP* preharvest canopy position treatments; *L*CP* interaction between orchard location and canopy position.

Table 3: Skewness of measured rind quality parameters.

	L*[a]	C*[b]	*h*° [c]	Rind DM[d] (%)	Mass loss (%)	RBD	Sucrose (mg/g DM)	Glucose (mg/g DM)	Fructose (mg/g DM)	Total sugars (mg/g DM)
Porterville	-1.85	-1.66	1.63	0.11	1.34	1.80	1.39	0.74	0.78	0.94
Paarl	-0.70	-0.97	0.41	-0.45	7.65	2.39	2.84	0.00	0.18	0.23
Citrusdal	-0.24	-0.95	0.62	0.15	1.27	2.16	3.46	-0.13	0.29	0.47
Citrusdal + Porterville	-1.27	-1.33	1.01	0.28	1.52	1.98	2.15	0.28	0.42	0.72
Citrusdal + Paarl	-0.47	-0.95	0.55	0.43	6.79	2.25	3.10	-0.02	0.31	0.45
Porterville + Paarl	-1.18	-1.28	0.93	-0.07	2.35	2.07	1.93	0.51	0.82	0.97
2012 season	-0.97	-1.18	0.82	0.44	2.70	2.09	2.32	0.27	0.51	0.75
2011 (Stellenbosch)	0.12	-0.46	0.64	0.17	1.50	1.94	0.84	0.21	0.28	0.33
All	-0.95	-1.09	0.80	0.40	2.44	2.41	2.47	0.50	0.71	1.24

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h* rind hue angle, [d]*Rind DM* rind dry matter content, [e]*RBD* rind breakdown disorder.

Table 4: Performance of PLS calibration models developed using data from Porterville in predicting rind parameters of fruit harvested from Paarl and Citrusdal.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R [h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Porterville		91	69.98	4.51	6.44	2	0.96	1.32		0.00	0.91	
		Paarl	94	71.34	4.37	6.13	2	0.91		1.88	-0.31	0.91	2.32
		Citrusdal	96	70.46	3.57	5.07	2	0.92		1.49	0.33	0.93	2.40
C* [b]	Porterville		91	61.09	5.61	9.18	5	0.89	2.13		0.00	0.85	
		Paarl	94	60.31	5.50	9.12	5	0.92		3.07	0.83	0.74	1.79
		Citrusdal	96	62.49	5.07	8.11	5	0.89		2.35	0.63	0.85	2.16
h* [c]	Porterville		95	79.22	8.14	10.27	3	0.92	3.10		0.00	0.85	
		Paarl	94	82.36	7.81	9.48	3	0.95		2.41	0.40	0.91	3.24
		Citrusdal	96	82.77	9.09	10.98	3	0.97		2.70	-1.35	0.99	3.37
Rind dry matter (%)	Porterville		92	31.95	3.55	11.11	6	0.86	1.77		0.00	0.85	
		Paarl	93	35.83	4.66	13.01	6	0.82		3.19	-1.69	0.76	1.46
		Citrusdal	90	31.00	3.23	10.42	6	0.65		2.10	-0.14	0.76	1.54
Rind fructose (mg/g DM)	Porterville		93	94.67	29.14	30.78	7	0.86	14.91		0.00	0.77	
		Paarl	91	87.23	18.36	21.05	7	0.78		13.88	6.15	0.83	1.32
		Citrusdal	90	105.4	22.09	20.96	7	0.72		19.84	-12.41	0.60	1.11
Rind glucose (mg/g DM)	Porterville		91	63.24	24.20	38.27	7	0.85	12.52		0.00	0.73	
		Paarl	94	63.56	17.95	28.24	7	0.68		14.16	-1.21	0.73	1.27
		Citrusdal	96	69.94	20.82	29.77	7	0.74		17.31	-10.28	0.79	1.20
Rind sucrose (mg/g DM)	Porterville		91	40.33	17.20	42.65	6	0.79	8.95		0.00	0.63	
		Paarl	94	35.61	11.88	33.36	6	0.64		10.04	3.91	0.83	1.18
		Citrusdal	96	32.77	9.06	27.65	6	0.83		12.19	-3.59	0.73	0.74
Rind total sugars (mg/g DM)	Porterville		91	197.74	66.84	33.80	9	0.86	34.17		0.00	0.74	
		Paarl	94	185.90	40.93	22.02	9	0.71		37.40	5.71	0.99	1.09
		Citrusdal	92	203.72	41.91	20.57	9	0.71		36.84	-12.04	0.82	1.14

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h°* rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 5: Performance of PLS calibration models developed using data from Paarl in predicting rind parameters of fruit harvested from Porterville and Citrusdal.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R[h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Paarl		96	71.22	4.79	6.73	2	0.93	1.73		0.00	0.87	
		Porterville	92	70.08	4.03	5.75	2	0.94		1.40	0.09	0.82	2.88
		Citrusdal	96	70.53	3.62	5.14	2	0.89		1.71	0.49	0.81	2.12
C* [b]	Paarl		96	60.31	5.50	9.12	5	0.90	2.29		0.00	0.80	
		Porterville	93	61.18	5.29	8.65	5	0.82		2.56	-0.06	0.69	2.07
		Citrusdal	95	62.48	5.07	8.11	5	0.87		2.60	-0.93	0.86	1.95
h* [c]	Paarl		96	82.36	7.81	9.48	3	0.95	2.69		0.00	0.89	
		Porterville	95	79.01	7.39	9.35	3	0.92		2.98	-0.47	0.87	2.48
		Citrusdal	94	82.66	8.35	10.10	3	0.97		2.17	-0.31	1.00	3.85
Rind dry matter	Paarl		94	35.83	4.66	13.01	6	0.91	1.78		0.00	0.65	
(%)		Porterville	92	31.95	3.55	11.11	6	0.93		1.44	1.05	0.77	2.47
		Citrusdal	93	31.00	3.23	10.42	6	0.69		1.93	0.99	0.63	1.67
Rind fructose	Paarl		94	86.29	19.51	22.61	7	0.91	7.57		0.00	0.83	
(mg/g DM)		Porterville	91	94.50	29.01	30.70	7	0.81		18.41	-0.24	0.45	1.58
		Citrusdal	93	105.4	22.09	20.96	7	0.75		14.46	-5.58	0.49	1.53
Rind glucose	Paarl		94	62.83	18.59	29.59	7	0.89	8.06		0.00	0.80	
(mg/g DM)		Porterville	91	63.24	24.05	38.03	7	0.72		17.43	8.91	0.46	1.38
		Citrusdal	96	69.94	24.05	34.39	7	0.67		16.87	1.81	0.59	1.43
Rind sucrose	Paarl		93	35.48	11.37	32.05	6	0.68	6.68		0.00	0.46	
(mg/g DM)		Porterville	92	40.00	17.35	43.38	6	0.71		13.49	-4.70	0.34	1.29
		Citrusdal	91	32.77	9.06	27.65	6	0.28		6.76	-0.58	0.20	1.34
Rind total sugars	Paarl		93	184.6	42.52	23.03	9	0.89	18.14		0.00	0.80	
(mg/g DM)		Porterville	96	94.50	66.87	34.38	9	0.84		40.43	2.51	0.47	1.65
		Citrusdal	91	208.10	49.09	23.59	9	0.58		30.15	36.15	0.26	1.63

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h°* rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 6: Performance of PLS calibration models developed using data from Citrusdal in predicting rind parameters of fruit harvested from Paarl and Porterville.

.	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R [h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Citrusdal		95	70.53	3.62	5.14	2	0.93	1.46		0.01	0.86	
		Paarl	92	71.30	3.91	5.48	2	0.93		1.63	-0.63	0.90	2.40
		Porterville	91	70.08	4.03	5.75	2	0.93		1.97	-1.20	0.78	2.05
C* [b]	Citrusdal		96	62.49	5.07	8.11	5	0.91	2.24		0.03	0.80	
		Paarl	93	60.31	5.50	9.12	5	0.83		2.97	1.20	0.72	1.85
		Porterville	90	61.33	5.16	8.41	5	0.89		2.60	-1.41	0.74	1.98
h* [c]	Citrusdal		98	82.77	9.09	10.98	3	0.97	2.39		0.01	0.95	
		Paarl	93	82.36	7.81	9.48	3	0.96		2.52	0.52	0.88	3.10
		Porterville	93	79.01	7.39	9.35	3	0.94		2.73	1.96	0.85	2.71
Rind dry matter	Citrusdal		96	31.19	3.23	10.36	6	0.86	1.68		0.01	0.76	
(%)		Paarl	94	35.76	4.34	12.14	6	0.84		2.39	-1.51	0.73	1.82
		Porterville	93	31.05	3.29	10.60	6	0.79		1.79	-1.02	0.75	1.84
Rind fructose	Citrusdal		97	105.40	22.09	20.96	7	0.82	12.56		-0.11	0.70	
(mg/g DM)		Paarl	90	86.29	19.51	22.61	7	0.77		20.49	15.99	0.72	0.95
		Porterville	93	94.50	29.01	30.70	7	0.61		23.29	4.18	0.38	1.25
Rind glucose	Citrusdal		94	69.94	20.82	29.77	7	0.83	11.52		-0.05	0.71	
(mg/g DM)		Paarl	93	62.83	18.59	29.59	7	0.65		16.62	8.28	0.54	1.12
		Porterville	92	63.24	24.05	38.03	7	0.57		20.68	5.79	0.35	1.16
Rind sucrose	Citrusdal		97	32.77	9.06	27.65	6	0.57	7.43		0.00	0.32	
(mg/g DM)		Paarl	89	35.48	11.37	32.05	6	0.63		13.94	-0.60	0.24	0.82
		Porterville	87	40.00	17.35	43.38	6	0.51		16.84	-7.14	0.16	1.03
Rind total sugars	Citrusdal		95	208.10	49.09	23.59	9	0.80	26.76		-0.31	0.66	
(mg/g DM)		Paarl	88	184.60	42.52	23.03	9	0.67		42.41	27.27	0.54	1.00
		Porterville	87	197.70	66.84	33.81	9	0.57		54.78	4.30	0.31	1.22

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h°* rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 7: Performance statistics of PLS calibration models developed using combined data from Citrusdal and Paarl to predict quality parameters of samples harvested from Porterville.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R [h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Citrusdal + Paarl		187	70.97	4.06	5.71	2	0.91	1.64		0.00	2.84	
		Porteville	91	69.88	4.43	6.34	2	0.92		2.39	-1.39	0.67	1.85
C* [b]	Citrusdal + Paarl		187	61.40	5.39	8.77	5	0.87	2.67		-0.01	0.74	
		Porteville	91	61.09	5.61	9.18	5	0.90		3.79	-2.84	0.77	1.48
h° [c]	Citrusdal + Paarl		184	82.57	8.45	10.24	3	0.96	2.51		0.00	0.91	
		Porteville	95	79.22	8.14	10.27	3	0.91		3.45	-0.32	0.75	2.36
Rind dry matter	Citrusdal + Paarl		187	33.33	4.41	13.25	4	0.86	2.26		-0.01	0.75	
(%)		Porteville	92	31.95	3.55	11.10	4	0.86		2.67	1.88	0.88	1.33
Rind fructose	Citrusdal + Paarl		187	96.28	22.93	23.81	6	0.83	12.09		-0.10	0.70	
(mg/g DM)		Porteville	90	94.50	29.01	30.69	6	0.71		19.46	4.28	0.41	1.49
Rind glucose	Citrusdal + Paarl		187	66.56	20.06	30.13	9	0.90	8.54		-0.01	0.81	
(mg/g DM)		Porteville	91	63.24	24.05	38.04	9	0.91		14.50	10.55	0.77	1.66
Rind sucrose	Citrusdal + Paarl		183	34.06	10.29	30.21	8	0.65	6.01		0.00	0.42	
(mg/g DM)		Porteville	91	40.00	17.35	43.38	8	0.79		13.83	-5.61	0.30	1.25
Rind total sugars	Citrusdal + Paarl		184	196.90	47.44	24.09	11	0.88	20.15		0.00	0.79	
(mg/g DM)		Porteville	89	194.50	67.33	34.62	11	0.90		36.00	10.20	0.55	1.87

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h°* rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 8: Performance statistics of PLS calibration models developed using combined data from Citrusdal and Porterville to predict quality parameters of samples harvested from Paarl.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R[h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Citrusdal + Porterville		185	70.22	4.02	5.73	3	0.93	1.47		-0.02	0.87	
		Paarl	94	71.41	4.42	6.19	3	0.91		1.92	-0.49	0.89	2.30
C* [b]	Citrusdal + Porterville		181	61.83	5.36	8.67	4	0.89	2.23		-0.04	0.79	
		Paarl	94	60.31	5.50	9.12	4	0.80		3.47	1.09	0.64	1.59
h° [c]	Citrusdal + Porterville		184	81.11	8.81	10.86	3	0.96	2.58		0.15	0.94	
		Paarl	93	82.36	7.81	9.48	3	0.95		2.56	0.99	0.9	3.05
Rind dry matter	Citrusdal + Porterville		141	31.31	2.93	9.35	4	0.87	1.40		-0.01	0.79	
(%)		Paarl	90	35.83	4.66	13.01	4	0.91		2.65	-1.74	0.74	1.76
Rind fructose	Citrusdal + Porterville		185	100.40	25.99	25.89	6	0.80	16.14		0.44	0.63	
(mg/g DM)		Paarl	87	86.29	19.51	22.60	6	0.84		14.73	-1.91	1.19	1.32
Rind glucose	Citrusdal + Porterville		185	66.87	22.55	33.72	9	0.79	13.74		0.54	0.64	
(mg/g DM)		Paarl	91	62.83	18.59	29.59	9	0.78		22.82	8.49	16.82	0.81
Rind sucrose	Citrusdal + Porterville		184	36.09	13.95	38.64	8	0.73	9.44		0.04	0.59	
(mg/g DM)		Paarl	94	35.48	11.37	32.06	8	0.58		10.77	-0.52	0.82	1.06
Rind total sugars	Citrusdal + Porterville		182	203.30	57.97	28.51	11	0.85	23.11		0.33	0.75	
(mg/g DM)		Paarl	93	184.60	42.52	23.03	11	0.87		28.68	-3.12	1.19	1.48

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h*° rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 9: Performance statistics of PLS calibration models developed using combined data from Porterville and Paarl to predict quality parameters of samples harvested from Citrusdal.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV % [f]	LVs [g]	R [h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	Porteville + Paarl		188	70.70	4.48	6.34	2	0.93	1.62		0.00	0.87	
		Citrusdal	93	70.53	3.62	5.14	2	0.93		1.64	0.61	0.89	2.22
C* [b]	Porteville + Paarl		186	60.67	5.55	9.15	5	0.88	2.62		0.00	0.75	
		Citrusdal	95	62.49	5.07	8.11	5	0.87		2.68	-0.88	0.84	1.89
h° [c]	Porteville + Paarl		186	80.89	8.10	10.01	3	0.95	2.57		0.00	0.90	
		Citrusdal	93	82.77	9.09	10.98	3	0.97		2.69	-1.64	0.99	3.38
Rind dry matter	Porteville + Paarl		188	34.47	4.67	13.56	4	0.93	1.17		0.02	0.88	
(%)		Citrusdal	96	31.00	3.23	10.42	4	0.77		1.96	-1.05	0.78	1.54
Rind fructose	Porteville + Paarl		187	90.25	24.82	27.50	6	0.83	13.53		-0.37	0.74	
(mg/g DM)		Citrusdal	98	105.40	22.09	20.96	6	0.88		11.20	-4.05	0.76	1.97
Rind glucose	Porteville + Paarl		187	63.03	21.34	33.86	9	0.91	8.28		-0.40	0.89	
(mg/g DM)		Citrusdal	96	69.94	20.82	29.77	9	0.90		12.66	8.89	0.74	1.64
Rind sucrose	Porteville + Paarl		183	37.66	14.70	39.03	8	0.73	9.91		0.00	0.53	
(mg/g DM)		Citrusdal	91	32.77	9.06	27.65	8	0.56	6.36	6.36	-1.95	0.60	1.42
Rind total sugars	Porteville + Paarl		187	190.90	55.83	29.25	11	0.92	22.14		-0.08	0.89	
(mg/g DM)		Citrusdal	95	208.10	49.09	23.59	11	0.88		34.38	25.23	0.78	1.43

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h°* rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 10: Model performance for all orchard locations in 2012 grouped together to predict individual quality parameter of fruit from Stellenbosch during 2011 season.

	Calibration	Prediction	n [d]	Mean	SD [e]	CV% [f]	LVs [g]	R [h]	RMSECV [i]	RMSEP [j]	Bias	Slope	RPD [k]
L* [a]	2012		288	70.64	4.20	5.94	3	0.92	1.61		0.00	0.85	
		2011	78	70.92	2.67	3.77	3	0.94		3.93	-3.82	0.82	0.68
C* [b]	2012		288	61.30	5.45	8.89	7	0.89	2.53		0.00	0.78	
		2011	78	66.05	3.82	5.79	7	0.65		4.02	-2.72	0.51	0.95
h° [c]	2012		288	81.55	8.48	10.40	3	0.94	2.87		0.00	0.89	
		2011	72	72.82	5.56	7.64	3	0.94		1.91	-0.12	0.86	2.91
Rind dry matter	2012		233	33.02	4.28	12.95	6	0.92	1.67		0.00	0.84	
(%)		2011	75	28.12	3.19	11.34	6	0.90		4.57	4.35	0.88	0.70
Rind fructose	2012		266	95.73	24.91	26.02	12	0.91	10.09		0.00	0.83	
(mg/g DM)		2011	74	120.6	35.09	29.10	12	0.92		14.03	-2.69	0.70	2.50
Rind glucose	2012		266	65.54	21.37	38.62	12	0.90	9.25		0.00	0.81	
(mg/g DM)		2011	73	80.70	31.09	38.53	12	0.91		15.24	5.09	0.64	2.04
Rind sucrose	2012		264	35.89	13.14	36.60	8	0.96	9.45		0.00	0.48	
(mg/g DM)		2011	72	92.68	41.22	44.47	8	0.69		59.96	-48.94	0.16	0.69
Rind total sugars	2012		264	197.20	54.02	27.40	10	0.85	27.26		0.00	0.72	
(mg/g DM)		2011	72	294.00	94.95	32.30	10	0.91		49.13	-26.60	0.57	1.93

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h*° rind hue angle, [d]*n* population size, [e]*SD* standard deviation, [f]*CV %* coefficient of variation, [g]*LV* latent variables, [h]*R* correlation coefficient between Vis/NIRS-predicted and measured values, [i]*RMSECV* root mean square error of cross validation, [j]*RMSEP* root mean square error of prediction, [k]*RPD* residual predictive deviation.

Table 11: Model performance for all orchard locations and seasons grouped together.

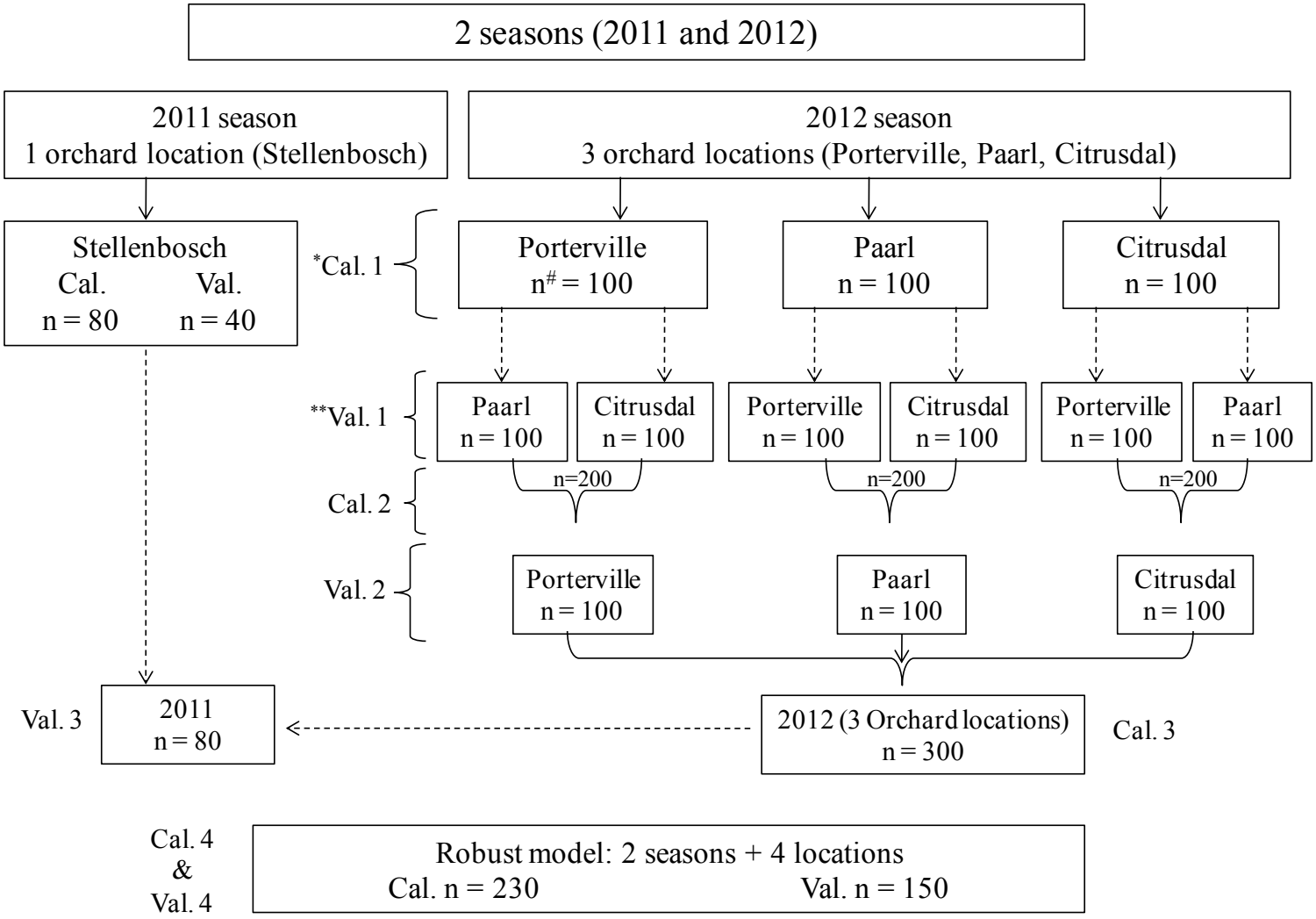
Quality parameter		Calibration model				Validation model					
	LVs [d]	n [e]	R_c [f]	RMSEC [g]	Slope	n	R_v [h]	RMSEP [i]	Slope	RPD [j]	Bias
L* [a]	3	216	0.91	1.55	0.83	151	0.91	1.69	0.84	2.32	0.02
C* [b]	5	212	0.89	2.36	0.80	151	0.87	2.63	0.86	2.09	0.14
h° [c]	3	203	0.96	2.42	0.93	144	0.93	2.62	0.94	3.32	0.33
Rind dry matter (%)	8	150	0.89	2.04	0.78	144	0.89	1.99	0.78	2.28	-0.22
Rind fructose (mg/g DM)	5	158	0.78	16.34	0.61	144	0.80	17.77	0.54	2.18	-1.47
Rind glucose (mg/g DM)	6	184	0.77	14.53	0.59	146	0.78	15.69	0.55	2.18	-1.01
Rind sucrose (mg/g DM)	7	184	0.78	15.63	0.62	144	0.78	18.88	0.56	1.63	1.00
Rind total sugars (mg/g DM)	8	185	0.81	38.05	0.75	140	0.84	41.49	0.59	2.02	-4.31

[a]*L** rind Lightness, [b]*C** rind chroma, [c]*h*° rind hue angle, [d]*LV* latent variables, [e]*n* population size, [f] R_c correlation coefficient between Vis/NIRS-predicted and measured values during calibration, [g] R_v correlation coefficient between Vis/NIRS-predicted and measured values during validation, [h]*RMSEC* root mean square error of calibration, [i]*RMESP* root mean square error of prediction, [j]*RPD* residual predictive deviation.

Table 12: Performance statistics of rind breakdown (RBD) PLS-DA calibration models for individual orchard, two orchards combined, and two season models. These models were based on binary RBD values, where 0 and 1 represented fruit without and with the disorder, respectively.

Calibration	Prediction	n [a]	Mean	SD [b]	CV % [c]	LVs [d]	R [e]	RMSECV [f]	RMSEP [g]	Bias	Slope	RPD [h]
Porterville		94	0.45	0.86	1.92	7	0.63	0.32		0.00	0.40	
	Paarl	93	0.33	0.82	2.45	7	0.64		0.30	-0.01	0.40	1.22
	Citrusdal	90	0.38	0.90	2.35	7	0.28		0.39	-0.10	0.14	1.11
Paarl		94	0.33	0.82	2.45	7	0.67	0.28		0.00	0.45	
	Porterville	86	0.45	0.86	1.92	7	0.47		0.42	-0.17	0.22	1.16
	Citrusdal	93	0.38	0.90	2.35	7	0.44		0.34	0.04	0.25	1.11
Citrusdal		93	0.38	0.90	2.35	7	0.56	0.31		0.00	0.31	
	Paarl	96	0.33	0.82	2.45	7	0.43		0.44	-0.24	0.37	1.22
	Porterville	91	0.45	0.86	1.92	7	0.18		0.58	-0.36	0.11	1.16
Citrusdal + Paarl		187	0.36	0.86	2.39	6	0.52	0.32		0.00	0.27	
	Porterville	93	0.45	0.86	1.92	6	0.43		0.42	-0.15	0.19	1.16
Citrusdal + Porterville		188	0.41	0.88	2.12	6	0.49	0.35		0.00	0.24	
	Paarl	92	0.33	0.82	2.45	6	0.52		0.32	0.00	0.33	1.22
Porterville + Paarl		186	0.39	0.84	2.16	6	0.52	0.35		0.00	0.27	
	Citrusdal	93	0.38	0.90	2.35	6	0.63		0.30	-0.04	0.38	1.11
2012		284	0.39	0.86	2.22	6	0.51	0.34		0.00	0.26	
	2011	79	0.51	0.97	190.01	6	-0.03		0.51	0.23	0.00	1.04

[a] *n* population size, [b] *SD* standard deviation, [c] *CV %* coefficient of variation, [d] *LV* latent variables, [e] *R* correlation coefficient between Vis/NIRS-predicted and measured values, [f] *RMSECV* root mean square error of cross validation, [g] *RMSEP* root mean square error of prediction, [h] *RPD* residual predictive deviation.



*Cal. = Calibration; **Val. = Validation; #n = number of samples

Fig. 1: Chronological sequence of experimental design and distribution of samples during model development.

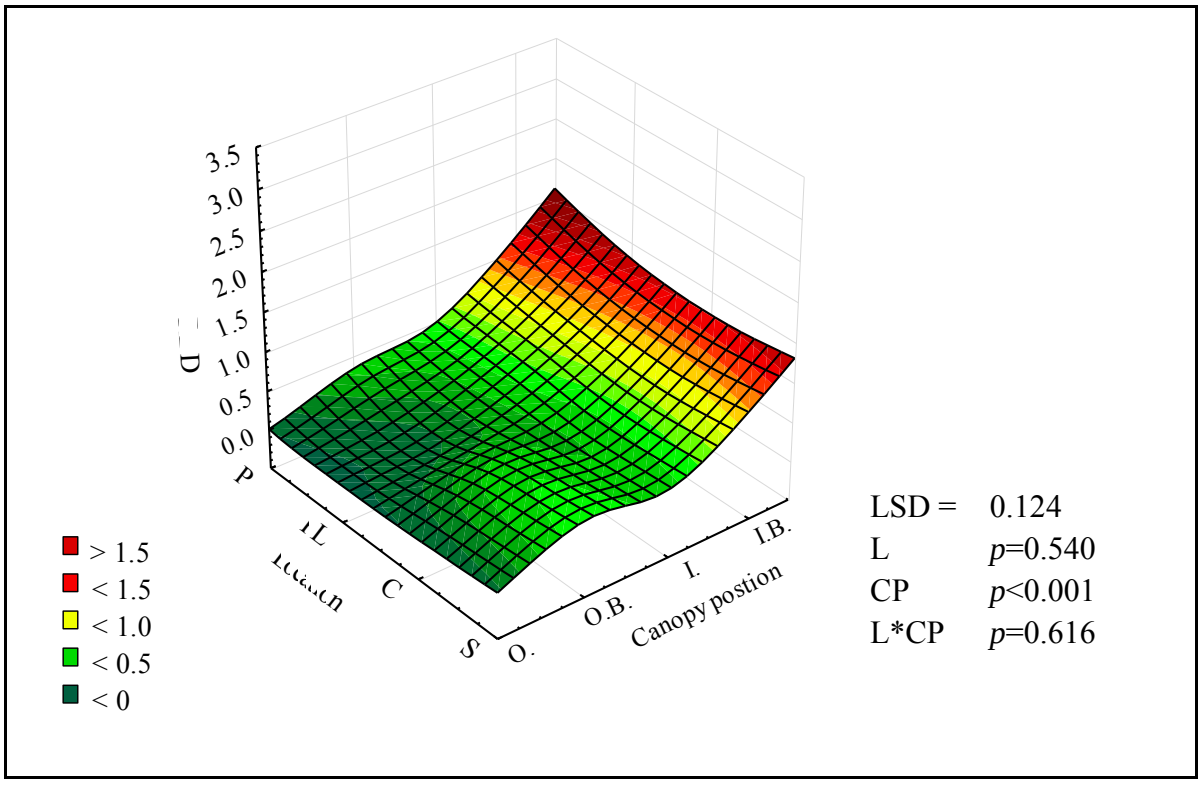


Fig. 2: Effect of fruit position within canopy (O. = outside; O.B. = outside bagged; I. = inside; I.B. = Inside bagged) and growing location (P = Porterville; PL = Paarl; C = Citrusdal; S = Stellenbosch) on the incidence of rind breakdown disorder (RBD) of 'Nules Clementine' mandarin during 2011 and 2012 seasons. RBD was measured from no (0), little (1), moderate (2) and severe RBD (3). *LSD*, least significant difference; *L*, orchard location; *CP*, preharvest canopy position treatments; *L*CP*, interaction between orchard location and preharvest treatments.

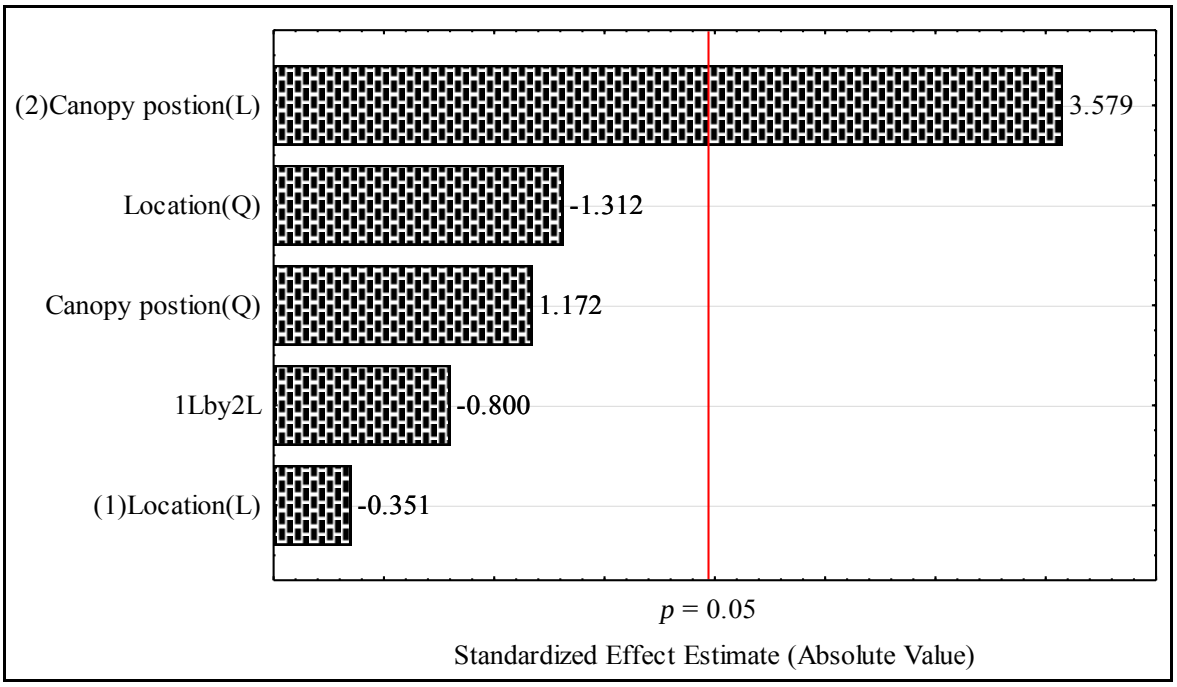


Fig. 3: Pareto chart demonstrating individual and interactive effect of fruit position within the canopy (2) and growing location (1) on the development of RBD of 'Nules Clementine' mandarin fruit observed after 8 weeks of storage. '*L*' and '*Q*' are linear and quadratic effect, respectively.

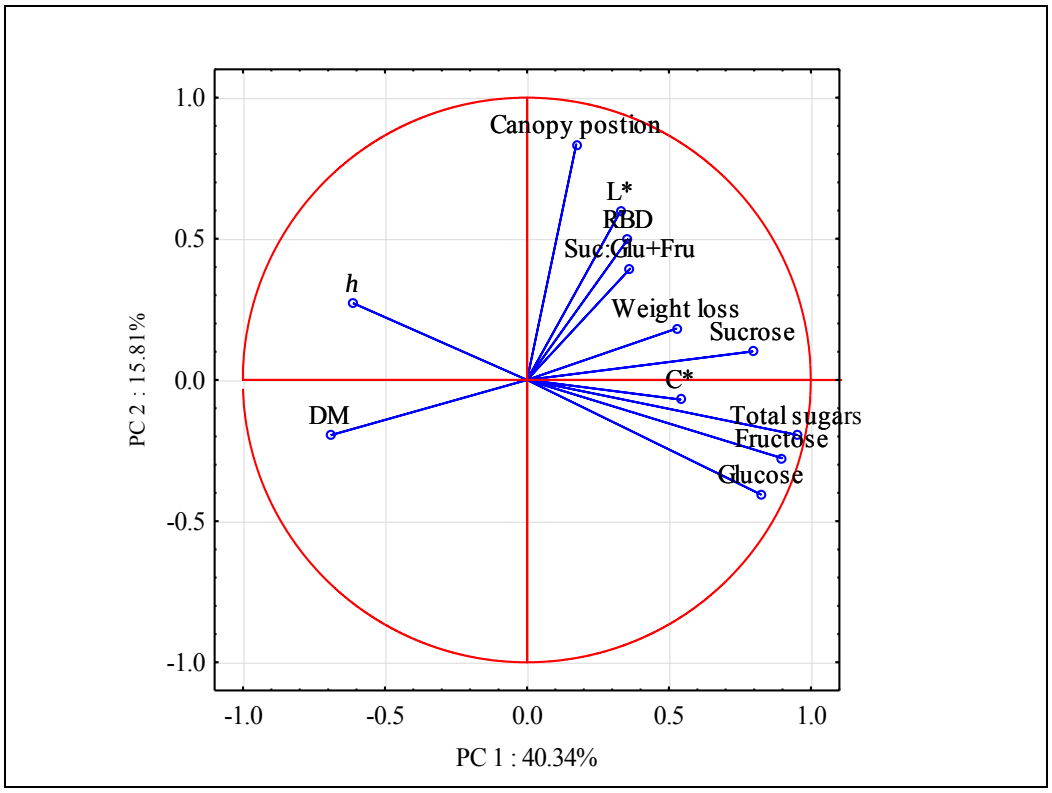


Fig. 4: Principal component analysis (PCA) similarity map of the first two principal components showing a correlation between measured rind physico-chemical properties and susceptibility of fruit to progressive rind breakdown (RBD) in 'Nules Clementine' mandarin. DM = dry matter content; L* = lightness; C* = Chroma; *h* = hue angle; Suc:Fru+Glu = non-reducing (sucrose) to reducing (glucose + fructose) ratio.

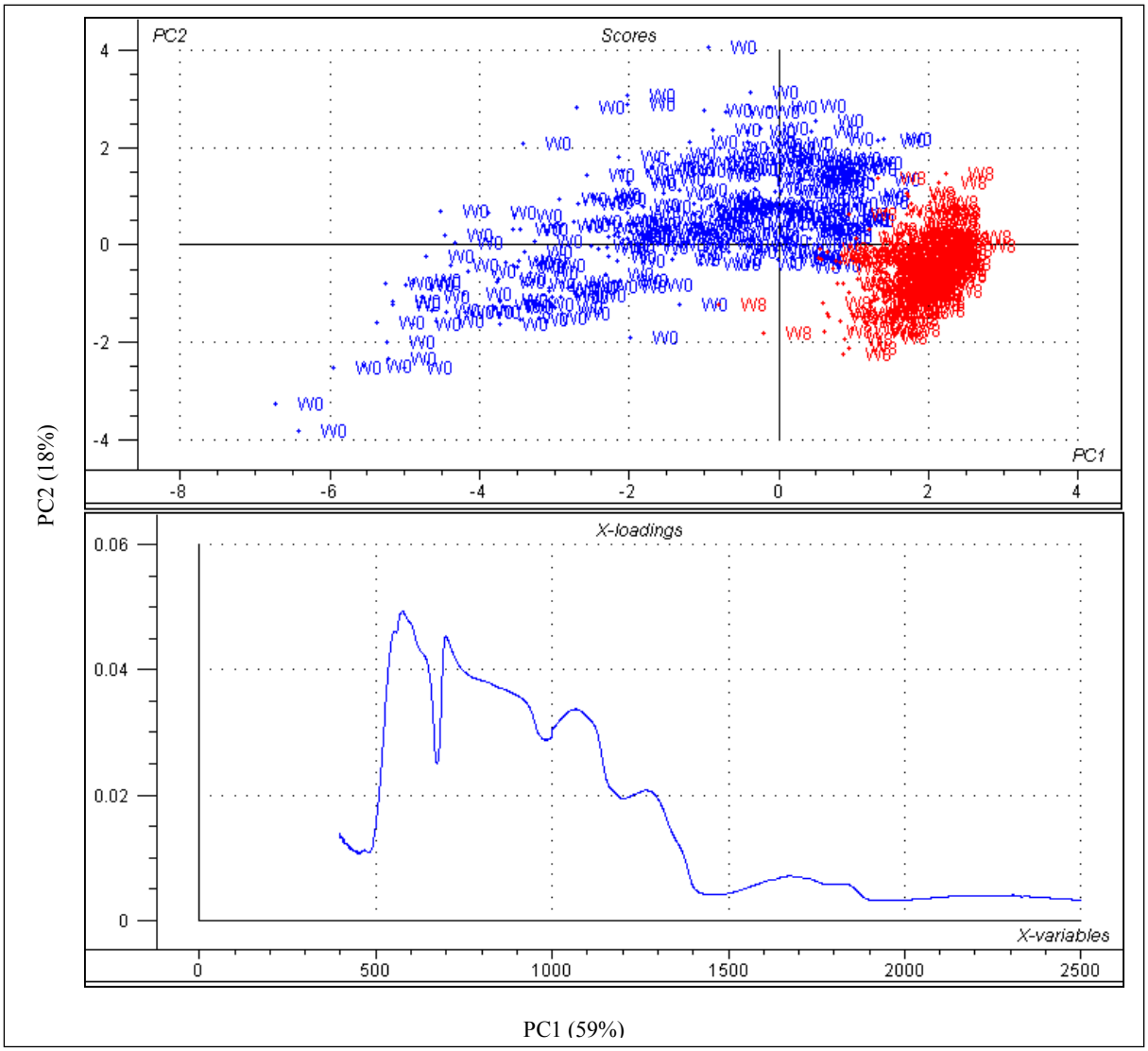


Fig. 5: Principal component analysis (PCA) similarity map for the first two principal components (PCs) (A) and spectral pattern 1 (B), showing 'Nules Clementine' mandarin fruit variations at harvest (W0, blue) and after 8 weeks of postharvest storage in 8±0.5°C (W8, red).

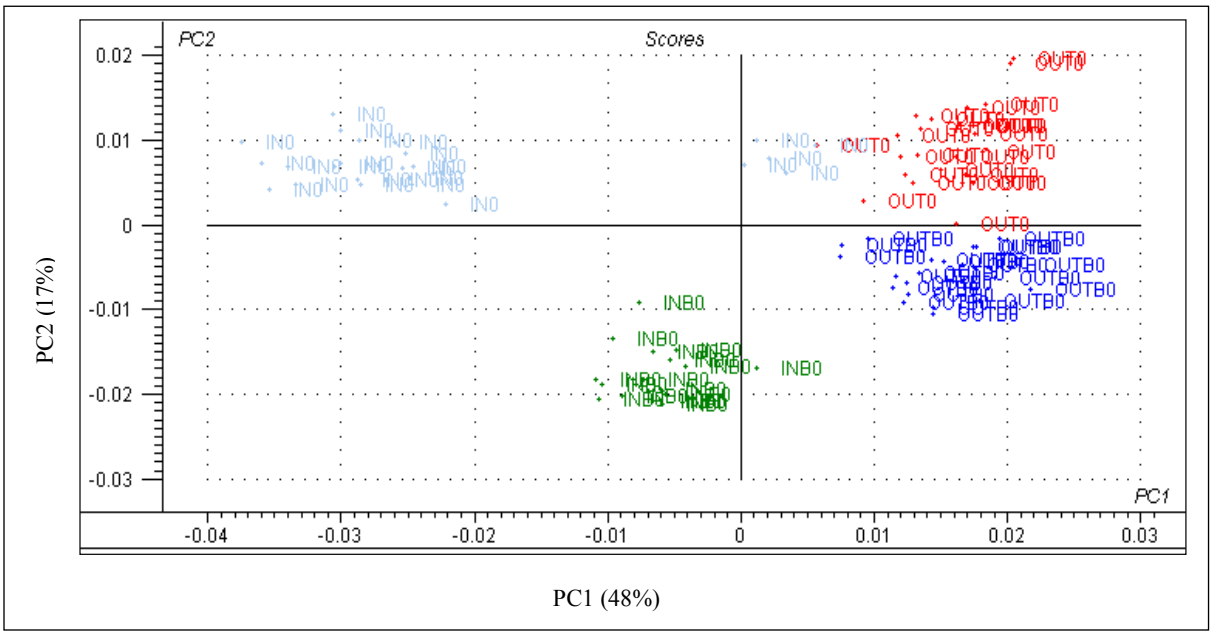


Fig. 6: Principal component analysis (PCA) similarity map determined by principal components (PCs) 1 & 2 showing clusters of fruit harvested from different canopy positions from Porterville. Out0 (red), OutB0 (blue), In0 (light blue), and InB0 (green), represent fruit from outside the canopy, bagged fruit from outside the canopy, fruit from inside the canopy and bagged fruit from inside the canopy, respectively.

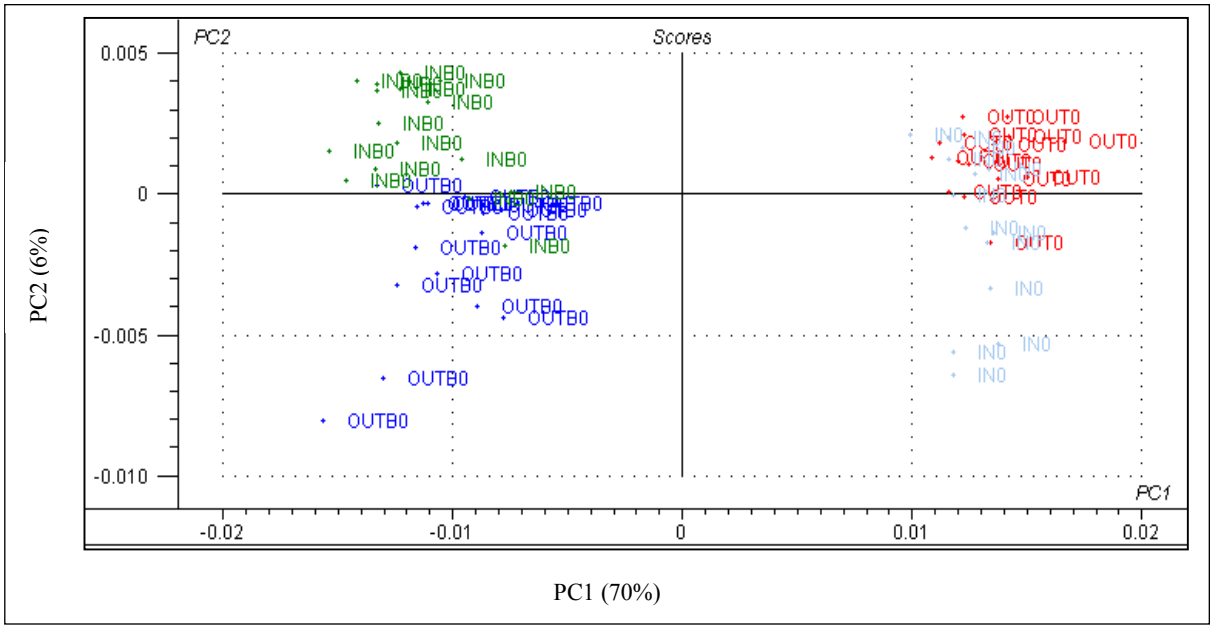


Fig. 7: Principal component analysis (PCA) similarity map determined by principal components (PCs) 1 & 2 showing clusters of fruit harvested from different canopy positions from Stellenbosch. Out0 (red), OutB0 (blue), In0 (light blue), and InB0 (green), represent fruit from outside the canopy, bagged fruit from outside the canopy, fruit from inside the canopy and bagged fruit from inside the canopy, respectively.

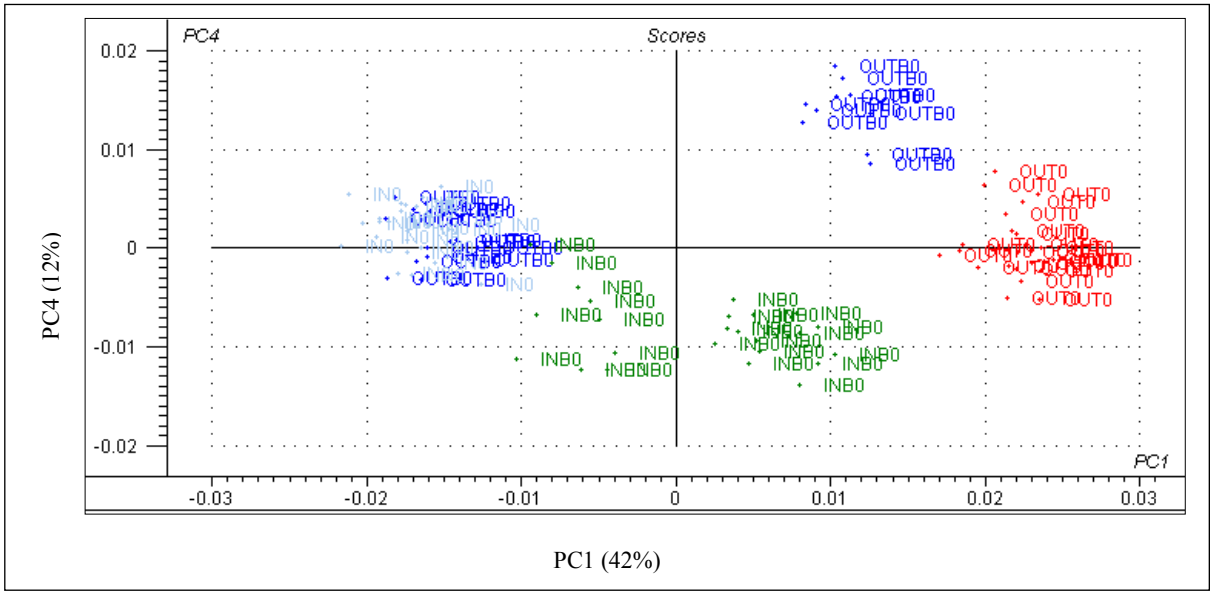


Fig. 8: Principal component analysis (PCA) similarity map determined by principal components (PCs) 1 & 2 showing clusters of fruit harvested from different canopy positions from Paarl. Out0 (red), OutB0 (blue), In0 (light blue), and InB0 (green), represent fruit from outside the canopy, bagged fruit from outside the canopy, fruit from inside the canopy and bagged fruit from inside the canopy, respectively.

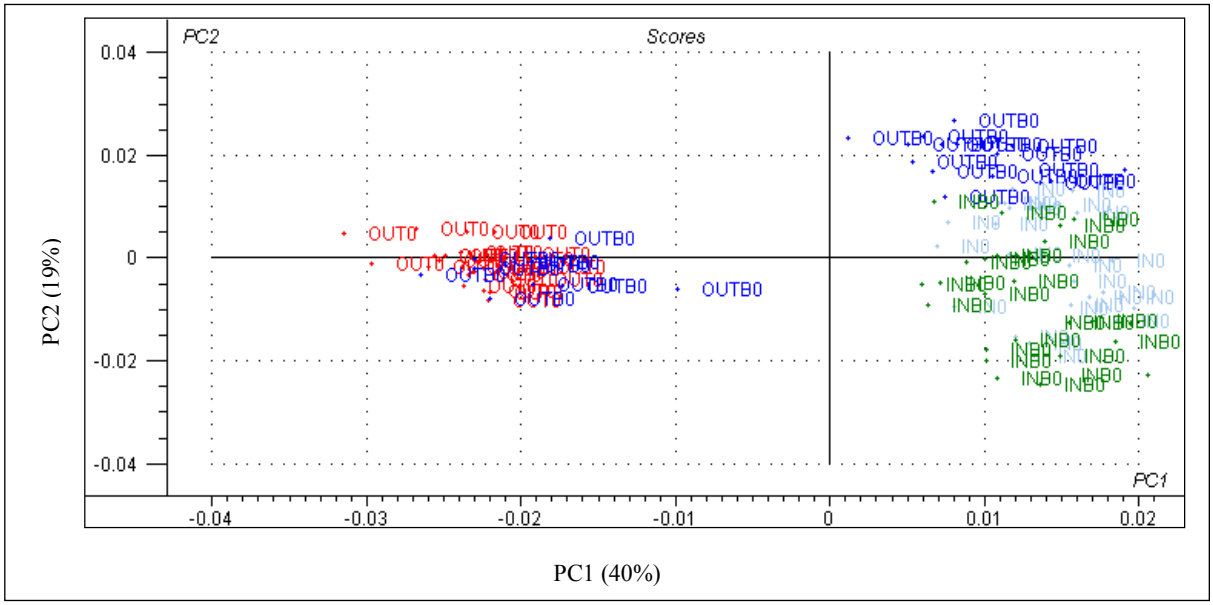


Fig. 9: Principal component analysis (PCA) similarity map determined by principal components (PCs) 1 & 2 showing clusters of fruit harvested from different canopy positions from Citrusdal. Out0 (red), OutB0 (blue), In0 (light blue), and InB0 (green), represent fruit from outside the canopy, bagged fruit from outside the canopy, fruit from inside the canopy and bagged fruit from inside the canopy, respectively.

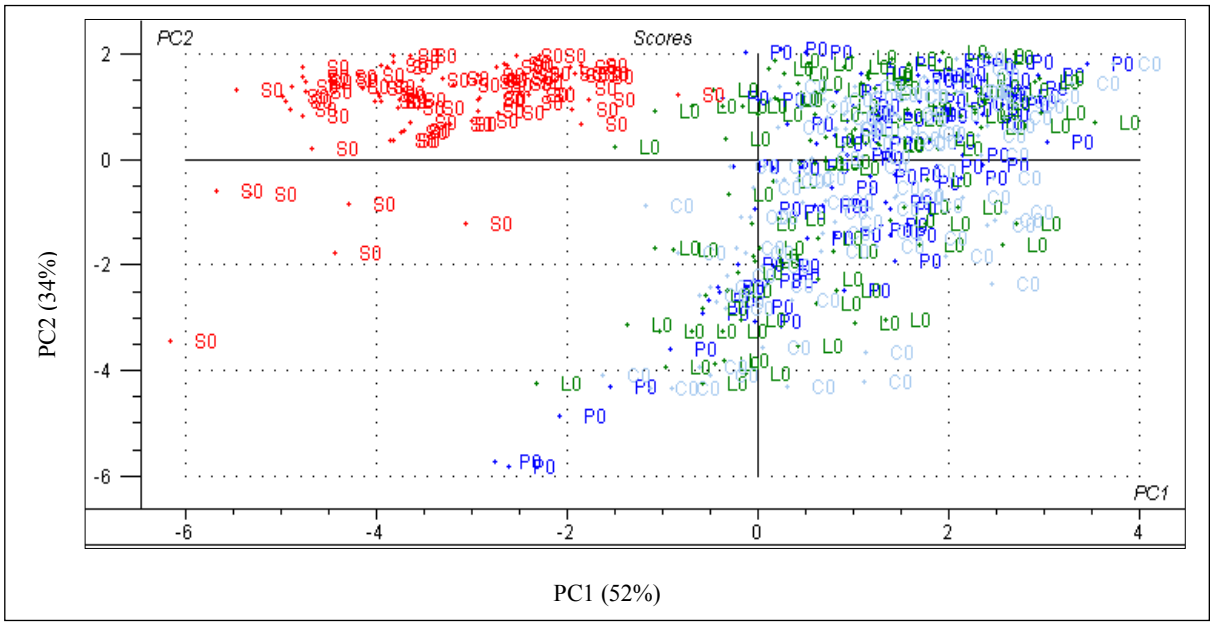


Fig. 10: Principal component analysis (PCA) similarity map determined by principal components (PCs) 1 & 2 showing clusters of fruit harvested during 2011 (Stellenbosch; S0) and 2012 (Porterville = P0; Paarl = L0; Citrusdal = C0) seasons).

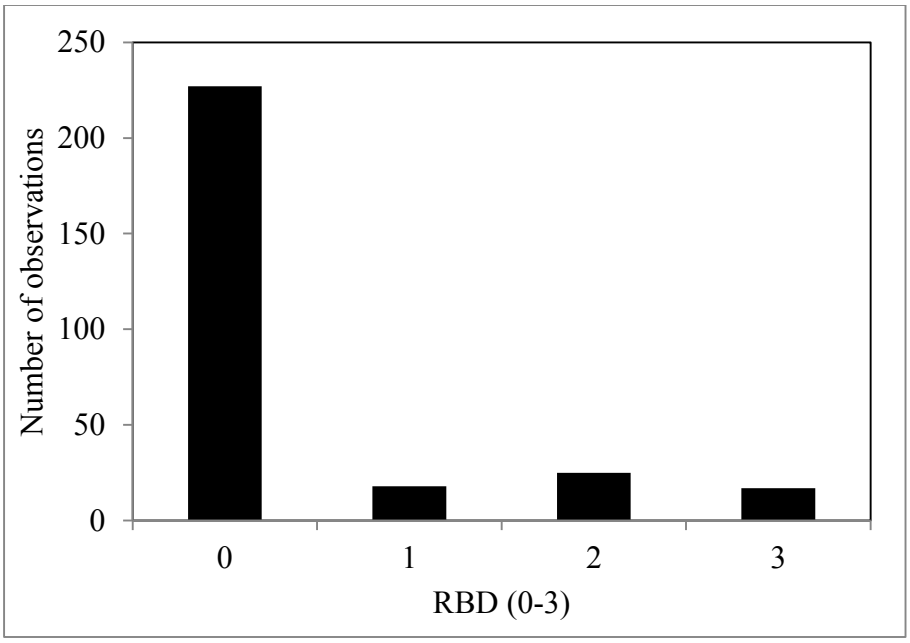


Fig. 11: Histogram of normal distribution of rind breakdown disorder (RBD) occurrence on 'Nules Clementine' mandarin after 8 weeks of postharvest storage. RBD was measured from no (0), little (1), moderate (2), and severe RBD (3).

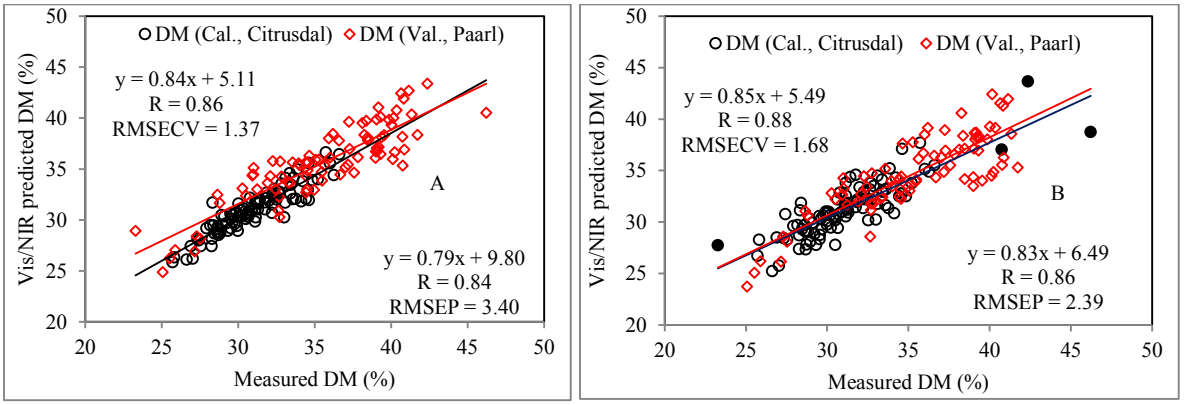


Fig. 12: Scatter plots of Vis/NIR predicted versus measure values of dry matter content (%). 'Nules Clementine' mandarins from Citrusdal were used for the model calibration (open black circles) while those from Paarl were used for model validation (red diamond). A displays the calibration model without spiking and in B, the calibration model was spiked with four data points from the validation (closed black circles).

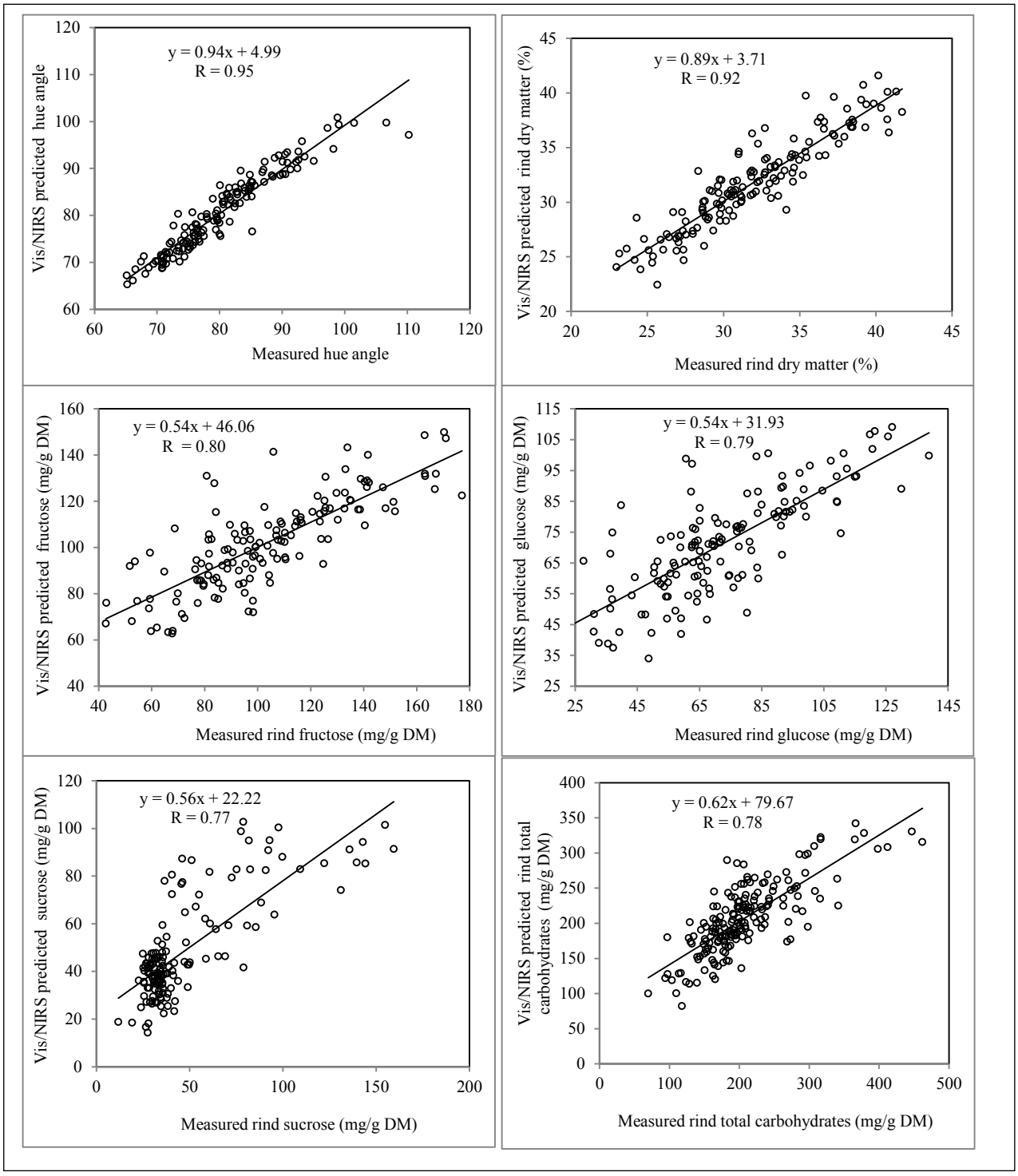


Fig. 13: Scatter plots for Vis/NIRS predicted versus measured values of hue angle (A), dry matter content (B), fructose (C), glucose (D), sucrose (E) and total carbohydrates (F).

CHAPTER 8

RESEARCH RESULTS

PAPER 7:

APPLICATION OF OPTICAL COHERENCE TOMOGRAPHY TO NON-DESTRUCTIVELY CHARACTERISE RIND BREAKDOWN DISORDER OF 'NULES CLEMENTINE' MANDARINS*

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APPLICATION OF OPTICAL COHERENCE TOMOGRAPHY TO NON-DESTRUCTIVELY CHARACTERISE RIND BREAKDOWN DISORDER OF 'NULES CLEMENTINE' MANDARINS

Abstract

A trend in postharvest biology and technology is the adoption of non-destructive techniques of quality assessment. Optical coherence tomography (OCT) is one such non-invasive analytical technique currently available to researchers and is suitable for examining the internal structure of plant tissues. In this study, the feasibility of OCT for imaging histological changes associated with the development of a progressive rind breakdown (RBD) disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco.) was investigated. The investigation utilised fruit with different levels of the disorder, carefully selected from a batch of fruit stored for eight weeks at 8±0.5°C. Images of healthy and RBD-affected intact mandarin fruit were acquired using a Thorlabs OCT system based on a broadband 930 nm source. OCT provided high resolution 2D images of fruit rind to a depth of about 1.1 mm. Immediate and non-destructive acquisition of images showing histological and microstructural features of intact rind tissues was demonstrated. The oil glands stayed intact in unaffected fruit and gradually collapsed on RBD affected fruit. At advanced stages of the disorder, the collapsed oil glands became increasingly deformed and flattened. The study showed that OCT is a promising technique for immediate, real-time and non-destructive acquisition of images showing histological and microstructural rind features of 'Nules Clementine' mandarin fruit.

Keywords: Citrus · Oil glands · Histological characteristics · Microstructural · Fruit quality.

1. Introduction

Fresh citrus fruit are prone to various types of postharvest physiological rind disorders, manifested by a multitude of symptoms during handling and storage (Magwaza et al., 2012a; Magwaza et al., 2013). A postharvest physiological rind disorder of 'Nules Clementine' mandarin, commonly referred to as rind breakdown (RBD) is among several commercially important defects affecting the citrus industry (Cronje et al., 2011a, b). RBD is characteristically progressive and starts showing symptoms during storage, about 3–5 weeks postharvest (Cronje et al., 2011b). In export terms, the development of visible symptoms usually coincides with commercial shipping period and/or point of sale. This is therefore extremely problematic as rind disorders can lead to tremendous financial losses and customer complaints (Cronje et al., 2011a).

During early stages, visual symptoms of RBD on 'Nules Clementine' mandarin are manifested as small, irregular, and slightly sunken patches of about 3–6 mm in diameter randomly scattered about the flavedo (the outer-most, pigmented part of citrus rind) of the fruit (Cronje et al., 2011a; Magwaza et al., 2013). The sunken areas associated with RBD occur directly above and among the oil glands of the affected flavedo. RBD is anatomically similar to other non-chilling rind physiological such as rind staining and breakdown of 'Navel' orange and mandarin (Agusti at el., 2001; Lafuente and Zacarìas, 2006), and noxan of 'Shamouti' orange (Ben-Yehoushua et al., 2001). In all these disorders, the affected areas coalesce, producing larger affected areas, turning reddish-brown to dark-brown, and becoming dry and necrotic in the severe stages of the disorder with extended storage period (Alférez et al., 2003; Alférez and Burns, 2004; Assimakopoulou et al., 2009).

Currently, the microstructural changes associated with these disorders on citrus fruit are evaluated using convensional methods such as light microscopy and transmission electron microscopy (Chikaizumi, 2000; Vitor et al., 2000; Cajuste et al., 2011). These microscopic techniques can be expensive and require laborious and specialised destructive sample preparation. Monitoring the internal structural changes of intact fruit using such techniques is therefore difficult and sometimes impossible. Recently, the trend has shifted towards developing reliable and cost effective technologies to non-destructively monitor fruit physiological disorders (Nicolaï et al. 2007; Magwaza et al., 2012b). Non-destructive instrument-based methods are

preferred because they allow the measurement and analysis of individual fruit, reduce waste and permit repeated measures on the same item over time.

The near infrared (NIR) spectroscopy-based imaging systems known as optical coherence tomography (OCT) is one such non-invasive and real-time analytical technique currently available to researchers and is suitable for examining the internal structure of plant tissues. The method is based on the interferometric measurements of NIR light back-scattering from the fruit (Huang et al., 1991; Sapozhnikova et al., 2003; Ford et al., 2012). The non-invasive NIR radiation used in an OCT system is suitable and safe for examining intact organs due to its non-ionising characteristics (Fercher et al., 2003; Kemsley et al., 2008). This provides the potential means to observe and monitor histological changes of plant tissues during postharvest development of rind physiological disorders (Loeb and Barton, 2003).

OCT can be used to visualise not only plant tissues and tissue boundaries but also the information on the shapes and sizes of individual cells (Sapozhnikova et al., 2004; Hrebesh et al., 2009; Meglinski et al., 2010). OCT technology enables non-destructive and contactless cross-sectional imaging of internal structures of biological objects producing 2-D or even 3-D images of plant tissues at a penetration depth of up to 2 mm from the surface with 5–20 μm resolution in scattering media such as fruit (Loeb and Barton, 2003; Sapozhnikova et al., 2003; Meglinski et al., 2010; Verboven et al., 2013). The aim of this study was to investigate the feasibility of OCT as a non-destructive technique for imaging histological changes associated with the development of RBD of 'Nules Clementine' mandarins.

2. Materials and methods

2.1. Plant material

One hundred and twenty 'Nules Clementine' mandarin (*Citrus reticulata* Blanco.) fruit were harvested from 15 uniform marked trees in a commercial orchard at Citrusdal, Western Cape Province, South Africa (32° 25' 22" South, 19° 00' 53" East). Fruit were harvested on 16 May 2012, at commercial maturity, and received all normal postharvest treatments, including fungicides and wax application according to industry practices. Fruit were packed in cartons and couriered by air at ambient temperature to Cranfield University in the United Kingdom, arriving

within 48 hours. Upon arrival fruit were stored for eight weeks in a temperature controlled room at 8±0.5°C; a temperature known to cause the highest incidence of RBD (Khumalo, 2006; Magwaza et al., 2013). During cold storage, fruit were visually examined and scored at weekly intervals for the development of RBD symptoms over 8 weeks. RBD was scored by visual inspection on a subjective scale from 0 = no breakdown to 3 = severe breakdown in order to quantify incidence as well as severity. RBD index was calculated according to the formula previously reported for chilling injury and rind pitting by (Alférez and Burns, 2004; Magwaza et al., 2012a).

At week 8, a total of 12 intact mandarin fruit with different levels of RBD were selected so that they could be examined with the OCT system. For these evaluations the fruit were sorted into the four severity levels (0–3) from which three representative fruit were used as replicates.

2.2. OCT scanning experimental system

A 'spectral radar' OCT system (Fig. 1), using a 930 nm broadband, near-infra-red super-luminescent diode source (Thorlabs Ltd., UK), was used for this study. The OCT system was based upon low-coherence interferometry; oscillating signals are produced, the frequency of which corresponds to path-length differences between structural discontinuities in the sample and the surface of a reference mirror (Ford et al., 2012; Landahl et al. 2012). The specified resolution of the system was 7 μm with maximum accessible optical depth of 1.6 mm in air, but lower in a high refractive-index medium. In the present study, horizontal scan lengths of 1–4 mm were used, with the horizontal sampling number fixed at a value of 1000 horizontally and 512 vertically. The samples were mounted on a translation stage which was scanned at a constant rate perpendicular to the image plane. In most cases, only one oil gland was contained within the imaging area. Initially, the translation stage was positioned just outside the range containing one end of the oil gland of interest, and was scanned until the other end of the gland just left the imaging area. The physical distance corresponding to this scan range was recorded from the micrometer screw gauge attached to the stage. Images were acquired continuously, at a constant rate, during the entire scan period. The scan distance was divided by the number of acquired images to obtain the distance step between images in a particular set.

2.3. Automatic and manual image processing

To achieve automatic image processing, techniques were developed using Matlab® (Mathworks® Inc., USA) to demonstrate appropriate techniques. However, automatic processing was only fully successful for images with a clear boundary between the oil gland and surrounding tissue. Therefore, manual processing techniques were also developed. A hybrid approach was adopted in this study whereby the perimeters of oil glands were defined manually using a 'point-and-click' method to define the path around the edge of each gland.

2.4. Area and volume uncertainty

A slight peak in intensity was seen approaching the boundary, followed by a rapid drop on moving into the gland interior. The drop from peak to near-zero intensity happened within the space of 4 pixels, or about 9 μm, which was slightly larger than the depth resolution of the instrument (7 μm), and implied that the boundary could be identified to within about ±2 pixels. In some images, and particularly at the bottom of deep glands, the uncertainty was closer to ±5 pixels. The average error in gland boundary position, whether calculated manually or automatically, was estimated at about 10% while the gland dimension exceeded 70 pixels, and decreased rapidly for larger glands. The typical average error in oil gland areas was estimated to be about 10–20% for ellipsoidal glands, and 20–30% for strongly flattened glands. The error in gland length was estimated to be about 5%, suggesting an uncertainty in volume of 15–25% for ellipsoidal glands, and 25–35% for flattened glands. Knowing the oil gland area for each image and the step size between successive images, the incremental volume corresponding to each slice was calculated and summation carried out to obtain the total volume of the gland.

The theoretical maximum imaging depth of the OCT system at a given centre wavelength is determined by the wavelength sampling interval. There is, however, a drop-off in sensitivity with depth, and in turbid media, depth penetration is limited in practice by scattering and absorption of the input light. The interior of the mandarin oil glands appeared almost completely transparent at the 930 nm OCT wavelength, allowing imaging to about 0.8 mm optical depth through large glands. However, the dense tissues of the surrounding flavedo and albedo were strongly scattering, resulting in data loss below an optical depth of about 0.4 mm for regions with no

glands. In calculating the physical dimension of oil glands from the images, we have assumed a refractive-index value of 1.47 for orange oil, taken from literature (Ahmad et al., 2006).

3. Results

3.1. Visualisation of fruit with different levels of RBD

Image sets were saved for unaffected mandarin samples and at the three stages of progression of the RBD symptom development. Each image, saved as a 1000 x 512 pixel bitmap, was processed to extract the shape and area of oil glands visible within the image area. Representative images of oil glands at different stages of RBD are shown in Fig. 2. The oil glands were intact with almost circular shape in unaffected fruit (Fig. 2a) and gradually collapsed in affected fruit (Fig. 2b–d), depending on the severity of the disorder. At advanced stages of the disorder, the collapsed oil glands became deformed and flattened (Fig. 2c and d).

3.2. Image processing

The automatic image analysis procedure was largely successful for images similar to that shown in Fig. 3. The image contained a single oil gland, the perimeter of which was well-matched by the automatic shape extraction. In results from healthy mandarin samples (Fig. 4), the oil glands were fully expanded, and signal-to-noise was rather poor in deeper regions of the image. The program was often unable to separate the gland from the background completely. For consistency, therefore, manual processing was ultimately employed for all images.

The resulting bitmaps for manual processing of images taken through the middle section of the oil glands are shown in Fig. 5, for an unaffected sample and a sample severely affected by RBD. The pixel areas within the gland images were calculated automatically by the software. From prior knowledge of the physical scanning dimensions, it was then straightforward to calculate gland area and volume.

3.3. Oil gland volume analysis

Volumes of glands obtained from fruit with different levels of the disorder were determined. A histogram was plotted, showing the volume of each oil gland as a function of the degree of RBD (Fig. 6). It is plain that the mean volume of the glands decreases considerably as the disorder progresses. The calculated volumes ranged from 12 nL in fruit with severe disorder to 150 nL for fruit without the disorder. Shrinkage of the oil glands was not homogeneous. In healthy mandarins, the glands were approximately ellipsoidal in shape. Most of the volume reduction occurred through shrinkage in the direction normal to the surface of the fruit, with the result that the glands became severely flattened in samples with advanced RBD. The gland surface also became increasingly irregular as the volume reduced. Re-entrant angles were seen in some of the cross-sections, implying that the tissue had crumpled around the perimeter of the gland. Three dimensional (3D) renderings were generated by stacking the bitmaps of the extracted areas. An iso-surface was fitted to the plots in Fig. 7 which gave the appearance of a solid 3D shape rather than a set of stacked slices.

4. Discussion

This study revealed that oil glands remained intact in unaffected fruit and gradually collapsed in affected fruit, depending on the severity of the disorder. RBD belongs to a group of several physiological disorders collectively referred to in the literature as non-chilling rind physiological disorders; including rind pitting, rind staining, rind breakdown, prteca spots, etc. (Agusti et al., 2001; Cronje et al., 2011a; Magwaza et al., 2013). Although the symptoms and the terminology is the same, the collapse of oil glands during the development of the disorder is one of the important deciding factors on the type of disorder. Results similar to those observed in the current study were reported for postharvest rind pitting on ‘Marsh’ grapefruit, ‘Navelina’ and ‘Navelate’ oranges and ‘Fallgold’ tangerine (Petracek et al., 1998a, b; Alférez and Burns, 2004). In these previous studies, the oil glands were identified as the primary sites of the damage associated with non-chilling disorders. In contrast, the oil glands of ‘Fortune’ (Almela et al., 1992), ‘Encore’ (Medeira et al., 1999; Vitor et al., 2000) and ‘Clementine’ (Assimakopoulou et al., 2009) mandarin affected by non-chilling rind spotting, remained intact and unaffected,

suggesting that these disorders might not be originating from the oil gland disruption. RBD is differentiated appreciably from these latter non-chilling physiological disorders of mandarins by its association with the collapse of the oil glands. Results of the present study supports the hypothesis that oil glands are the primary sites of damage in RBD and that rupture of these oil bodies could, in turn, release phytotoxic oil into the surrounding cells, causing the collapse of the oil gland and damage to adjacent cellular structures (Petracek et al., 1998b; Alférez and Burns, 2004; Cronje et al., 2011a).

Individual cells and cellular structures surrounding the oil glands could not be clearly distinguished on OCT images as previously shown on light, scanning and electron micrographs (Bosabalidis and Tsekos, 1982; Agustí et al., 2001; Knight et al., 2001; Voo et al., 2012). Similarly, in onions (Hrebesh et al., 2009) and apples (Verboven et al., 2013), lateral resolution of OCT images has also been previously reported to be lower than those obtained with transmission and confocal microscopy, respectively. From the two studies, it was concluded that the OCT images were not as detailed as conventional microscopy, due to the longer depth of focus for OCT system. The fundamental limitation of the OCT resolution in the current study was imposed by the source bandwidth, which was about 7000 nm. The dense tissues of intact flavedo and albedo are strongly scattering, resulting in the attenuation of OCT signal, hence, reduced image contrast at greater depth.

Although images from conventional microscopy are known to provide higher resolution than OCT for identification of rind morphological structures (Knight et al., 2001; Cronje et al., 2011a; Voo et al., 2012), it should be noted that both methods have their advantages and drawbacks. OCT is a non-destructive technique that could open new possibilities for monitoring plant physiology and morphology, because repeated measurements can be taken during development. Furthermore, OCT covers a considerably large field of view in both lateral and axial dimensions of the fruit rind, while providing similar information to confocal microscopy (Verboven et al., 2013).

Previous studies have shown that variations in microclimatic conditions during the growing season and within an orchard and even canopy may influence fruit biochemical profile of the rind and subsequently play a significant role in fruit susceptibility to RBD (Cronje et al. 2011a, b; Magwaza et al. 2012a). The ability of OCT to non-destructively visualise oil gland changes associated with RBD also opens new possibilities to monitor changes in gland structure, gland

size, and the number of oil glands per surface area in developing fruit. Understanding gland development and growth rate on rinds of fruit located on different positions of the canopy might be useful in understanding why shaded fruit inside the canopy are more susceptible to RBD than outside fruit (Cronje et al., 2011a; Magwaza et al., 2012a). To further understand the mechanism of RBD, the next logical step in future studies would be targeting specific spots on fruit with potential of developing the disorder and follow it over time.

The inability to automatically process images was not unique to healthy samples. In some RBD affected fruit, there were also difficulties in identifying oil gland areas. Cronje et al. (2011a) reported that once oil leaks out of the gland it tends to cause so much damage to the cells around it that it could become difficult to demarcate the periphery of the oil gland. Therefore, although we successfully demonstrated the potential of OCT in imaging rind microstructural features associated with RBD, the sensitivity of the technique still needs to be enhanced to increase the signal-to-noise ratio, and thereby improve the image resolution. This could be achieved by using a higher wavelength to reduce scattering, and/or a broader spectrum. Essentially, the use of different optical parameters and improved image processing techniques are crucial to improve the performance of this system.

The oil gland volume observed in this study is in agreement with that previously reported on grapefruit by Knight et al. (2001) and Voo et al. (2012). These results show there are strong indications that the gland volume starts to decrease at an early stage during the progression of RBD in the fruit, and that more than an order of magnitude of change in volume reduction occurs during the course of the disorder.

5. Conclusion

OCT is a promising technique for immediate, real-time, and non-destructive acquisition of images showing histological and microstructural rind features of intact ‘Nules Clementine’ mandarin fruit. OCT provided high resolution 2D images of fruit rind to a depth of about 1.1 mm. Using conventional microscopes, these results would require complex sample preparation procedures and take possibly several hours to acquire similar images. The study further demonstrated the power of image processing procedures to compute volume and 3D models of oil glands. RBD was shown to be associated with the progressive collapse of oil

glands and flattening of the flavedo tissue. However, it was observed that the OCT images appeared to have lesser resolution compared to microscopic images reported in the literature, due to the light scattering properties of intact fruit. OCT application as an imaging technique for plant tissues is currently in its infancy and therefore only available to researchers for experimenting purposes. It is therefore critical to note that OCT is currently not a replacement for light, electron or confocal microscopy, but a potential alternative for non-destructive evaluation and monitoring of changes associated with RDB and other rind physiological disorders of citrus fruit.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415–422.

Ahmad, M.M., Rehman, S-R., Anjum, F.M., Bajwa, E.E., 2006. Comparative physical examination of various citrus peel essential oils. Int. J. Agric. Biol. 8, 186–190.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65.

Assimakopoulou, A., Tsougrianis, C., Elena, K., Fasseas, C., Karabourniotis, G., 2009. Pre-harvest rind-spotting in 'Clementine' mandarin. J. Plant Nutr. 32, 1486–1497.

Ben-Yehoshua, S., Peretz, J., Moran, R., Lavie, B., Kim. J.J., 2001. Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*Citrus sinensis*, Osbeck). Postharvest Biol. Technol. 22, 19–27.

Bosabadalidis, A., Tsekos, I., 1982. Ultrastructural studies on the secretory cavities of *Citrus deliciosa* ten. II. Development of the essential oil-accumulating central space of the gland and process of active secretion. Protoplasma 112, 63–70.

Cajuste, J.F., García-Breijo, F.J., Armiñana, R., Lafuente, M.T., 2011. Ultrastructural and histochemical analysis reveals ethylene-induced responses underlying reduced peel collapse in detached citrus fruit. Microsc. Res. Tech. 74, 970–979.

Chikaizumi, S., 2000. Mechanism of rind-oil spot development in 'Encore' (*Citrus nobilis* Lour. X *C. Deliciosa* Ten.) fruit. J. Japan. Soc. Hort. Sci. 69, 149–155.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Fercher, A.F., Drexler, W., Hitzenberger, C.K., Lasser, T., 2003. Optical coherence tomography-principles and applications. Rep. Prog. Phys. 66, 239–303.

Ford, H.D., Tatam, R.P., Landahl, S., Terry, L.A., 2012. Investigation of disease in stored onions using optical coherence tomography. Acta Hort. 945, 247–254.

Hrebesh, M.S., Dabu, R., Sato, M., 2009. In vivo imaging of dynamic biological specimen by real-time single-shot full-field optical coherence tomography. Opt. Commun. 282, 674–683.

Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., Hee, M.R., Flotte, T., Gregory, K., Puliafito, C.A., Fujimoto, J.G., 1991. Optical Coherence Tomography. Science 254, 1178–1181.

Kemsely, E.K., Tapp, H.S., Binns, R., Mackin, R.O., Peyton, A.J., 2008. Feasibility study of NIR diffuse optical tomography on agricultural produce. Postharvest Biol. Technol. 48, 223–230.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Knight, T.G., Klieber, A., Sedgley, M., 2001. The relationship between oil glands and fruit development in Washington Navel orange (*Citrus sinensis* L. Osbeck). Ann. Bot. 88, 1039–1047.

Lafuente, M.T., Zacarìas, L., 2006. Postharvest physiological disorders in citrus fruit. Stewart Postharvest Rev. 2, 1–9.

Landahl, S., Terry, L.A., Ford, H.D., 2012. Investigation of diseased onion bulbs using data processing of optical coherence tomography images. Acta Hort. 969, 261–270.

Loeb, G., Barton, J.K., 2003. Imaging botanical subjects with optical coherence tomography: A feasibility study. Trans. Am. Soc. Agric. Eng. 46, 1751–1757.

Magwaza, L.S., Opara, U.L., Cronje, P.J.R., Landahl, S., Nicolaï, B.M., Terry, L.A., 2013. Non-chilling physiological rind disorders in citrus fruit. Hort. Rev. 41, (in press).

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H., Mouazen, A.M., Saeys, W., Nicolai, B.M., 2012a. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using vis/NIR spectroscopy. Postharvest Biol. Technol. 74, 1–10.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J.R., Saeys, W., Nicolaï, B.M., 2012b. NIR spectroscopy applications for internal and external quality analysis of citrus fruit - a review. Food Bioprocess Tech. 5, 425–444.

Medeira, M.C, Maia, M.I., Vitor, R.F., 1999. The first stages of preharvest peel pitting development in 'Encore' mandarin: a histological and ultrastructural study. Ann. Bot. 83, 667–673.

Meglinski, I.V., Buranachai, C., Terry. L.A., 2010. Plant photonics: application of optical tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I.K., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Petracek, P.D., Dou, H., Pao, S., 1998a. The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biol. Technol. 14, 99–106.

Petracek, P.D., Montalvo, L., Dou, H., Davis, C., 1998b. Postharvest pitting of 'Fallgold' tangerine. J. Am. Soc. Hort. Sci. 123, 130–135.

Sapozhnikova, V.V., Kamenskii, V.A., Kuranov, R.V., 2003. Visualization of plant tissues by optical coherence tomography. Russ. J. Plant Physiol. 50, 282–286.

Sapozhnikova, V.V., Kamensky, V.A., Kuranov, R.V., Kutis, I., Snopova, L.B., Myakov, A.V., 2004. In vivo visualization of Tradescantia leaf tissue and monitoring the physiological and

morphological states under different water supply conditions using optical coherence tomography. Planta 219, 601–609.

Verboven, P., Nemeth, A., Abera, M.K., Bongaers, E., Daelemans, D., Estrade, P., Herremans, E., Hertog, M., Saeys, W., Vanstreels, E., Verlinden, E., Leitner, M., Nicolaï, B., 2013. Optical coherence tomography visualizes microstructure of apple peel. Postharvest Biol. Technol. 78, 123–132.

Vitor, R.F., Lidon, M., Barreiro, M.D., Maia, M.I., Medeira, M.C., Guerreiro, A., 2000. 'Peel pitting' of 'Encore' mandarin fruits: etiology, control and implications in fruit quality. Fruits 56, 27–35.

Voo, S.S., Grimes, H.D., Lange, B.M., 2012. Assessing the biosynthetic capabilities of secretory glands in *Citrus* peel. Plant Physiol. 159, 81–94.

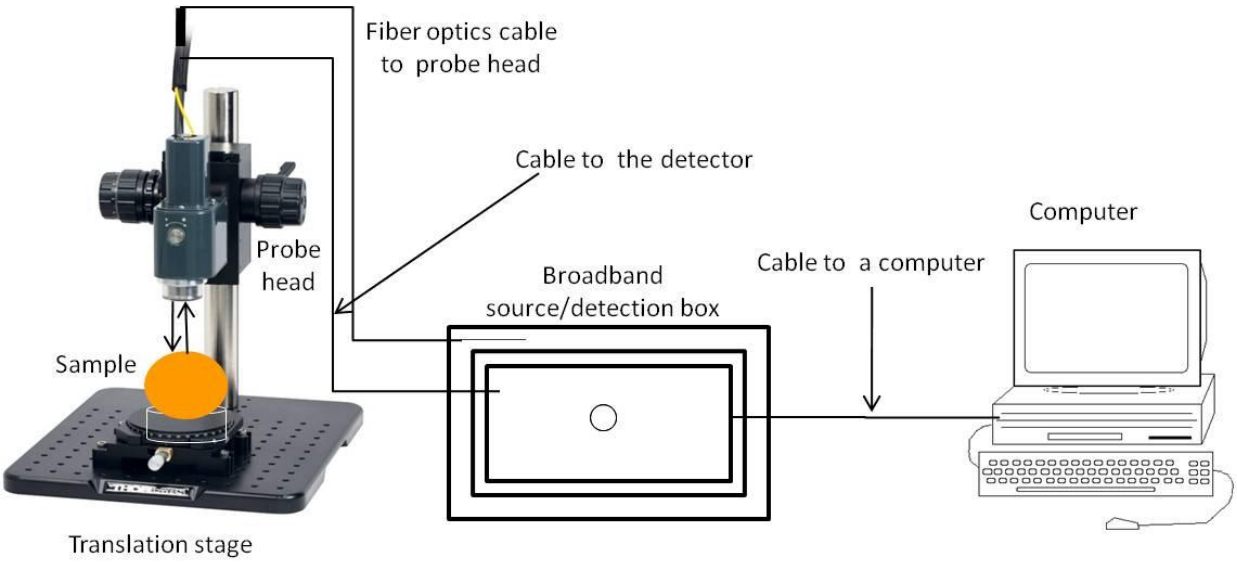

Fig. 1: Schematic diagram of the OCT system used in the experiment.

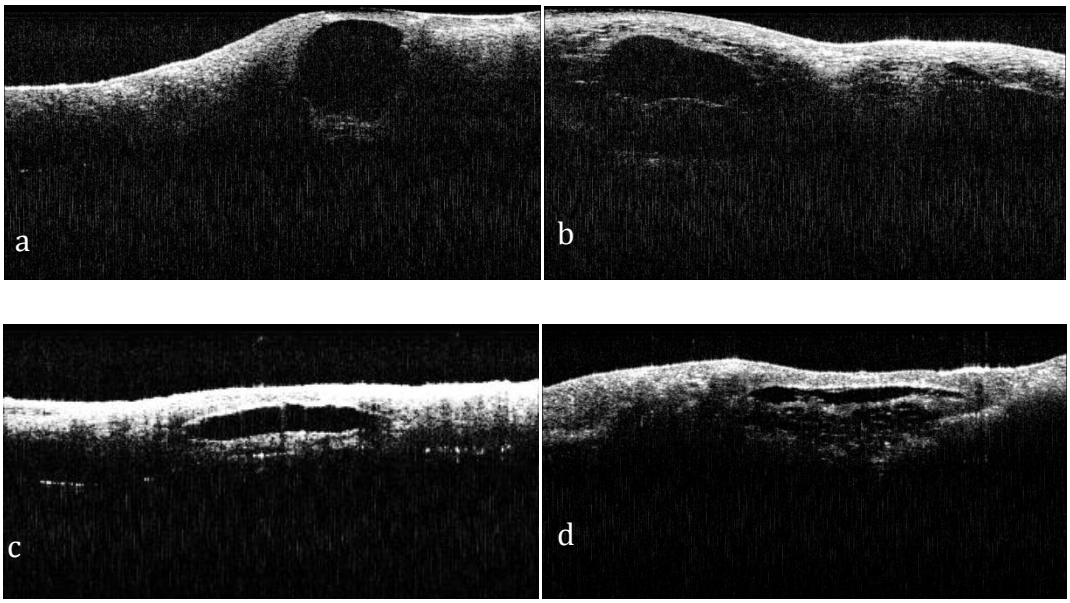

Fig. 2: Cross-sectional OCT images from mandarin rind at four different stages of RBD, with the degree of RBD increasing from 0, unaffected fruit (a); 1, little (b), 2, moderate (c) and 3 severe RBD (d). The oil gland of unaffected fruit (a) was almost circular in shape and flattened as the disorder developed. Images are 2 mm wide x 1.1mm deep.

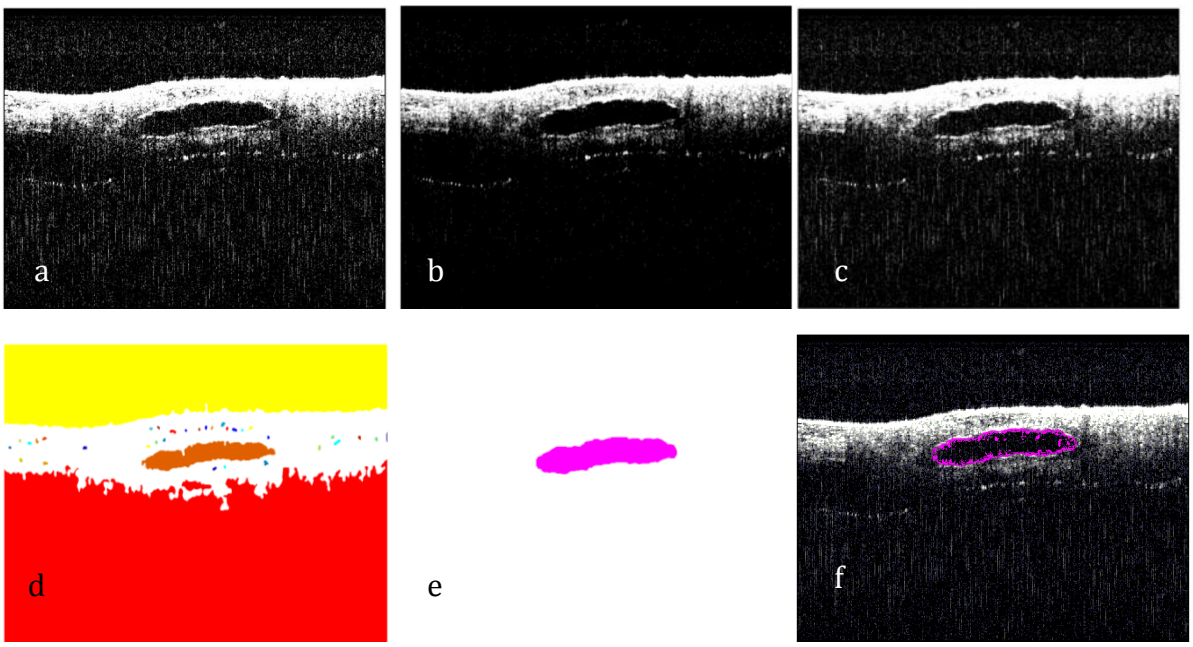

Fig. 3: Automatic image-processing for mandarin rind sample with stage 2 RBD. (a) Raw bitmap image. (b) 'Opened' image. (c) averaging filter applied. (d) 'Extended minima' function applied, holes filled and objects labelled. (e) object size limits imposed, erosion applied to smooth perimeter. (f) adjust size and extracted gland shape superimposed on original image.

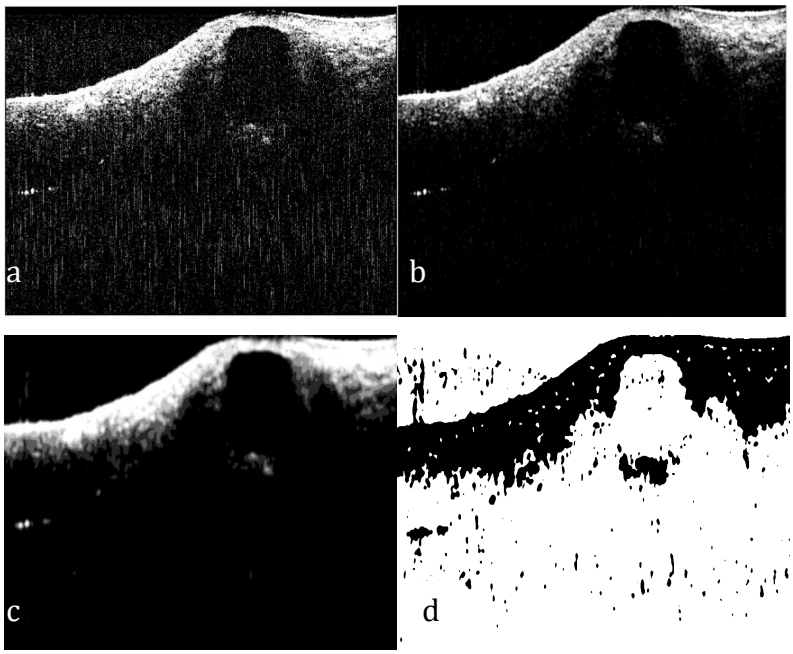

Fig. 4: Automatic image-processing for sample with no RBD. (a) Raw bitmap image. (b) 'Opened' image. (c) Averaging filter applied. (d) 'Extended minima' function applied but oil-gland not successfully separated from background.

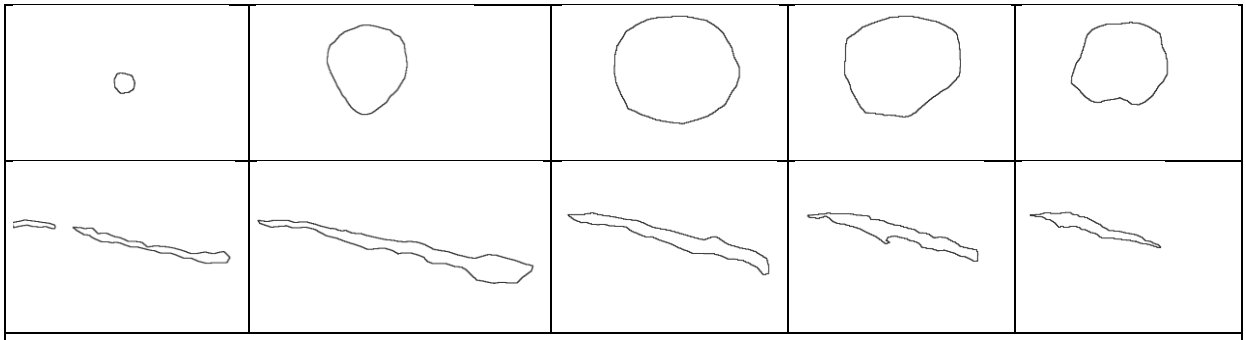

Fig. 5: Representative sets of oil-gland perimeters from cross-sectional images for a mandarin sample with no RBD (above) and a sample with severe RBD (below). All images were taken through the middle section of the oil glands.

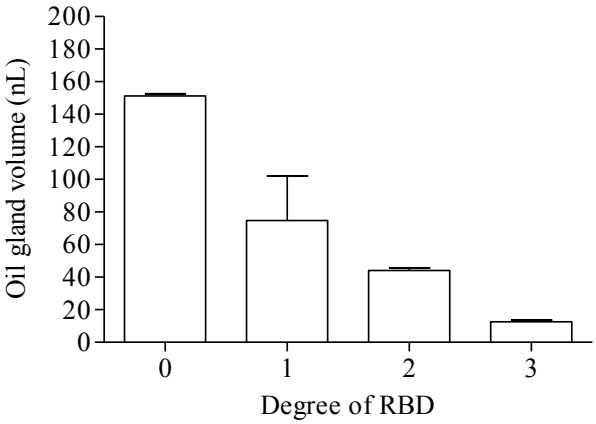

Fig. 6: Gland volume as a function of degree of progression of RBD from no (0), little (1), moderate (2) and severe RBD (3).

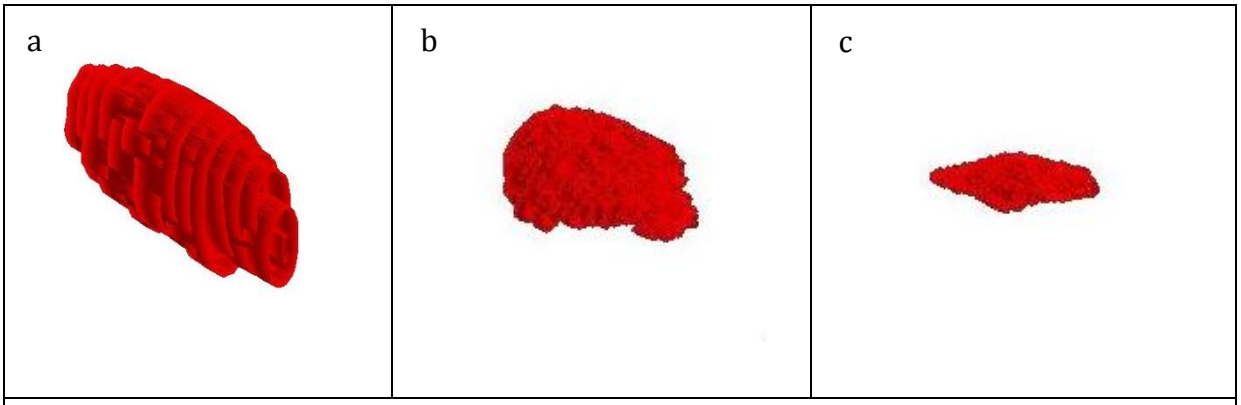

Fig. 7: 3D representations of oil glands with (a) no (b) moderate and (c) severe RBD.

SECTION III

BIOCHEMICAL AND PHYSIOLOGICAL PROFILE OF FRUIT LOCATED AT DIFFERENT POSITIONS OF THE CANOPY IN RELATION TO RIND BREAKDOWN DISORDER OF 'NULES CLEMENTINE' MANDARIN

A. Paper 8: Relationship between Canopy Position and Biochemical Composition of 'Nules Clementine' Mandarin Rind.
B. Paper 9: Canopy position effect on rind biochemical profile of 'Nules Clementine' mandarin fruit during postharvest storage.

CHAPTER 9

RESEARCH RESULTS

PAPER 8:

RELATIONSHIP BETWEEN CANOPY POSITION AND BIOCHEMICAL COMPOSITION OF 'NULES CLEMENTINE' MANDARIN RIND

RELATIONSHIP BETWEEN CANOPY POSITION AND BIOCHEMICAL COMPOSITION OF 'NULES CLEMENTINE' MANDARIN RIND

Abstract

This research was conducted to examine the effects of fruit position within the tree canopy and sunlight exposure on the biochemical composition of rind in 'Nules Clementine' mandarin during storage. Fruit position within the canopy and bagging treatments had significant effects on rind biochemical composition. At harvest, the concentration of ρ-coumaric and ferulic acids in rind tissue of fruit from unbagged outside fruit was double that in bagged fruit from inside the canopy. The sum of individual phenolic acids decreased by approximately 20% during storage, while the sum of individual phenolic compounds increased by a third due to an equal increase in the concentration of flavanones. Unbagged fruit from the outside position had lower rind sucrose (45.5 mg/g DM) compared to inside bagged fruit (89.1 mg/g DM) but higher glucose concentration (83.2 mg/g DM) compared to inside fruit (60.35 mg/g DM). This was consistent on both freshly harvested fruit and eight weeks postharvest. The concentration of non-structural carbohydrates (sucrose, glucose, fructose and total sugars) increased 1.7-fold during storage. Ascorbic acid was higher in the rind of sun exposed outside fruit. These changes in rind biochemical composition could lead to a reduced rind antioxidant status which might predispose such fruit to physiological rind disorders such as progressive rind breakdown (RBD) during stressful postharvest storage conditions.

Keywords: Phenolics · Organic acids · Nonstructural carbohydrates · Postharvest physiological disorder · Citrus

1. Introduction

Citrus fruit are prone to develop various types of physiological rind disorders, manifested by a multitude of symptoms during handling and storage (Alquezar et al., 2010). The incidence of fruit affected by these disorders can reach up to 60% of total production (Agustí et al., 2001). Although these physiological disorders affect the rind and do not compromise the edible internal portion of the fruit, they decrease fruit market value since external appearance is the primary specification used to evaluate quality of citrus fruit and they are a major consumer complaint (Alquezar et al., 2010). Limited knowledge of the physiological mechanism underlying these disorders affects supply and profits. The challenge is significant regarding citrus physiological rind disorders such as rind breakdown of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.), (Cronje et al., 2011a, b) rind breakdown of 'Navel' orange [*C. sinensis* L. (Osb.)] (Agustí et al., 2001) and non-chilling postharvest rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Alférez and Burns, 2004), which characteristically do not manifest during harvest grading but develop about one to five weeks after harvest. The symptom development is unpredictable and if fruit are shipped over long distances, symptom development usually coincides with the commercial shipping period and/or point of sale. This is therefore extremely problematic as the disorder can lead to financial losses at this late stage of the logistical supply chain.

Several groups of researchers have established that different microclimates within the tree canopy can influence the sensitivity of citrus fruit to various physiological rind disorders (Almela et al., 1992; Duarte et al., 1995; Chikaizumi, 2000; Agustí et al., 2001). A recent study revealed that 'Nules Clementine' mandarin fruit inside the canopy had an increased susceptibility to postharvest rind breakdown (Cronje, 2009; Cronje et al., 2011a). Higher air temperature in the exposed canopy has been reported to influence the biochemical profile of the rind and hence rind condition. These authors further quantified aspects of different light levels within the canopy and their influence on rind concentration of carbohydrate and rind condition in general. They reported that the concentrations of the main sugars (sucrose, glucose and fructose) in the flavedo of 'Nules Clementine' mandarin was lower on fruit located under low light conditions inside the canopy compared to outside exposed fruit. In addition to sugars, organic acids and phenolic

compounds are among the major compounds in citrus fruits are thus warrant further investigation (Kelebek, 2010).

The main non-volatile organic acids of citrus fruits are citric, ascorbic, malic, tartaric, and succinic acids (Kelebek, 2010). A high proportion of the antioxidant activity in citrus fruit is associated with organic acids, particularly ascorbic acid (Sdiri et al., 2012). The profile and concentration of phenolic compounds in citrus fruit rinds has received scientific interest in recent years due to the important role they might play on the antioxidant capacity (Manthey and Grohmann, 1996; Li et al., 2006; Xu et al., 2008a; Khan et al., 2010; Sun et al., 2010). Citrus fruit have numerous phenolic compounds such as coumarins, psoralens, phenolic acids and flavonoids (Benavente-García et al., 1998; Bocco et al., 1998). Flavonoids in citrus fruit are characteristically represented by two classes of compounds referred to as polymethoxylated flavones (PMFs) and flavanone glycosides (FGs) (Benavente-García et al., 1998). These two classes of flavonoids are found only in citrus fruits, and their presence or absence is specific for each species and therefore could be used as taxonomic markers and be related to postharvest physiology (Manthey and Grohmann, 1996). The PMFs exhibit higher biological activity even though they occur in much lower concentrations (Benavente-García et al., 1998). The two primary groups of phenolic compounds in citrus rind are FGs and phenolic acids (Ma et al., 2008; Simonne and Ritenour, 2011; Ye et al., 2011). In 'Satsuma' (*Citrus Unshiu* Marc.) and 'Ponkan' (*Citrus poonensis* Hort. Ex Tanaka) mandarin rinds, a total of 11 phenolic compounds including four hydroxybenzoic acids (sinapic, protocatechuic, ρ-hydroxybenzoic, and vanillic), three hydrocinnamic acids (caffeic, ρ-coumaric and ferulic), two PMFs (nobiletin and tangeretin) and two FGs (narirutin and hesperidin) were identified (Xu et al., 2008b). Very limited literature is available quantifying fruit position effects on rind phenolic compounds (McDonald et al., 2000). The influence of different light levels within the canopy on rind organic acids on citrus fruit has not been quantified.

Based on the above information, this study was conducted to determine FGs, phenolic acids, sugars, organic acids composition and antioxidant capacity in rind of 'Nules Clementine' mandarins harvested from different positions within the tree canopy. This study also aimed to relate rind biochemistry to the influence of preharvest microclimatic factors on appearance and non-chilling physiological rind disorders of citrus fruit.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), metaphosphoric acid, organic acids (citric acid, L-ascorbic acid, malic acid, tartaric acid and oxalic acid), sugars (sucrose, D-glucose and D-fructose) and phenolic compounds (ρ-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, ρ-coumaric acid, ferulic acid, sinapic acid, naringin, and hesperidin) standards were purchased from Sigma Aldrich (Dorset, UK). Narirutin and didymin standards were purchased from Extrasynthese (Lyon, France). Acetonitrile, methanol, and formic acid were all of HPLC grade, dimethyl sulfoxide (DMSO) was analytical grade and purchased from Fisher Scientific Chemicals (Leics., UK). Solutions and solvents were prepared with Milli-Q water (Milipore Inc.; $\sigma = 18$ M Ω cm^{-1}).

2.2. Plant Material and Sample Preparation

One hundred and twenty 'Nules Clementine' mandarin (*Citrus reticulata* Blanco.) fruit were harvested from marked trees in an orchard at Stellenbosch University Experimental Farm, Western Cape Province, South Africa (33°53'04.56"S, 18°37'36.84"E). To evaluate the effect of light exposure on rind biochemical composition, fruit from inside shaded and outside sun-exposed positions of the tree were selected and tagged during January 2011, after physiological drop about four months before commercial maturity. Half of the selected fruit from each of the canopy positions was covered with brown opaque paper bags without removing or covering subtending leaves. The study therefore consisted of four preharvest treatments; outside, outside bagged, inside and inside bagged.

Fruit were harvested at commercial maturity, on 16 May 2011, and received postharvest treatments according to industry practices. Postharvest treatments, included drenching (Thiabendazole, 500 mg/L; Imazalil, 500 mg/L and 2,4-dichlorophenoxyacetic acid, 125 mg/L) and waxing (polyethylene citrus wax, Citrashine®, Johannesburg, South Africa). After which they were brought to the postharvest evaluation laboratory, where they were sorted, separately

packed in cartons and couriered at ambient temperature the next day to Cranfield University (CU) in the United Kingdom, arriving within 48 hours, and stored for eight weeks at 8±0.5°C.

Upon arrival at CU, a total of 40 fruit (10 fruit from each preharvest treatment) were selected, weighed, peeled and the rind snap-frozen in liquid nitrogen and stored at -40°C. Frozen samples were then freeze-dried in a Labogene ScanVac CoolSafe Freeze Dryer System (CS55-4, Lynge, Denmark) for 7 days at 0.015 kPA and -55°C. Lyophilised samples were ground using a pestle and mortar into fine powder. To achieve standard particle size, the ground material was sieved through a 1 mm metal sieve. Large particles remaining on the sieve were further ground until all the material passed through the sieve. Ground samples were returned into the freezer until extraction and further analysis.

2.3. Storage Conditions

The remaining 80 fruit were stored in a cold room with delivery air temperature of 8±0.5°C (Cronje et al. 2011a). During cold storage, fruit were scored weekly, for the rind disorders such as chilling injury and rind breakdown for eight weeks. Different rind disorders were scored on a subjective scale from 0 = no defects to 3 = severe defects. Rind breakdown was then expressed as rind breakdown index (RBDI) estimate as previously described (Alférez and Burns, 2004) and calculated according to the following formula for chilling injury and peel pitting:

$$RBDI = \frac{\sum\{RBD(0-3) \times \text{No.of fruit in each class}\}}{\text{Total number of fruit}} \qquad (1)$$

After eight weeks, fruit samples were peeled and the rind segregated from the rest of the fruit. The pulp was juiced and the juice used for fresh total soluble solids (TSS) analysis on a digital hand-held refractometer (Palette, PR-32α, Brix 0.0-32.0, Atago, Co. LTD, Japan) using 1 mL of freshly squeezed juice. The rind from each sample was snap frozen in liquid nitrogen and stored in a -40°C freezer until further sample preparation as described in section 2.2. above.

2.4. Extraction and HPLC Quantification of Phenolic Compounds

Three different extraction solvent combinations and three extraction times were compared. The extraction methods included 80:20 (v/v) aqueous ethanol (Xu et al., 2008a), 70:29.5:0.5 (v/v/v), methanol:H_2O:HCL (Crespo et al., 2010) and 50:50 (v/v) DMSO:methanol as described elsewhere (Tomás-Barberán et al., 2003; Xu et al., 2008b). Freeze dried citrus rind powder (150 ± 0.5 mg) was extracted in 5 mL solvent following the optimum solvent to solid ratio of citrus fruit prescribed by Sun et al. (2010) and put into an ultrasonic water bath (Ma et al., 2008) at 35°C for 10, 20 or 30 minutes. Samples were agitated for 30s every 5 minutes, centrifuged at 16000g force for 10 minutes before the flocculate was filtered through a 0.2 µm syringe-driven filter (Millipore Corporation, Billerica, MA). Extraction method comparison (please refer to Appendix 1) showed that phenolic acids were optimally extracted using 70:29.5:0.5 (v/v/v), methanol:H_2O:HCL for 30 minutes and FGs concentrations were optimal on samples extracted using 50:50 (v/v) DMSO:methanol for 10 minutes. Based on these findings, phenolic acids and FGs were extracted using these two methods, respectively.

2.4.1. Extraction recovery and preparation of standard solution

The recovery of different phenolic compounds was evaluated using a pooled rind sample extracted as above. Briefly, freeze-dried samples were prepared, spiked with specific concentration of naringin and cinnamic acid (16 µg/mL) and extracted in triplicates. The recoveries were calculated based on a method described elsewhere (Chang et al., 1997). The recovery of these phenolic compounds ranged from 94.3–103.7%.

A mixed standard solution (5 mg/mL) was prepared by transferring all measured phenolic compounds into the extraction solvent. Eight concentration levels of the mixed standard solution were prepared by serial dilution of the stock solution. Concentrations of phenolic acids were determined from linear standard calibration curves ($R^2 = 0.994$).

2.4.2. Development of HPLC Method for Quantification of Phenolic Compounds

Determination of phenolic compounds were executed in triplicate on a HPLC (1200 series, Agilent Technologies, Santa Clara, CA, USA) binary pump system equipped with a diode array detector (DAD) with multiple wavelength detector, degasser and cooled autosampler. The

system was operated by Windows XP-based ChemStation© software (Agilent Technologies), which was also used for data processing. Citrus rind extracts (20 µL) were injected into a Poroshell 120 column (4.6 x 150 mm and 2.7 µm particle size, Agilent), which was held at 40°C. The flow rate of the mobile phase was set at 1 mL/min. The mobile phases consisted of two solvents, 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile:water (B). The DAD UV detection of all phenolic acids and FGs was carried out at 280 nm. The solvent gradient conditions for phenolic acids in volume ratios were as follows: 0-5% B during 5 min, 5-10% B up to 10 min; 10-12% B up to 16 min, 12-15% up to 25 min, 15-100% B up to 27 min. For FGs, the solvent gradient conditions were 0-15% during 5 min, 15-20% up to 10 min, 20-60% up to 25 min and 60-100% up to 27 min. FGs were quantified using naringin (an FG not present in 'Nules Clementine' mandarin) as an internal standard. The identification of phenolic compounds was accomplished by comparing the retention times and HPLC spectra of each compound of the peaks in the sample to those of the phenolic compound standards.

2.5. Extraction and HPLC Quantification of Non-structural Carbohydrates

Non-structural carbohydrates were extracted from 150 ± 0.5 mg of lyophilised fruit rind powder using 62.5% (v/v) aqueous methanol as described elsewhere (Terry et al., 2007). Following extraction, concentrations of fructose, glucose and sucrose were determined using a HPLC binary pump system (Agilent Technologies, UK), equipped with a refractive index detector (RID, G1362A), based on that previously described (Crespo et al., 2010). Briefly, sample extracts were diluted in HPLC grade water (1:10), and injected into a Rezex RCM monosaccharide Ca^{+} (8%) column of 7.8 mm diameter x 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} guard column of 3 mm x 4mm x (Phenomenex). Temperature of the column was set at 80°C using a thermostated column compartment (G1316A, Agilent). The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min. The presence and concentration of the selected non-structural carbohydrates was calculated by comparing peak area of samples against peak area of known standard concentrations (0.05-1.25 mg/mL; average $R^2 = 0.991$).

2.6. Extraction and Quantification of Non-volatile Organic Acids

Extraction and determination non-volatile organic acids were performed using a method described elsewhere (Crespo et al., 2010) with slight modification. A 150 ± 0.5 mg of freeze dried sample was dissolved in 6 mL of 3% aqueous metaphosphoric acid (Uckoo et al., 2011). Samples were kept on ice for 5 minutes and the flocculate filtered through a 0.2 µm syringe filter. Extraction was performed in triplicate. Citric, L-ascorbic, malic, tartaric and oxalic acid concentrations were determined using a HPLC system equipped with a DAD (G1315B/G1365G, Agilent Technologies, London, UK) with multiple wavelength detector. Sample extract (20 µL) was injected into a Prevail organic acid column (4.6 mm diameter x 250 mm, 5 µm particle size; Alltech, UK) with an organic acid guard column (part no. 96429, Alltech, UK). The mobile phase used was HPLC-grade 0.2% aqueous metaphosphoric acid at a flow rate of 1.0 ml/min and the column temperature was set to 35°C. Non-volatile organic acids were detected at 210 nm except for ascorbic acid which was detected at 245 nm. The quantity of each acid was calculated against the linear standard curves (5–250 µg/mL; average $R^2 = 0.996$).

2.7. Determination of Antioxidant Capacity

Supernatants of samples extracted using the three solvent combinations reported in section 2.4.2 above were tested for antioxidant capacity following the method by Brand-Williams et al. (1995) with slight modification by Xu et al. (2010). Briefly, 100 µL of extracts diluted 5 times in phosphate buffer saline (PBS) were added to 3.9 mL of a 0.2 mg/100 mL methanolic DPPH solution stirred well and left to react in the dark for 60 minutes. The absorbance of prepared sample solutions was measured spectrophotometrically at 515 nm using a UV/vis spectrophotometer against the blank (M501, Camspec, UK). Antioxidant capacity was estimated from a linear standard curves ($R^2 = 0.997$) of percentage inhibition plotted against Trolox concentration expressed in µmol Trolox equivalents (TE)/g DM.

2.8. Statistical Analysis

Statistical analyses were carried out using SPSS 10.0 for Windows (SPSS Inc. Chicago, IL, USA). Data was subjected to analysis of variance (ANOVA). Duncan's multiple-range tests were used to compare the significant differences of the mean values ($P \leq 0.05$). The limit of detection (LOD) and limit of quantification (LOQ) for phenolic compounds were calculated by repeatedly ($n = 10$) injecting known concentration of a mixture of standard solution. The LOD and LOQ values were calculated as the amount of each individual phenolic required to give the signal to noise ratio of 3 to 1 and 10 to 1, respectively (Bressolle et al., 1996).

3. Results and discussion

3.1. Canopy Position and Bagging Effect on Fruit Physico-chemical Properties

Fruit position within the canopy and bagging treatments had a significant effect on fruit physico-chemical properties (Table 1). Similar to results reported earlier (Cronje et al., 2011a; Khalid et al., 2012), outside fruit had higher (6.12) color index compared to 3.65 recorded for inside fruit. The greener rind color of inside fruit at harvest indicated a reduced expression of carotenoids during colour break (Khalid et. al., 2012; Cronje et al., 2013a). Fruit from the bagged treatments, both inside and outside of the canopy, were characterised by high postharvest mass loss, and this was essentially due to water loss by transpiration, as this accounts for 90% of total mass loss (Ben-Yehoshua et al., 1983). Water loss from the fruit resulted from a water pressure gradient prevailing between the fruit rind, which is close to saturation with water, and the less saturated outer atmosphere. The results in the current study are in accordance with literature stating that changes in water status of the rind is the primary postharvest factor contributing to the susceptibility of citrus fruit to non-chilling rind physiological disorders (Alférez et al., 2003, 2005, 2010; Alférez and Burns, 2004; Alquezar et al., 2010). Fruit position within the canopy also had a significant influence on rind dry matter content. Outside fruit had higher rind dry matter content compared to other preharvest treatments. The higher content of dry matter (30.0 g/100g) in outside fruit indicated a close relationship between sunlight level within the tree canopy and the concentration of stored carbohydrates. In kiwifruit, Tombesi et al. (1993) reported that radiation interception can affect carbohydrate and dry matter accumulation through subtending leaves. However, in the current study radiation interception by the fruit on its own

may also have played an important role, since reduced PAR in bagged fruit inhibits fruit photosynthesis and reduce fruit sink strength (Thorpe et al., 1974; Yen and Koch, 1990; Hiratsuka et al., 2012). Another hypothesis worth considering is that bagging fruit with brown paper bags reduced water vapour pressure and caused fruit to have higher moisture content at harvest which is lost during storage.

Shading fruit also resulted in higher incidence of RBD (Table 1). Symptoms of the disorder were visible on affected fruit three weeks postharvest. Sun-exposed, outside fruit were least sensitive to RBD compared to other preharvest treatments (Table 1). In addition, results indicated that exclusion of sunlight by bagging fruit resulted in increased fruit susceptibility to RBD (Table 1). These findings concur with previous results (Cronje, 2009) where it was reported that bagged fruit borne on the inside position of the tree canopy had the highest incidence of RBD. In earlier studies, it had been established that the sensitivity of fruit to development of non-chilling rind disorders related to rind breakdown was influenced by different microclimates (Almela et al., 1992). It is hypothesised that rind photosynthesis contributes significantly to rind condition. Reduction in photosynthetically active radiation on shaded portions of the canopy reduced fruit photosynthesis rate and osmotic potential, which is thought to have contributed to RBD (Agustí et al., 2001; Cronje et al., 2011a). The results presented above are in accord with the notion that low light levels around an individual fruit reduces rind condition and increase susceptibility to physiological rind disorders (Cronje et al., 2011a, b). It is therefore hypothesized that reduced sink strength and rind photosynthesis of inside and bagged fruit reduced dry matter accumulation, which contributed to susceptibility to RBD, due to premature senescence of the rind.

Comparing the positional and light exclusion effect on antioxidant capacity, results showed that antioxidant capacities were generally higher on sun exposed outside fruit and decreased with shading (Table 1). It has been reported previously that the presence of antioxidant species or the lack thereof could be implicated in the development of various postharvest disorders including non-chilling peel pitting in ‘Navelate’ oranges (Cajuste et al., 2007). The higher antioxidant capacity of sun-exposed fruit rind might indicate that fruit physiological defense mechanisms to the stress are induced by sun exposure during growth and maturation on the tree.

3.2. Effect of Sunlight Exposure on Changes in Fruit Rind Biochemistry

3.2.1. Phenolic Compounds

Fruit position and additional exclusion of sunlight by bagging showed significant differences in concentrations of some of the rind phenolic compounds (Table 2). In this study, the positional effects on the concentration of phenolic compounds were only significant for ρ-coumaric, ferulic and vanillic acids (Table 2). On fruit sampled at harvest, the concentration of ρ-coumaric acid was higher in the rind from outside fruit (13.84 µg/g DM) whilst bagged fruit inside the canopy had lower (4.17 µg/g DM) levels. This trend was reversed on fruit sampled after storage with corresponding concentrations for ρ-coumaric acid of 11.60 and 19.04 µg/g DM, respectively. There were no significant differences in the levels of chlorogenic and caffeic acids among fruit from the two canopy positions i.e. inside and outside.

Sunlight has been previously reported as one of the most important factors contributing to synthesis of phenolic compounds (Awad et al., 2001). The observation that shaded fruit inside the canopy receive lower sunlight may explain the lower concentration of ρ-coumaric, ferulic and vanillic acids observed in this study. According to results by Xu et al. (2008a), rind phenolic acids decrease greatly with fruit maturity. In accordance with this statement, the results herein showed that less mature bagged fruit inside the canopy had higher total phenolic acids compared at harvest and after 8 weeks in storage. The discrepancy between results in this study and those obtained by Xu et al. (2008a) could be due to different tissues tested (pulp vs. rind). The higher concentration of these phenolic acids in the rind of bagged and inside fruit could also be a stress related response resulting from reduced photosynthesis during growth. The effect of bagging (reducing radiation interception by fruit) on total phenols appears contrary to the previous findings where exterior canopy fruit had higher levels of total sum of individual phenolics and flavonoids (McDonald et al., 2000). Similar results were reported by Xie et al. (2013) whereby higher flavanoids were observed on bagged compared to unbagged fruit. Considering that high PAR on outside position of the tree is known to stimulate production of phenylalanine ammonia lyase (PAL), which induces production of phenolic compounds (phytoalexins) (Ben-Yehoshua et al., 1992), one would expect phenolic acids and flavanones to be higher on sun-exposed fruit. The reason for this discrepancy is not yet understood, but it could be speculated that response to PAR might be cultivar dependant.

The concentration of flavanones such as narirutin and didymin at harvest were not significantly different between treatments but were significantly higher in rinds of fruit harvested from bagged inside fruit and lowest on exposed outside fruit post-storage (Table 2). FGs measured at harvest were not affected by preharvest treatments but significantly increased in fruit sampled after eight weeks of storage. Such increase was clear for hesperidin and total flavanones for all preharvest treatments. In agreement to results obtained in the current study, a similar increase in the contents of FGs and othe phenolic compounds after prolonged storage has been reported by other researchers (Del Caro et al., 2004; Contreras-Oliva et al., 2011; Rojas-Argudo et al., 2012). With the exception of outside fruit, the total concentration of individual phenolic acids decreased during storage while the total of phenolic compounds increased due to an increase in the concentration of FGs as the rind senesces. One may therefore propose that such increase in phenolic compounds could be due to the stimulation of the PAL activity during postharvest storage, which is indicative of tissue response to overcome postharvest stress associated with senescence (Del Caro et al., 2004; Contreras-Oliva et al., 2011). However, antioxidant capacity in the rind tissue of 'Nules clementine' mandarins did not correlate well with phenolic compounds. Lack of correlation between antioxidant activity and phenolic compounds may be the fact that the method employed to measure antioxidant capacity takes into account the total action of all antioxidants present in tissue extract. These findings may indicate that the high proportion of the antioxidant activity in rinds of these fruit is contributed by other compounds such as carotenoids as well as organic acids, particularly ascorbic acid (Sdiri et al., 2012).

3.2.2. Non-structural Carbohydrates

The three main non-structural carbohydrate components evaluated were sucrose, glucose and fructose. In freshly harvested fruit rinds, sucrose was the least abundant carbohydrate (Fig. 2). The carbohydrate concentration of the citrus rind is hypothesised to affect rind condition and fruit susceptibility to various physiological rind disorders. In grapefruit, it was suggested that reducing sugar levels in the rind of grapefruit is negatively correlated to fruit susceptibility to chilling injury (Purvis and Grierson, 1982). In contrast, sugar accumulation in 'Fortune' mandarin did not precede fruit resistance to chilling injury. In this study, 'Nules Clementine'

mandarin fruit from the outside position had lower rind sucrose (45.5 mg/g DM) and higher glucose (83.2 mg/g DM) concentration at harvest and increased to 95.3 and 92.0 mg/g DM, respectively, after the storage period (Fig. 2). In comparison, bagged fruit borne inside the canopy had higher sucrose of 89.1 mg/g DM at harvest and the concentration of this sugar correspondingly increased after postharvest storage to 114.9 mg/g DM. With the exception of sucrose, these results do not support the notion that a positive relationship exists between canopy microclimates, particularly sunlight levels and temperature, and fruit carbohydrates accumulation in the pulp (Khalid et al., 2012) as well as in the rind of the fruit (Holland et al., 1999; Cronje et al., 2011a).

Reduced radiation interception has previously been reported to have reduced sink strength effect on fruit (Thorpe et al., 1974). In this study, the opposite was observed in that bagged fruit from inside position of the canopy had higher sucrose concentration than sun-exposed fruit. Concomitant to this, non-reducing (sucrose) to reducing (glucose + fructose) ratio was lower (0.29) on fruit exposed to sunlight and higher (0.61) on bagged fruit borne inside the canopy. These soluble carbohydrates are frequently the primary metabolites associated with osmotic adjustment (Zhang and Archbold, 1993). Bagging and shading might have affected sucrose concentration through other effects such as increased relative humidity, reduced temperature, lower vapor pressure deficit and therefore reduced fruit metabolism. Higher sucrose concentrations in the rind of bagged inside fruit is possibly a stress response, triggered by low light levels, to maintain osmotic potential in the shaded rind tissue. In ‘Satsuma’ mandarins, reduced osmotic potential as a result of net accumulation of sucrose has been reported to attract water into the cells (Yakushiji et al., 1998). This may also explain lower dry matter content in bagged fruit inside the canopy and higher mass (or water) loss during postharvest storage (Table 1). In shaded ‘Nules Clementine’ mandarin fruit, high potassium (K) concentration in flavedo of inside fruit has been reported supporting the need to maintain osmotic potential in the shaded rind tissue (Cronje et al. 2011b). The proposed underlying mechanism of how higher sucrose concentration at harvest affected susceptibility to RBD is based on reduced osmotic potential resulting from sucrose accumulation, a carbohydrate with the lowest relative contribution to osmotic/solute potential, leading to water accumulation, reduced rind dry matter content (Morgan, 1992; Zhang and Archbold, 1993), premature senescence (Cronje et al., 2011a, b), which manifests through developement of RBD.

The average concentration of sucrose increased significantly eight weeks postharvest and was approximately 1.7-fold higher than at harvest. A similar increase in sucrose concentration was reported on 'Navelate' and 'Pinalate' oranges stored for 30-45 days (Holland et al., 2005). Glucose and fructose also increased during storage but their increases were lower than that of sucrose, with 1.3-fold and 1.5-fold, respectively. The sum of the three main carbohydrates components also increased 1.5-fold. A similar increasing pattern in sugar concentration over the postharvest period was observed even when concentration was expressed on fresh mass (FM) basis indicating that the increase in concentration was not due to mass loss observed in Table 1. During postharvest storage, citrus fruit would be expected to show higher consumption of carbohydrates reserves for respiration instead of increased levels (Holland et al., 2005). Results in this study suggest that sucrose synthetic enzymes (sucrose synthase and sucrose phosphate synthase) might have been activated in response to postharvest stress such as water loss and senescence, resulting in sucrose accumulation during storage (Kader and Arpaia, 2002). However, the fact that glucose and fructose concentrations were not decreasing during storage, suggest that starch (not quantified herein) reserves or another alternative sources of carbon for sucrose synthesis may have been used (Vu et al., 1995; Holland et al., 2005).

Holland et al. (2005) reported that higher sucrose concentration in the rind of 'Fortune' mandarin was the result of stressed-induced response to chilling injury. However, it should be taken into consideration that fruit from different canopy positions had a similar increasing pattern in sucrose concentration over the storage period, although the level of increase was not equal. This indicates that increase in carbohydrate concentrations during postharvest storage might not be involved in the susceptibility of 'Nules Clementine' mandarin to rind physiological disorders, but initial levels of sucrose at harvest might be playing a more significant role.

3.2.3. Non-volatile Organic Acids

Five non-volatile organic acids were identified and quantified, including citric, ascorbic, malic, tartaric, and oxalic acid (Fig. 2). Similar to previous studies (Del Caro et al., 2004; Palma et al., 2005), ascorbic acid concentration remained constant except for outside fruit which showed a significant decrease after eight weeks postharvest storage. Tartaric acid decreases from harvest to eight weeks postharvest. In contrast, oxalic acid increased considerably from a

minimum of 2.17 mg/g DM at harvest to a maximum of 4.13 mg/g DM after storage. The sum of acids ranged from 47.75 to 58.04 mg/g DM at harvest and significantly decreased to between 38.65 and 49.34 mg/g DM after storage. Similar changes in organic acid concentration during postharvest storage have been observed in different citrus cultivars and were reported to depend on the storage conditions and cultivar (Sdiri et al., 2012). Pérez et al. (2005) reported an increasing concentration of ascorbic acid in 'Clementine' mandarin stored at 1°C whilst Rapisarda et al. (2008) reported a significant increase in ascorbic acid during postharvest storage. The differences between previous studies and our results might be due different fruit structure tested (rind vs. pulp), cultivars and storage conditions.

The overall composition of non-volatile organic acids was slightly affected by the preharvest treatments. Citric acid, a predominant organic acid was neither significantly affected by fruit position nor exclusion of light by bagging. However, ascorbic acid was significantly higher in the rind of sun-exposed fruit. Ascorbic acid is a potent antioxidant and accounts for a great proportion of the antioxidant capacity of citrus fruit (Sdiri et al., 2012). The high concentration of ascorbic acid observed in sun-exposed fruit from the outside position of the canopy could also explain the increased tolerance of these fruit to RBD. There was no significant difference in malic acid between the preharvest treatments. Oxalic acid was slightly lower on outside fruit but due to high variation between samples at harvest, it only showed a significant difference over the storage period. In general, the amounts of total acids were considerably higher in the inside shaded than outside fruit. The effect of canopy position on fruit pulp acidity was documented by Khalid et al. (2012) who reported that fruit from the South Western top part of the canopy (Northern hemisphere) had significantly lower titratable acid content and higher ratio than fruit borne in the north east bottom position.

4. Conclusion

The overall results indicated that exposing fruit to sunlight enhances postharvest rind quality by accumulating enough rind dry matter during on-tree development processes such as cell enlargement, pigment synthesis, then secondary metabolites, such as phenolics. Associated with higher mass loss and lower rind dry matter content, bagged fruit inside the canopy had a significantly higher incidence of RBD compared to sun-exposed outside fruit, indicating a close

relationship of the parameters with fruit susceptibility to RBD. Furthermore, the results herein may also suggest that the rind of shaded fruit might not have been conditioned or acclimatized to stresses compared to fruit that were exposed the sun. During storage, the fruit will be dependent on adequate rind dry matter accumulation for cellular respiration and metabolism in order to maintain cellular integrity during stress conditions, such as fluctuation in humidity and temperature. The concentration of non-structural carbohydrates (sucrose, glucose, fructose and total sugars) increased 1.7-fold during storage. Ascorbic acid was higher in the rind of sun exposed outside fruit. These changes in rind biochemical composition could lead to a reduced solute potential and rind antioxidant capacity which might predispose such fruit to physiological rind disorders such as RBD during stressful postharvest storage conditions.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterisation of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415–422.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarìas, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges. I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222–231.

Alférez, F., Alquezar, B., Burns, J.K., Zacarìas, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65.

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Awad, M.A., Wagenmakers, P.S., de Jager, A., 2001. Effects of light on flavonoid and chlorogenic acid levels in the skin of `Jonagold' apples. Sci. Hort. 88, 289–298.

Benavente-García, O., Castillo, J., Marin, F.R., Ortunõ, A., J.A., 1998. Del Río. Uses and properties of *Citrus* flavonoids. J. Agric. Food Chem. 45, 4505–4515.

Ben-Yehoshua, S., Shapiro, B., Chen, Z.E., Lurie, S., 1983. Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress. Plant Physiol. 73, 87–93.

Ben-Yehoshua, S., Rodov, V., Jin, K. J., Carmeli, S., 1992. Performed and induced antifungal materials of citrus fruits in relation to enhancement of decay resistance by heat and ultraviolet treatment. J. Agric. Food Chem. 40, 1217−1221.

Bocco, A., Cuvelier, M., Richard, H., Berset, C., 1998. Antioxidant Activity and Phenolic Composition of Citrus Peel and Seed Extracts. J. Agric. Food Chem. 46, 2123-2129.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie. 28, 25–30.

Bressolle, F., Bromet-Petit, M., Audran, M., 1996. Validation of liquid chromatographic and gas chromatographic methods, applications to pharnacokinetics. J. Chromat. B. 686, 3–10.

Cajuste, J.F., Lafuente, T.M., 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. Postharvest Biol. Technol. 45, 193–203.

Chang, C.W., Hsiu, S.L., Wu, P.P., Kuo, S.C., Chao, P.D.L., 1997. HPLC Assay of Naringin and Hesperidin in Chinese herbs and Serum. J. Food Drug Anal. 5, 111–120.

Chikaizumi, S., 2000. Mechanism of rind-oil spot development in 'Encore' (*Citrus nobilis* Lour. X *C. Deliciosa* Ten.) fruit. J. Japan. Soc. Hort. Sci. 69, 149–155.

Contreras-Oliva, A., Perez-Gago, M.B., Palou, L., Rojas-Argudo, C., 2011. Effect of insecticidal atmosphere and low dose X-ray irradiation in combination with cold quarantine storage on bioactive compounds of clementine mandarins cv. 'Clemenules'. Int. J. Food Sci. Technol. 46, 612–619.

Crespo, P., Giné Bordonaba, J., Terry, L.A., Carlen, C., 2010. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. Food Chem. 122, 16–24**.**

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementines' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of 'Nules Clementine' mandarin fruit, thereby affecting rind condition. J. Am. Soc. Hort. Sci. 138, 217–244.

Del Caro, A., Piga, A., Vacca, V., Agabbio, M., 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84, 99–105.

Duarte, A.M.M., Guardiola, J.L., 1995. Factors affecting rind pitting in the mandarin hybrids 'Fortune' and 'Nova'. The influence of exogenous growth regulators. Acta Hort. 379, 59–67.

Hiratsuka, S., Yokoyama, Y., Nishimura, H., Miyazaki, T., Nada, K., 2012. Fruit photosynthesis and phosphoenolpyruvate carboxylase activity as affected by lightproof fruit bagging in Satsuma mandarin. J. Am. Soc. Hort. Sci. 137, 215–220.

Holland, N., Menezes, H.C., Lafuente, M.T., 2005. Carbohydrate metabolism as related to high-tempearture conditioning and peel disorders occurring during storage of citrus fruit. J. Agric. Food Chem. 53, 8790–8796.

Holland, N., Sala, J.M., Menezes, H.C., Lafuente, M.T., 1999. Carbohydrate content and metabolism as related to maturity and chilling sensitivity, of cv. Fortune mandarins. J. Agric. Food Chem. 47, 2513–2518.

Kader, A.A., Arpaia, M.L., 2002. Postharvest handling systems: Subtropical fruits. In Postharvest Technology of Horticultural Crops, 3rd edition; Kader, A.A. Ed.; Regents of the University of California, Division of Agricultural and Natural Resources, Oakland, CA, pp. 375–384.

Kelebek, H., 2010. Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (*Citrus paradisi*) cultivars grown in Turkey. Ind. Crops Prod. 32, 269–274.

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M., 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of 'Kinnow' mandarin (*Citrus nobilis* Lour x *Citrus deliciosa*Tenora). Sci. Hort. 135, 137–144.

Khan, M.K., Albert-Vian, M., Fabiano-Tixier, A., Dangles, O., Chemat, F., 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides0 from prange (*Citrus sinensis* L.) peel. Food Chem. 119, 851–858.

Li, B.B., Smith, B., Hossain, M.M., 2006. Extraction of phenolics from citrus peels II. Enzyme-assisted exctraction method. Sep. Purification Technol. 48, 189–196.

Ma, Y-Q., Ye, X-Q., Fang, Z-X., Chen, J-C., Xu, G-H., Liu, D.H., 2008. Phenolic compounds and antioxidant activity of exctracts from ultrasonic treatment of Satsuma mandarin (*Citrus unshiu* Marc.) peels. J. Agric. Food Chem. 56, 5682–5690.

Manthey, J.A., Grohmann, K., 1996. Concentration of hesperidin and other orange peel flavonoids in citrus processing byproducts. J. Agric. Food Chem. 44, 811–814.

McDonald, R.E., Miller, W.R., McCollum, T.G., 2000. Canopy position and heat treatments influence gamma-irradiation-induced changes in phenylpropanoid metabolism in grapefruit. J. Am. Soc. Hort. Sci. 125, 364–369.

Morgan, J. M., 1992. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol., 1992, 19, 67–76.

Palma, A., DiAquino, S., Agabbio, M., Schirra, S., 2005. Changes in flavonoids, ascorbic acid, polyphenol content and antioxidant activity in cold stored 'Fortune' mandarin. Acta Hort. 682, 617–622.

Pérez, A.G., Luaces, P., Oliva, J., Rios, J.J., Sanz, C., 2005. Changes in vitamin C and flavour components of mandarin juice due to curing of fruits. Food Chem. 91, 19–24.

Purvis, A.C., Grierson, W., 1982. Accumulation of reducing sugars and resistance of grapefruit peel to chilling injury as related to winter temperature. J. Am. Soc. Hort. Sci. 107, 139–142.

Rapisarda, P., Lo Bianco, M., Pannuzzo, P., Timpanaro, N., 2008. Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. Postharvest Biol. Technol. 49, 348–354.

Rojas-Argudoa, C., Paloua, L., Bermejob, A., Canob, A., del Ríoa, M.A., González-Masb, M.C., 2012. Effect ofX-ray irradiation on nutritional and antifungal bioactive compounds of 'Clemenules' clementine mandarins. Postharvest Biol. Technol. 68, 47-53.

Sdiri, S., Navarro, P., Monterde, A., Benabda, J., Salvador, A., 2012. Effect of postharvest degreening followed by a cold-quarantine treatment on vitamin C, phenolic compounds and antioxidant activity of early-season citrus fruit. Postharvest Biol. Technol. 65, 13–21.

Simonne, A.H., Ritenour, M.A., 2011. Citrus (Orange, Lemon, Mandarin, Grapefruit, Lime and Other citrus fruits). In Health-Promoting Properties of Fruits and Vegetables, 1st edition; Terry, L.A., Ed.; CAB International: Oxfordshire, UK, pp, 90–117.

Sun, Y., Wang, J., Gu, S., Liu, Z., Zhang, Y., Zhang, X., 2010. Simultaneous determination of flavonoids in different parts of *Citrus reticulata* 'Chachi' fruit by high performance liquid chromagraphy-photodiode array detection. Molecules 15, 5378–5388.

Terry, L.A., Chope, G.A., Giné Bordonaba, J., 2007. Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (Fragaria x ananassa) fruit quality. J. Agric. Food Chem. 55, 10812–10819.

Thorpe, M.R., 1974. Radiant heating of apples. J. Appl. Ecol. 11, 755–760.

Tomás-Barberán, F.A., Gil-Izquierdo, A., Ferreres, F., Gil, M.I., 2003. Analysis and putification of flavanones, chalcones and dihydrochalcones. In Methods in Polyphenol Analysis, 1st edition; Santos-Buelga, C., Williamson, G., The Royal Society of Chemistry: Cambridge, UK, pp, 359–371.

Tombesi, A., Antognozzi, E., Palliotti, A., 1993. Influence of light exposure on characteristics and storage life of Kiwifruit. New Zeal. J. Crop Hort. Sci. 21, 87–92.

Uckoo, R.M., Jayaprakasha, G.K., Nelson, S.D., Patil, B.S., 2011. Rapid simultaneous determination of amines and organic acids in citrus fruit using high-performance liquid chromatography. Talanta. 83, 948–954.

Vu, J.C.V., Niedz, R.P., Yelenosky, G., 1995. Activities of sucrose metabolism enzymes in glycerol-grown suspention cultures of sweet oranges (*Citrus sinensis* L. Osbeck). Environ. Exp. Bot. 35, 455–463.

Xie, R.J., Zheng, L., Jing, L., He, S.L., Xi, W.P., Lv, Q., Yi, S.L., Zheng, Y.Q., Deng, L., 2013. The effect of cultivar and bagging on physicochemical properties and antioxidant activity of three sweet orange cultivars (*Citrus sinensis* (L.) Osbeck). American-Eurosian J. Agric. Environ. Sci. 13, 139–147.

Xu, C., Zhang, Y., Cao, L., Lu, J., 2010. Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Food Chem. 119, 1557–1565.

Xu, G.H., Ye, X.Q., Liu, D.H., Ma, Y.Q., Chen, J.C., 2008a. Composition and distribution of phenolic acids in Ponkan (*Citrus poonensis* Hort. Ex Tanaka) and Hoyou (*Citrus paradisi* Macf. Changshanhuyou) during maturity. J. Food Comp. Anal. 21, 382–389.

Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., Jiang, P., Ye, X.Q., 2008b. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73, C11–C18.

Yakushiji, H., Morinaga, K., Nonami, H., 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. J. Am. Soc. Hort. Sci. 123, 719–726.

Ye, X.Q., Chen, J.C., Liu, D.H., Jiang, P., Shi, J., Xue, S., Wu, D., Xu, J.G., Kakuda, Y., 2011. Identification of bioactive composition and antioxidant activity in young mandarin fruits. Food Chem. 124, 1561–1566.

Yen, C-R., Hoch, K.E., 1990. Developmental changes in translocation and localization of ^{14}C-labbeled assimilates in grapefruit: light and dark CO_2 fixation by leaves and fruit. J. Am. Soc. Hort. Sci. 115, 815-819.

Zhang, B., Archbold, D.D., 1993. Solute accumulation in leaves of a *Fragaria chiloensis* and a *E virginiana* selection responds to water deficit stress. J. Am. Soc. Hort. Sci. 118, 280–285.

Table 1: Physico-chemical properties of fruit from different canopy positions and bagging treatments. Different letters next to figures (mean and standard error of the mean) in the same row are significantly different. The data presented in this table was measured after eight weeks of storage.

Quality Parameter	Canopy position			
	Outside	Outside bagged	Inside	Inside bagged
Color index	6.12±0.19b	3.62±0.38a	3.65±0.57a	3.99±0.27a
Mass at harvest (g)	107.63±4.68b	104.89±4.28b	89.29±4.95a	107.61±6.38b
RBD Index (% incidence)	0.05	0.45	0.20	0.75
Incidence of RBD (%)	5	20	10	45
Water loss (g)	9.51±0.42ab	13.02±1.76bc	7.76±0.58a	13.62±1.67c
TSS (°Brix)	13.71±0.24b	11.89±0.28a	12.11±0.27a	11.94±0.25a
Dry matter (g/100g FM)	30.00±0.78b	26.98±0.51a	28.780.96a	26.99±0.79ab
AC (µmol trolox eq./g DM)	75.53±4.69c	52.72±3.52a	64.35±3.12b	53.47±2.10a

RBD, Rind breakdown; TSS, total soluble solids; AC, Antioxidant capacity; FM, fresh mass; DM, dry mass.

Table 2: Effect of fruit position, bagging and postharvest storage on concentration of rind phenolic compounds (µg/g DM). Means in the row with different letters are significantly different ($P \leq 0.05$).

	Outside		Outside bagged		Inside		Inside bagged	
Phenolic compound	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
Hydroxybenzoic acids								
ρ-Hydrobenzoic acid	29.18±2.38a	32.04±3.98a	28.33±1.33a	29.67±1.14a	30.57±1.00a	29.77±2.29a	30.10±1.75a	30.06±2.06a
Vanillic acid	27.97±2.79ab	23.04±1.90ab	27.209±4.51ab	22.20±1.21a	44.70±5.45c	20.52±1.27a	33.27±4.15b	24.28±1.33ab
Hydroxycinnamic acids								
Chlorogenic acid	33.54±1.58ab	38.72±3.09b	32.18±1.73a	32.95±1.08a	36.19±1.19ab	35.88±1.00ab	33.25±1.04a	34.79±1.31ab
Caffeic acid	26.70±1.93ab	30.26±3.12c	23.27±2.02a	24.21±2.18a	23.83±1.29a	26.30±1.52ab	24.41±1.73a	27.22±2.15ab
ρ-Coumaric acid	13.84±2.55bc	11.60±1.58b	2.54±0.48a	24.59±2.41d	7.69±0.41ab	14.33±1.48bc	4.17±1.79a	19.04±3.13cd
Ferulic acid	21.60±2.18c	13.03±1.69ab	13.68±1.81ab	6.96±1.91a	17.98±2.22ab	9.03±2.33a	12.12±2.14ab	17.47±4.64ab
Sinapic acid	33.88±3.91a	52.94±4.64ab	74.52±9.59bc	26.60±5.35a	100.2±9.24c	24.81±5.51a	143.6±9.91d	60.22±10.40ab
Total phenolic acids	186.7±17.59a	201.6±6.86a	201.7±19.6a	167.1±8.38a	261.1±22.63b	170.4±10.55a	280.9±28.11b	213.1±15.60a
Flavanones								
Narirutin	1194±94.77a	1479±84.06ab	1283±91.22a	1644±117.9ab	1606±284.4ab	1316±135.7ab	1512±175.78ab	1921±171.45b
Didymin	468.9±38.74a	619.0±15.22ab	509.2±74.46ab	613.0±27.69ab	563.2±65.07ab	587.4±31.53ab	576.4±26.60ab	657.8±31.29b
Hesperidin	34323±1896a	45163±864b	35550±3048ab	43000±1605ab	39439±2332ab	43479±1345ab	37872±1308ab	42828±1682ab
Total flavanones	35982±1863a	47262±911b	37339±3253ab	45259±1690ab	41605±2565ab	45383±1371ab	39958±1448ab	45407±1790ab
Total phenolics	36142±1855a	47463±912b	37516±3236ab	45425±1693ab	41827±2559ab	45553±1372ab	40210±1435ab	45620±1779ab

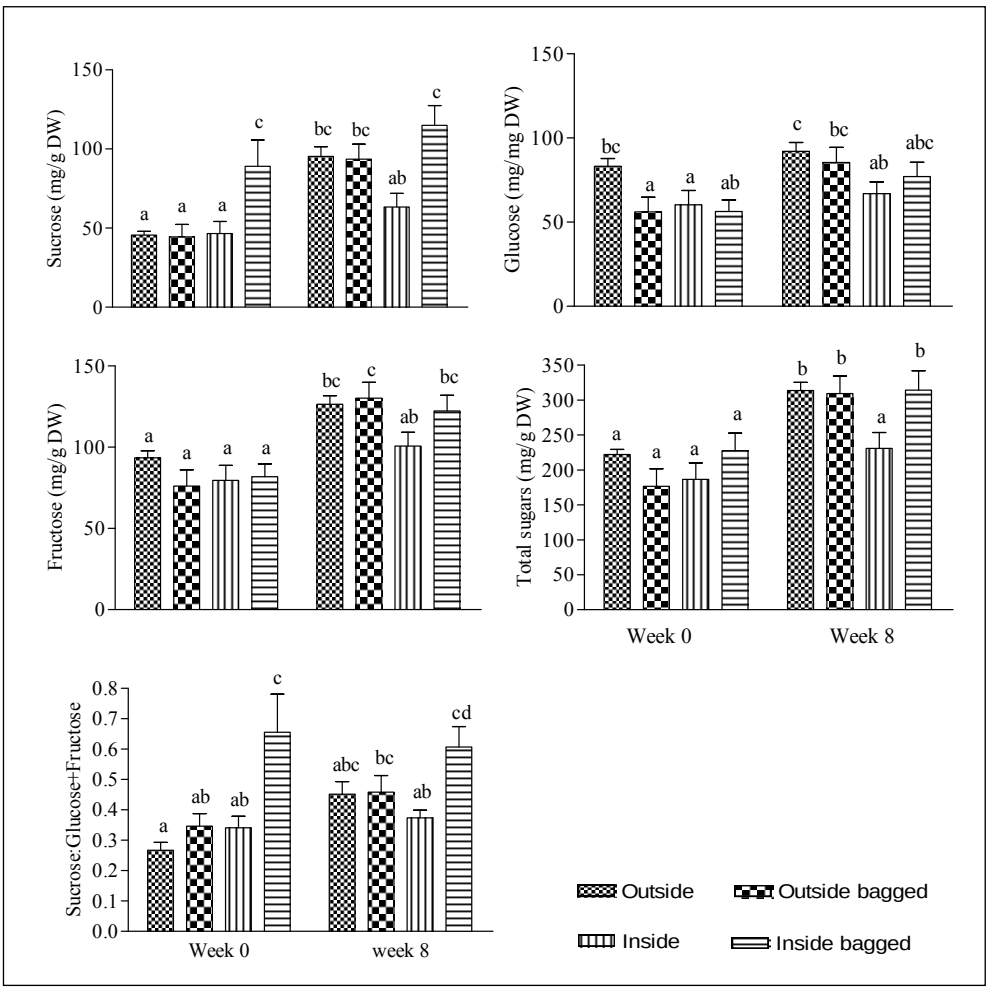

Fig. 1: Positional and bagging effects on carbohydrates composition in rinds of 'Nules Clementine' mandarins. Means in the same plot with different letters are significantly different ($P \leq 0.05$).

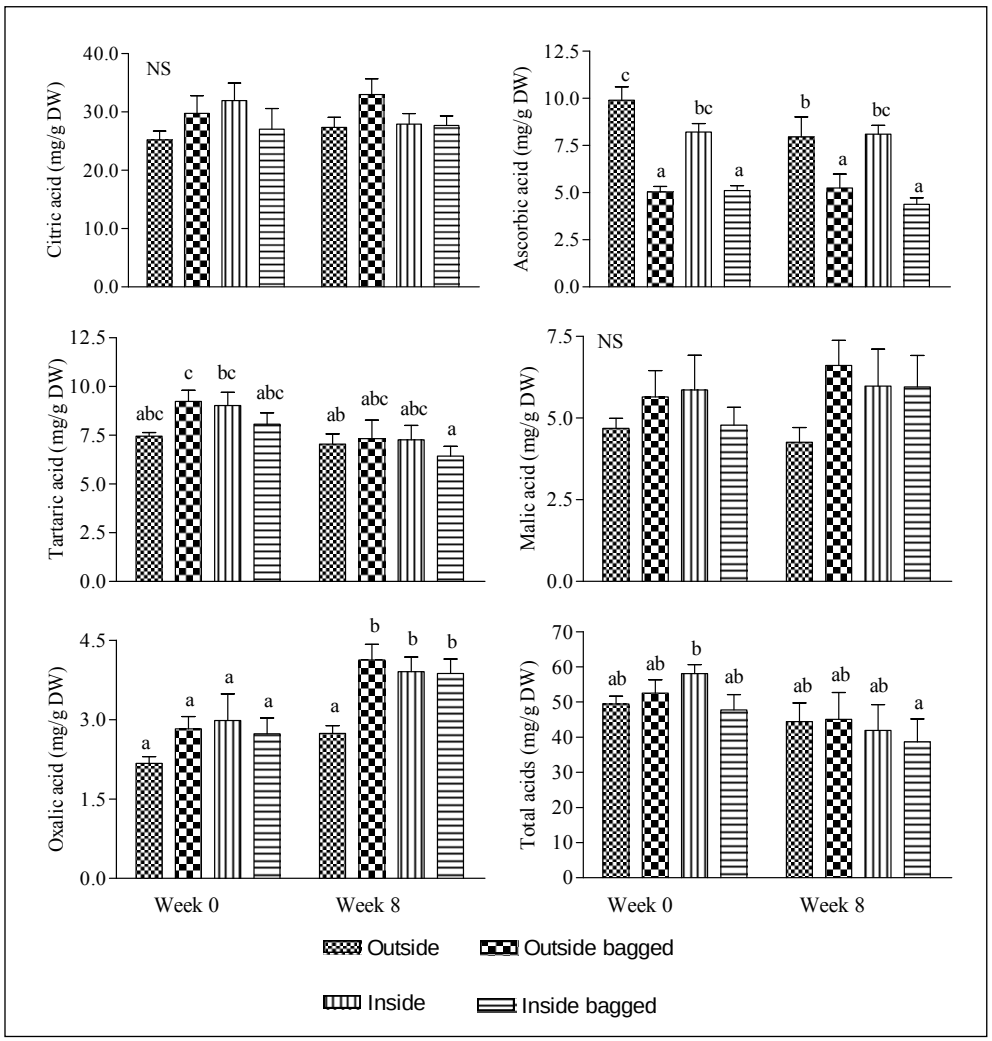

Fig. 2: Positional and bagging effects on organic acids composition in rinds of 'Nules Clementine' mandarins. Means in the same plot with different letters are significantly different ($P \leq 0.05$). NS, non-significant.

CHAPTER 10

RESEARCH RESULTS

PAPER 9:

CANOPY POSITION AFFECTS RIND BIOCHEMICAL PROFILE OF 'NULES CLEMENTINE' MANDARIN FRUIT DURING POSTHARVEST STORAGE*

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CANOPY POSITION AFFECTS RIND BIOCHEMICAL PROFILE OF 'NULES CLEMENTINE' MANDARIN FRUIT DURING POSTHARVEST STORAGE

Abstract

This study was conducted to investigate the effects of preharvest canopy position and bagging treatments on rind physiological and biochemical properties of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit. Before storage, the respiration rate of unbagged outside fruit was significantly higher (21.6 mL CO_2 kg^{-1} h^{-1}) than bagged inside fruit (16.3 mL CO_2 kg^{-1} h^{-1}). Unbagged fruit outside the canopy had 1.4-fold higher carbohydrates, and 1.1-fold higher dry matter (DM) content than bagged inside fruit. Bagged fruit inside the canopy had higher (24%) mass loss than outside sun-exposed fruit (14%). This corresponded with a higher rind breakdown (RBD) index for bagged inside fruit, compared to sun-exposed fruit which did not develop the disorder. During postharvest storage, rind fructose levels of bagged fruit inside the canopy increased from 62.4 mg/g DM at harvest to 81.3 mg/g DM after 8 weeks, while those of unbagged outside fruit increased from 97.9 to 108.4 mg/g DM. Concomitant with the increase in fructose, sucrose in rind tissue of bagged inside fruit decreased from 42.6 to 27.7 mg/g DM and from 49.3 to 33.4 mg/g DM for unbagged outside fruit. Rind glucose of unbagged inside fruit decreased from 90.6 to 76.2 mg/g DM. Ascorbic acid concentrations remained almost constant during storage, with levels between 3.3 and 6.7 mg/g DM for inside bagged and unbagged outside fruit, respectively. Hesperidin was the major flavanone detected, with concentrations between 35 and 45 mg/g DM followed by narirutin (1.1–2.8 mg/g DM). At harvest, the rind of fruit harvested from outside the canopy had lower hesperidin concentration (38.1 mg/g DM) compared to shaded fruit (44.2 mg/g DM). Overall, the results suggest that variations in microclimatic conditions inside the tree canopy during the growing season affect biochemical profile of the fruit rind, which in turn influences fruit response to postharvest stresses associated with senescence and susceptibility to RBD.

Keywords: Senescence · Sugars · Physiological disorder · Rind breakdown · Citrus · Flavanone glycosides.

1. Introduction

Citrus fruit is botanically classified as a hesperidium, which consists of two morphologically distinct parts; the pericarp (rind) and the edible endocarp (pulp) (Swingle, 1943). The pericarp can be further divided into the exocarp or flavedo (the outer-most, pigmented part of citrus rind) and the mesocarp or albedo (the inner, white part of the rind) (Iglesias et al., 2007). The marketability of citrus fruit, especially for fresh consumption is highly dependent on external appearance (Khalid et al., 2012) and therefore not only free of physical blemishes but physiological disorders too. Rind quality of citrus fruit is the sum of many physical and chemical components including primary and secondary metabolites which determine the rind susceptibility to disorders. The predominant primary metabolites in citrus rind are carbohydrates, contributing 30 to 50% of rind dry mass (Liu et al., 2012) and non-volatile organic acids, while carotenoids and phenolic compounds are the major secondary compounds (Iglesias et al., 2007). Citrus rind also contains characteristic essential oils sequestered into specialised oil cavities called oil glands (Iglesias et al., 2007).

The final concentrations of the primary and secondary metabolites at harvest depend on the regulation of the metabolic processes during fruit growth and development (Poiroux-Gonord et al., 2013). Among many environmental factors that affect citrus rind quality, canopy position occupies a predominant role because it is thought to exert a very strong influence on the biochemical makeup of rind and whole fruit. Microclimatic conditions during the growing season and within the canopy may influence the biochemical profile of the fruit rind and play a role in fruit sensitivity to physiological rind disorders. Cronje et al. (2011a, b; 2013) and Magwaza et al. (2012a, b) observed differences in rind quality on fruit harvested from different canopy positions. These studies showed that fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affect the rind content of sugars during fruit development (Cronje et al. 2011a). The flavedo from fruit borne on the outside of the canopy had significantly higher sugar content than fruit borne inside the canopy. The incidence of rind breakdown (RBD) in 'Nules Clementine' mandarin was also greater on inside fruit compared with the outside fruit and this was consistent from season to season; this being attributed to the exclusion of adequate sunlight during development (Cronje et al., 2011a, b; 2013; Magwaza et al., 2012a, b). Lower osmotic potential due to higher rind sucrose, an osmoregulatory compound in plant cells (Yakushiji et al., 1998; Huang et al., 2000), has previously been reported, demonstrating a possible link between fruit

position, rind soluble non-structural carbohydrates content and ultimately development of RBD (Cronje et al. 2011a).

During postharvest storage, sugar substrates are catabolised during senescent processes in citrus rind (Ingram and Bartels, 1996; Sun et al., 2012). Senescence is energy expensive, and a lack of adequate supply of carbohydrates for postharvest respiration in the rind may lead to a series of physiological rind disorders including RBD (Holland et al., 1999; Cronje et al., 2011a). Carbohydrate supply is also thought to be the most important factor influencing biosynthesis of secondary metabolites (Poiroux-Gonord et al., 2013), through its positive influence on precursor availability for the synthesis of organic acids, glycosides, carotenoids and other isoprenoid compounds (Cunningham, 2002).

Citrus fruit rinds are known for their high content of non-volatile organic acids, including citric, ascorbic, malic, tartaric, succinic, and oxalic (Sun et al., 2012). Ascorbic acid (AsA) contributes significantly to the antioxidant capacity of citrus fruit rinds (Mathur et al., 2011). However, the types and concentrations can vary significantly between citrus cultivars. Besides AsA, citrus fruit are also rich in phenolic compounds, which contribute to high antioxidant capacity (Manthey and Grohmann, 1996). The phenolic composition of citrus fruit comprises numerous compounds such as coumarins, psoralens, phenolic acids and flavonoids (Benavente-García et al., 1998). Flavonoids reported to occur in citrus rind include flavanone glycosides (FGs) and polymethoxylated flavones (Bocco et al., 1998). These two groups of compounds, not only play a physiological role, but are also of commercial interest because of their potent antimicrobial activity against bacteria and fungus (Mathur et al., 2011). FGs, which include hesperidin, neohesperidin, naringin, narirutin and didymin are the most abundant flavonoid group found in different parts of citrus fruit (Khan et al., 2010). FGs are unique to citrus and are characteristic of some species and varieties (Tomás-Barberán et al., 2003). For instance, hesperidin is a major component in oranges and mandarins where it is mostly found in rind tissue, whereas naringin is a predominant FG in grapefruit (Kalt, et al., 1999). Differences in concentrations of FGs may also occur as a result of differences in fruit maturity, environmental conditions during growth and development, postharvest treatments, and storage conditions (Abad-García et al., 2012). Thus, FGs have a potential to be used as biochemical markers to determine fruit origin within the canopy and perhaps susceptibility to rind physiological disorders. Very limited literature is available quantifying fruit position effects on rind phenolic compounds during postharvest storage (McDonald et al., 2000). To the best of the authors' knowledge, the influence of different

light levels within the canopy on rind organic acids and FGs on citrus fruit has not been quantified.

Detailed biochemical changes, especially regarding secondary metabolites that occur in citrus rind during storage are largely unknown. Therefore, the aim of the current study was to investigate the effects of canopy position on rind physico-chemical properties of 'Nules Clementine' mandarin fruit and analyse the changes in sugars, organic acids, and FGs in rind tissue during postharvest storage in relation to the development of RBD.

2. Materials and methods

2.1. Reagents and standards

Dimethyl sulfoxide (DMSO) was of analytical grade, while methanol, acetonitrile and formic acid were HPLC grade. All were purchased from Thermo Fisher Scientific (Leics, UK). Water was purified in a Milli-Q Integral Water Purification System (Merck Millipore corporation, Billerica, MA, USA; σ = 18 MΩ cm^{-1}). Narirutin and didymin were purchased from Extrasynthese (Genay, France). Standards for organic acids (citric, L-ascorbic, malic, tartaric and oxalic), carbohydrates (sucrose, D-glucose and D-fructose) and phenolic compounds (naringin, and hesperidin) were purchased from Sigma-Aldrich Company Ltd. (Dorset, UK).

2.2. Plant material and sample preparation

'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit were harvested from eight (8) uniformly marked trees in a commercial orchard at Citrusdal, Western Cape Province, South Africa (32° 25' 22" South, 19° 00' 53" East). Ten fruit each from inside (shaded) and outside (sun-exposed) positions in the tree canopy were selected and tagged during January 2012, after physiological drop, about four months before commercial maturity. To determine the effect of blocking sunlight on rind biochemical profile and fruit susceptibility to RBD, half of the fruit sample (5) from inside and outside position of the canopy were enclosed in brown paper bags on the same day, about 4 months before harvest, as previously discussed by Magwaza et al. (2012a). The study therefore consisted of four preharvest treatments; i.e. outside, outside bagged, inside and inside bagged. During each destructive sampling date,

each treatment was replicated five times. For continuous non-destructive evaluation of RBD, 100 single fruit replicates were used.

Fruit were harvested on 16 May 2012 at commercial maturity and received all normal postharvest treatments, including fungicides and wax application according to industry practices (Magwaza et al., 2012a). Thereafter, fruit were packed in ventilated cartons and couriered at ambient temperature to Cranfield University (CU) in the United Kingdom, arriving within 48 hours. Upon arrival, fruit were stored for eight weeks in a cold room at 8±0.5 °C; a temperature known to increase the incidence of RBD (Khumalo, 2006). During cold storage, fruit were visually examined and scored at weekly intervals for the development of RBD symptoms over 8 weeks. RBD incidence was scored by visual inspection on a subjective scale from 0 = no breakdown to 3 = severe breakdown, in order to quantify incidence as well as severity and RBD index calculated as follows (Magwaza et al., 2012a). Fruit respiration, sugars, and organic acids were analysed upon arrival at CU Plant Science Laboratory (referred to as week 0), week 3, week 6 and week 8.

2.3. Respiration rate measurements

Respiration rate was measured on each sampling date using the method described by Collings et al. (2012) with slight modifications. Gas measurements were taken directly from two fruit per treatment in a 3 L jar using a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA), which was calibrated with a certified standard (10 % CO_2, 2 % O_2, 88 % N_2) from Brin's Oxygen Company (BOC) industrial gases supplies (Surrey, UK). Three sets of respiration rate measurements were acquired from each treatment. Sequencing was controlled by a flow multiplexer (hardware version 4, firmware version 1.05, Sable Systems International, NV, USA) and air was subsampled at 1.5 L min^{-1} from each jar via a continuous flow 'push mode' set up using a subsampler (SS4, firmware version 2.0). The universal interface (UI-2), connected to the subsampler, multiplexer and detectors, allowed data to be analysed and interpreted by the computer software (ExpeData Release 1.3.8, Version: PRO). The subsampled air from each jar was analysed over a 5 min period, and repeated three times to provide one average reading for each sampling date. After measuring fruit mass, respiration rate values were calculated as mL CO_2 kg^{-1} h^{-1}.

2.4. Physico-chemical measurements

2.4.1. Fruit mass and colour

All fruit were weighed weekly using a calibrated balance (Mettler Toledo, ML3002E / 01, Switzerland). Rind colour components were measured in the CIE lightness (L^*), chroma (colour saturation: C^*) and hue angle ($h°$) colour space using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using a standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203) (Terry et al., 2007; Pathare et al., 2013). The mean of three readings at three equidistant points around the equatorial axis of the fruit was automatically calculated.

2.4.2. Sample preparation and rind dry matter measurements

The rind (flavedo and albedo) was peeled from the rest of the fruit, snap frozen in liquid nitrogen, stored at -40 °C prior to freeze drying for seven days in a freeze dryer system (Labogene ScanVac CoolSafe CS55-4, Lynge, Denmark) at 0.015 kPa and -55 °C. Dried samples were weighed and the dry matter content was expressed as a percentage of fresh mass. Lyophilised samples were then ground into fine powder using pestle and mortar and returned to the freezer (-40 °C) prior to being used for carbohydrate, organic acids and FGs determination by high performance liquid chromatography (HPLC).

2.4.3. Extraction and quantification of sugars

Extraction and quantification of sugars was based on a method described by Terry et al. (2007) and modified for citrus as described in Magwaza et al. (2012a). Briefly, a 150 ± 0.5 mg of fruit rind powder was extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentration of fructose, glucose and sucrose were determined in an HPLC binary pump system (1200 series, Agilent Technologies, UK). Twenty micro litres (20 µL) of a diluted sample solution (1:10) was injected into a Rezex RCM monosaccharide Ca^{+} (8%) column of 7.8 mm diameter x 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} guard column of 3 mm x 4mm (Phenomenex). The thermostated column compartment (G1316A, Agilent) temperature was set at 80 °C. The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min and the presence of carbohydrates was detected on a refractive index detector (RID, G1362A, Agilent Technologies). Sugars were quantified from a linear standard curve (0.05 to 1.25 mg/mL; average $R^2 = 0.997$).

2.4.4. Extraction and quantification of non-volatile organic acids

Non-volatile organic acids were extracted and determined using a method described by Crespo et al. (2010) with slight modifications by Uckoo et al. (2011) and described in Chapter 9. Briefly, 150 ± 0.5 mg of freeze dried sample was cold extracted for 5 min in 6 mL of 3% aqueous metaphosphoric acid. The flocculate was filtered through a 0.2 µm syringe filter before HPLC analysis. Citric, ascorbic, malic, tartaric and oxalic acid concentrations were determined on a HPLC binary pump system equipped with a diode array detector (DAD) with multiple wavelength detector, degasser and cooled autosampler. The filtered sample extract (20 µL) was injected into a Prevail organic acid column (4.6 mm diameter x 250 mm, 5 µm particle size; Alltech, UK) with an organic acid guard column (part no. 96429, Alltech). Temperature of the column was set to 35°C using a thermostated column compartment (G1316A, Agilent). The mobile phase used was 0.2% HPLC-grade aqueous metaphosphoric acid at a flow rate of 1.0 ml/min. Non-volatile organic acids were detected at 210 nm except for AsA which was detected at 245 nm and quantified using linear standard curves (5 to 250 µg/mL; average $R^2 = 0.99$).

2.4.5. Extraction and quantification of flavanone glycosides

Flavanone glycosides were extracted using a method described by Tomás-Barberán et al. (2003) and Xu et al. (2008) with modification described in Chapter 9. Briefly, freeze dried citrus rind powder (150 ± 0.5 mg) was extracted in 5 mL 50:50 (v/v) DMSO:methanol solvent and put into an ultrasonic water bath at 35 °C for 10 min. Samples were agitated for 30 s every 5 min, centrifuged at 16000 g for 10 min before the supernatant was filtered through a 0.2 µm syringe filter (PTFE 25mm, Cronus™, SMI-Labhut Ltd., UK).

Determination of FGs was executed on an HPLC (1200 series, Agilent Technologies) binary pump system equipped with a diode array detector (DAD) with multiple wavelength detector, degasser and cooled autosampler (Magwaza et al., 2012a). Rind extracts (20 µL) of 'Nules Clementine' mandarin were injected into a Poroshell 120 column (4.6 x 150 mm and 2.7 µm particle size, Agilent), held at 40 °C. The mobile phases used were, 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile: water (B) at a flow rate of 1.0 mL/min. The solvent gradient conditions in volume ratios were as follows: 0-15% during 5 min, 15-20%

up to 10 min, 20-60% up to 25 min and 60-100% up to 27 min. FGs were detected by the DAD UV detector at 280 nm. The identification of FGs was accomplished by comparing the retention times of the peaks and HPLC spectra of each compound in the sample to those of the standards.

2.5. Statistical analysis

Statistical analyses of fruit physico-chemical properties were carried out using statistical software (STATISTICA, Vers. 11.0, StatSoft Inc. USA). Data was subjected to analysis of variance (ANOVA) and means were separated by least significant difference (LSD; p=0.05) (2 factors). In order to gain better insight into how measured physico-chemical properties correlate with each other and RBD, data was subjected to principal component analysis (PCA) as well as two-tailed Pearson's correlation (p=0.05, p=0.01). Graphical presentations were made using GraphPad Prism software version 4.03 (GraphPad Software, Inc. San Diego, CA, USA).

3. Results and discussion

3.1. Rind breakdown disorder (RBD)

RBD symptoms were first visible on affected fruit after two weeks of storage (Fig. 1). In addition, after week 4-5, significant differences due to preharvest treatments were evident. Bagged fruit borne inside the canopy were more susceptible to RBD compared to outside fruit. The interaction between preharvest canopy position and storage time was significant, indicating that preharvest treatment affects the incidence of RBD during postharvest storage. This increase in RBD confirmed the progressive nature of this physiological disorder (Cronje et al., 2011a; Magwaza et al., 2013b). These results are similar to findings by Cronje et al. (2011a, b) and Magwaza et al. (2012a; b) reinforcing the hypothesis that canopy microclimate affect rind development via accumulation of nutrients and carbohydrates (Cronje et al., 2011a,b; 2013) which could contribute to the rind condition and susceptibility to RBD.

3.2. Mass loss

Fruit mass loss was significantly affected by preharvest canopy position and bagging treatment (Fig. 2). After eight weeks of postharvest storage, bagged fruit inside the canopy had higher (24.3%) mass loss than outside fruit (14.3%). Changes in water status of the rind is a primary postharvest factor in determining the susceptibility of citrus fruit to non-chilling rind pitting (Alférez et al., 2003, 2005, 2010; Alférez and Burns, 2004; Alquezar et al., 2010). In the present study, bagged inside fruit with higher mass loss (Fig. 2) were more susceptible to RBD (Fig. 1). In accordance with observations in Figs. 1 and 2, results in the PCA plot (Fig. 3) and Pearson's correlation table (Table 1) also showed a positive correlation between mass loss and RBD, confirming a significant contribution of this parameter to the development of the disorder. The low correlation coefficient (0.22) (Table 1) between the RBD and mass loss observed in the current study and that reported by Alférez and Burns (2004) in 'Marsh' grapefruit suggests that development of the disorder might be attributed to other factors in addition to mass loss during postharvest storage. Loss of moisture from the rind observed during postharvest storage in the current study resulted primarily from differences in vapour pressure deficit between flavedo and the atmosphere surrounding the fruit (Alférez et al., 2010).

3.3. Rind colour

All rind colour parameters were significantly affected by fruit position, bagging treatments and storage time (Fig. 4A, B and C). Throughout the storage period, L^* (Fig. 4A) and $h°$ (Fig. 4C) of shaded fruit inside the canopy were higher than outside fruit. At the onset of storage, values for L^* and $h°$ were higher and showed a gradually declining trend while C^* increased during postharvest storage. The decrease in L^*, $h°$ and increase in C^* during postharvest storage indicated an improvement in rind colour; from green to deep orange for outside fruit and from green to pale yellow for inside and bagged fruit. As previously observed by Cronje et al. (2011a), the effects of fruit canopy position and bagging treatments on rind colour may be associated with the role of radiation and temperature on pigment production. In 'Satsuma' mandarins, Hiratsuka et al. (2012) reported similar results where bagging fruit with paper bags inhibited chlorophyll degradation and reduced carotenoid formation in the rind resulting in pale yellow fruit. Similar to results observed by Cronje et al.

(2011a), although the rind colour of all treatments was improved during storage, inside fruit remained yellow compared to orange outside fruit.

3.4. Fruit respiration

Before storage, the respiration rate of sun-exposed outside fruit was significantly higher (21.6 mL CO_2 kg^{-1} h^{-1}) compared to bagged fruit from inside the canopy (16.3 mL CO_2 kg^{-1} h^{-1}) (Fig. 5). These results agree with observations by Hiratsuka et al. (2012) who reported bagged fruit to have about 10% lower respiration rate than unbagged fruit. However, after eight weeks of storage, bagged outside fruit showed significantly higher (28.1 mL CO_2 kg^{-1} h^{-1}) respiration rate than inside bagged fruit (24.5 mL CO_2 kg^{-1} h^{-1}). In 'Nules Clementine' mandarins stored at 7.5 °C, Cronje et al. (2011a) observed higher respiration rate of inside fruit at the onset of postharvest storage but after 4 weeks, this trend changed, showing higher respiration on outside fruit (Cronje et al., 2011a). On 'Satsuma' mandarins stored at 5 and 15 °C, Izumi et al. (1990) observed similar results, with outside fruit having a higher respiration rate compared to shaded fruit inside the canopy. The results in the current study showed a gradual and almost linear increase in respiration during storage and as the fruit senesced.

3.5. Rind sugars and dry matter

The concentrations of sucrose, glucose and fructose in the rind of bagged fruit from inside and outside canopy positions were, 0.7-fold and 0.9-fold lower compared to unbagged fruit, respectively (Fig. 6). Before storage, inside bagged fruit had the lowest concentration (188.2 mg/g DM) of total sugars, while sun-exposed outside fruit had the highest (239.9 mg/g DM). During postharvest storage, rind sucrose concentration declined in all four treatments, from between 42.5 and 49.3 mg/g DM at harvest to 27.7 and 38.1 mg/g DM at week 8 (Fig. 6C). A similar declining trend in sucrose was reported in 'Navelate' and 'Pinalate' oranges stored at 2 and 12 °C (Holland et al., 2005) and may be due to its hydrolysis to monosaccharides, glucose and fructose. Rind fructose concentration gradually increased during storage (Fig. 6A) whereas glucose declined by approximately 0.8-fold (Fig. 6B), indicating the possibility of its use as a substrate during respiration. These results contrast those reported by Holland et al. (2002), who reported that sucrose was a preferred respiratory substrate in 'Fortune' mandarins. Considering that respiration rate increased during storage

(Fig. 5), a consumption of carbohydrates reserves explains the steady decline in glucose and sucrose. The overall change of rind carbohydrates reserves during postharvest storage, as illustrated by total sugars, was not statistically significant (Fig. 6D). This may suggest that in addition to sugars an alternative source of carbon such as starch reserves (not measured in the current study) (Holland et al., 2002, 2005) may have been hydrolysed, although whole rind and flavedo physiology are different.

It is important to note that assimilates absorbed by the fruit are not only used in the fruit energy pool but also for DM accumulation (Hiratsuka et al., 2012). Before storage, rind DM of fruit harvested from the outside position of the canopy had a significantly higher value (32.8%) than bagged inside fruit (30%) (Fig. 7). However, after 8 weeks, the trend was slightly different showing higher DM (31.1%) on inside bagged fruit than that of sun-exposed outside fruit (30.0%). The increase in dry matter as a proportion of fresh mass after 8 weeks may be partly due to the increase in water loss as observed in Fig. 2.

These results strengthen the hypothesis by Cronje et al. (2011a) that lower carbohydrates content in the rind tissue of 'Nules Clementine' mandarins from inside part of the canopy could result in increased susceptibility to RBD. The majority of citrus fruit assimilates are produced by leaves during photosynthesis, but young citrus fruit also contain chlorophyll and are capable of carrying on photosynthesis (Yen and Koch, 1990; Hiratsuka et al., 2012). The low concentration of sugars in the rind of bagged fruit in this study could be the result of inhibited fruit photosynthesis (Thorpe et al., 1974; Yen and Koch, 1990) or reduced fruit sink strength (Hiratsuka et al., 2012). Another hypothesis worth considering when explaining higher sugars concentration on rinds of outside fruit is that of sugar accumulation (osmotic adjustment) induced by dehydration after transpiration from stomata of sun exposed fruit surfaces (Yakushiji et al., 1998). Lower osmotic potential due to net accumulation of sucrose in the cells in response to reduced water potential (Yakushiji et al., 1998; Huang et al., 2000) has previously been reported to attract water into the cells. This hypothesis is also supported by results obtained by Morgan (1992) and Zhang and Archbold (1993), where sucrose had the lowest relative contribution to osmotic potential followed by fructose, while glucose contributed the most. In shaded fruit, Rojas-Lara and Morrison (1989) reported an increase in potassium (K) associated with the reduction of sugars on outside grapes. Mpelasoka et al. (2003) hypothesised the increase in K during preharvest growth as a mechanism to maintain osmotic potential and turgor thereby decreasing the impact of reduced sucrose accumulation on shaded fruit. This may also explain how the decline in sucrose and increase in fructose during postharvest storage in the current coincided with high RBD. It is therefore proposed

that during postharvest storage, higher osmotic potential due to increased fructose resulted in cellular water loss, leading to progressive shrinkage of the vacuole, which in turn induced cell and oil gland collapse (Magwaza et al., 2013b), allowing RBD to develop.

3.6. Non-volatile organic acids

Citric acid was the major non-volatile organic acid followed by malic acid; therefore the total organic acid content followed the trend of citric acid (Fig. 8). Oxalic and malic acids were not significantly affected by preharvest treatments but they increased significantly during postharvest storage (Fig. 8A and B). Oxalic acid concentration increased from 0.4 to 1.4 mg/g DM in the rind of fruit from outside the canopy and from 0.5 to 1.5 mg/g DM in bagged inside fruit (Fig. 8A). Malic acid concentration in the rind decreased during the first three weeks and then increased to higher levels on week 8 compared to week 0. Tartaric acid concentration in the rind increased from a minimum of 6.7 mg/g DM at harvest to a maximum of 9.2 mg/g DM after removal from cold storage (Fig. 8B). Reasons for the observed responses of oxalic, malic and tartaric acids are unclear since preharvest canopy position effects on postharvest organic acids profile of stored citrus fruits remain largely unknown. There were observed changes in non-volatile organic acids during postharvest storage, yet it is unknown what role they might have in the development of RBD.

The concentration of citric acid was affected by fruit canopy position. Before storage, the concentration of citric acid was higher (140.4 mg/g DM) in the rinds of bagged fruit from inside the canopy compared to that of sun-exposed outside fruit (98.8 mg/g DM) (Fig. 8C). Citrus fruit are characterised by their ability to accumulate high levels of citric acids in the vacuoles (Shimada et al., 2006). It is therefore proposed that the accumulation of citric acid into the vacuoles of inside fruit was accompanied by an influx of protons such as H^+ (Muller et al., 1997), which reduced vacuolar pH (Muller and Taiz, 2002). Higher pH in turn provided a driving force for additional citrate uptake into the vacuole to buffer the vacuolar acidic pH. In general, citric acid and AsA showed no significant change during storage. These results are in contrast to those reported by Izumi et al. (1990) who reported a steady decline in flavedo AsA content during storage. A mechanism for this discrepancy could be due to different tissues analysed; whole rind in the present study and flavedo by Izimu et al. (1990). Similar results of no reduction in AsA during storage were obtained in flesh of oranges (Rapisarda et al., 2008) and ‘Satsuma’ mandarin (Izumi et al., 1990).

The concentration of AsA was also affected by preharvest canopy position and shading treatments (Fig. 8D), with inside bagged fruit having lower (3.3 mg/g DM) AsA compared to unbagged outside fruit (6.2 mg/g DM). Previously reported results demonstrated that climatic conditions such as light and temperature have a strong influence on AsA content of horticultural crops (Klein and Perry, 1982). Lee and Kader (2000) stated that the amount of light available to the plant organs during growth have an influence on the amount of AsA formed. According to the literature, AsA is synthesised from carbohydrates, therefore the lower sugar content of shaded inside fruit could have contributed to these lower levels compared to outside fruit (Valpuesta and Botella, 2004; Zhan et al., 2013). A positive correlation between AsA and sucrose and glucose, confirmed the role of these sugars, especially glucose as primary substrate for AsA synthesis pathway (Table 1 and Fig. 3) (Valpuesta and Botella, 2004). Exposure of outside fruit to sunlight may have led to an up regulation of the ascorbate glutathione cycle, which is an important pathway for the recycling of AsA (Ma and Cheng, 2004). As a potent antioxidant, which accounts for a large proportion of the antioxidant capacity of citrus fruit (Bocco et al., 1998), higher concentration of AsA observed in rinds of fruit from the outside position of the canopy may also suggest a defense mechanism of these fruit against preharvest stress.

3.7. Flavanone glycosides (FGs)

Hesperidin was the major FGs detected with its concentration ranging from 35 to 45 mg/g DM followed by narirutin (1.1–2.8 mg/g DM) and didymin (0.7–0.8 mg/g DM) in the fruit rind (Fig. 9). These observations were similar to previously reported results by Xu et al. (2008) and Khan et al. (2010). Didymin was neither influenced by fruit preharvest canopy position and bagging treatments nor postharvest storage (Fig. 9A). At harvest, rinds of 'Nules Clementine' mandarin fruit harvested from outside the canopy had lower hesperidin concentration (38.1 mg/g DM) compared to shaded inside fruit (44.2 mg/g DM) (Fig. 9C). Narirutin concentration on the rinds of inside bagged fruit was 1.7-fold higher than outside fruit indicating the positive effect of reduced light availability on the concentration of this polyphenolic compounds (Fig 9B). These results are similar to those previously reported by Xie et al. (2013) who observed higher flavanoids on bagged fruit compared to un-bagged. Given that high PAR on unbagged fruit on the outside position of the canopy stimulates production of phenylalanine ammonia lyase (PAL), which induces production of phenolic compounds (phytoalexins), flavanones would be expected to be higher on sun-exposed fruit

(Ben-Yehoshua et al., 1992). However, this was not the case in the current study, and a mechanism for this discrepancy still needs to be investigated. Similar to results obtained in the previous study (Chapter 9), total sum of FGs (hesperidin+narirutin+didymin) increased significantly during postharvest storage (p=0.035). Manthey (2004) and Xu et al. (2008) reported that phenolics contribute a major proportion to the total antioxidant capacity in citrus rind. Therefore, results in the current study strengthen the hypothesis that the elevation of FGs could possibly be involved in the defence mechanism of the fruit due to stressful postharvest storage conditions (Chapter 9).

4. Conclusion

Fruit may be exposed to different microclimates within the tree canopy and bagging of individual fruit is a common orchard practice to influence appearance quality. This study has demonstrated important effects of fruit position in the canopy and bagging on rind physico-chemical properties, such as colour, dry matter content as a proportion of fresh mass, mass loss, sugars, organic acids, and FGs in relation to fruit susceptibility to RBD. These results confirmed that postharvest water loss could be an important factor inducing rind breakdown of 'Nules Clementine' mandarin fruit. The results further showed that concentration of biochemical properties on fruit rinds is dependent on microclimatic factors as they affect fruit photosynthesis, respiration, water movement, and sink strength. The rind of fruit located in the outside part of the canopy, which developed under high sunlight conditions, were better coloured (orange) compared to inside fruit which were yellow. The results also showed that rind sugars, ascorbic, citric, and malic acids concentrations of outside fruit were higher than inside fruit while FGs were higher on shaded fruit. Inside fruit with low carbohydrates concentration were more susceptible to RBD. Overall, the results support the hypothesis that reducing PAR levels around an individual fruit reduced fruit photosynthesis rate, which in turn reduced accumulation of solutes, osmotic potential, decreased rind condition and ultimately increased fruit susceptibility to RBD. The incidence of RBD can therefore be reduced by ensuring sufficient light penetration within the canopy by pruning and maintain high water potential during postharvest storage, conditions which are known to reduce the rate of fruit senescence.

References

Abad-García, B., Berrueta, L.A., Garmón-Lobato, S., Urkaregi, A., Gallo, B., Vicente, F., 2012. Chemometric characterization of fruit juices from Spanish cultivars according to their phenolic compound contents: I. citrus fruits. J. Agric. Food Chem. 60, 3635−3644.

Alférez, F., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarìas, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges. I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222–231.

Alférez, F., Alquezar, B., Burns, J.K., Zacarìas, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49.

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Benavente-García, O., Castillo, J., Marin, F.R., Ortunõ, A., Del Río, J.A., 1998. Uses and properties of Citrus flavonoids. J. Agric. Food Chem. 45, 4505–4515.

Ben-Yehoshua, S., Rodov, V., Jin, K. J., Carmeli, S., 1992. Performed and induced antifungal materials of citrus fruits in relation to enhancement of decay resistance by heat and ultraviolet treatment. J. Agric. Food Chem. 40, 1217−1221.

Bocco, A., Cuvelier, M., Richard, H., Berset, C., 1998. Antioxidant activity and phenolic composition of citrus peel and seed extracts. J. Agric. Food Chem. 46, 2123–2129.

Collings, A., García Cas, J.A., Ordaz Ortiz, J.J., Terry, L.A., 2012. A new real-time automated method for measuring in-situ respiration rates of fresh produce. Acta Hort. (In Press).

Crespo, P., Giné Bordonaba, J., Terry, L.A., Carlen, C., 2010. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. Food Chem. 122, 16–24.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of 'Nules Clementine' mandarin fruit, thereby affecting rind condition. J. Am. Soc. Hort. Sci. 138, 217–244.

Cunningham, F.X., 2002. Regulation of carotenoid synthesis and accumulation in plants. Pure Appl. Chem. 74, 1409–1417.

Hiratsuka, S., Yokoyama, Y., Nishimura, H., Miyazaki, T., Nada, K., 2012. Fruit photosynthesis and phosphoenolpyruvate carboxylase activity as affected by lightproof fruit bagging in Satsuma mandarin. J. Am. Soc. Hort. Sci. 137, 215–220.

Holland, N., Sala, J.M., Menezes, H.C., Lafuente, M.T., 1999. Carbohydrate content and metabolism as related to maturity and chilling sensitivity of cv. Fortune mandarin. J. Agric. Food Chem. 47, 2513–2518.

Holland, N., Menezes, H.C., Lafuente, M.T., 2002. Carbohydrate as related to the heat-induced chilling tolerance and respiratory rate of 'Fortune' mandarin fruit harvested at different maturity stages. Postharvest Biol. Technol. 25, 181–191.

Holland, N., Menezes, H.C., Lafuente, M.T., 2005. Carbohydrate metabolism as related to high-tempearture conditioning and peel disorders occurring during storage of citrus fruit. J. Agric. Food Chem. 53, 8790–8796.

Huang, X-M., Huang, H-B., Gao, F-F., 2000. The growth potential generated in citrus fruit under water stress and its relevant mechanisms. Sci. Hort. 83, 227–240.

Iglesias, D.J., Cercós, M., Colmenero-Flores, J.M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-Rivero, Omar, Lliso, I., Morillon, R., Tadeo, F.R., Talon, M., 2007. Physiology of citrus fruiting. Braz. J. Plant Physiol. 19, 333–362.

Ingram, J., Bartels, D., 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 377–403.

Izumi, H., Ito, T., Yoshida, Y., 1990. Changes in fruit quality of Satsuma mandarin during storage, after harvest from exterior and interior canopy of trees. J. Jpn. Soc. Hort. Sci. 58, 885–893.

Kalt, W., Forney, C.F., Martin, A., Prior, R., 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanin after fresh storage of small fruit. J. Agric. Food Chem. 47, 4638–4644.

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M., 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of 'Kinnow' mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). Sci. Hort. 135, 137–144.

Khan, M.K., Albert-Vian, M., Fabiano-Tixier, A., Dangles, O., Chemat, F., 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides from orange (*Citrus sinensis* L.) peel. Food Chem. 119, 851–858.

Khumalo, N.P., 2006. Factors affecting post-storage quality of 'Nules Clementine' mandarin fruit with special reference to rind breakdown. MSc Thesis, Department of Horticultrual Science, Stellenbosch University, Stellenbosch, South Africa.

Klein, B.P., Perry, A.K., 1982. Ascorbic Acid and Vitamin A Activity in Selected Vegetables from Different Geographical Areas of the United States. J. Food Sci. 47, 941–945.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20, 207–220.

Liu, Y-Q., Heying, E., Tanumihardjo, S.A., 2012. History, global distribution, and nutritional importance of citrus fruits. Compr. Rev. Food Sci. Food Saf. 11, 530–545.

Ma, F., Cheng, L., 2004. Exposure of the shaded side of the apple fruit to full sun leads up to up-regulation of both the xanthophyll cycle and the ascorbate gluthione cycle. Plant Sci. 166, 1479–1486.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J.R., Nieuwoudt, H., Mouazen, A.M., Saeys, W., Nicolai, B.M., 2012a. Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest Biol. Technol. 74, 1–10.

Magwaza, L.S., Opara, U.L. Cronje, P.J.R. Nieuwoudt, H.H. Landahl, S., Terry, L. A., 2012b. Quantifying the effects of fruit position in the canopy on physical and biochemical properties and predicting susceptibility to rind breakdown disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) Using Vis/NIR spectroscopy. Acta Hort. 1007, 83–91.

Magwaza, L.S., Ford, H.D., Cronje, P.J.R., Opara, U.L., Landahl, S., Tatam, R.P., Terry, L.A., 2013b. Application of optical coherence tomography to non-destructively

characterise rind breakdown disorder of 'Nules Clementine' mandarins. *Postharvest Biol. Technol. 84, 16–21.*

Manthey, J.A., Grohmann, K., 1996. Concentration of hesperidin and other orange peel flavonoids in citrus processing byproducts. J. Agric. Food Chem. 44, 811–814.

Manthey, J.A., 2004. Fractionation of orange peel phenols in ultrafilterd molasses and mass balance studies of their antioxidant levels. J. Agric. Food Chem. 52, 7586–2193.

Mathur, A., Verma, S.K., Purohit, R., Gupta, V., Dua, V.K., Prasad, G.B.K.S., Mathur, D., Singh, S.K., Singh, S., 2011. Evaluation of *in vitro* antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. IJPI's J. Biotechnol. Biotherapeutics 1, 1-17.

McDonald, R.E., Miller, W.R., McCollum, T.G., 2000. Canopy position and heat treatments influence gamma-irradiation-induced changes in phenylpropanoid metabolism in grapefruit. J. Am. Soc. Hort. Sci. 125, 364–369.

Morgan, J. M., 1992. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol., 1992, 19, 67–76.

Mpelasoka, B.S., Schachtman, D.P., Treeby, M.T., Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape Wine Res. 9, 154–168.

Muller, M.L., IrkensKiesecker, U., Kramer, D., Taiz, L., 1997. Purification and reconstitution of the vacuolar H+-ATPases from lemon fruits and epicotyls. J. Biol. Chem. 272, 12762–12770.

Muller, M.L., Taiz, L., 2002. Regulation of the lemmon-fruit V-ATPase by variable stoichiometry and organic acids. J. Membr. Biol. 185, 209–220.

Pathare, P.B., Opara, U.L., Al-Said, F.A., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Tech. 6, 36–60.

Poiroux-Gonord, F., Fanciullino, A-L., Poggi, I., Urban, L., 2013. Carbohydrate control over carotenoid build-up is conditional on fruit ontogeny in clementine fruits. Physiol. Plant. 147, 417–431.

Rapisarda, P., Lo Bianco, M., Pannuzzo, P., Timpanaro, N., 2008. Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. Postharvest Biol. Technol. 49, 348–354.

Rojas–Lara, B.A., Morrison, J.C., 1989. Differential effects of shading fruit or foliage on the development and composition of grape berries. Vitis 28, 199–208.

Shimada, T., Nakano, R., Shulaev, V., Sadka, A., Blumwald, E., 2006. Vacuolar citrate/H+ symporter of citrus juice cells. Planta 224, 472–480.

Sun, X-H., Xiong, J-J., Zhu, A-D., Zhang, L., Ma, Q-L., Xu, J., Cheng, Y-J., Deng, X-X-., 2012. Sugars and organic acids changes in pericarp and endocarp tissues of pumelo fruit during postharvest. Sci. Hort. 142, 112–117.

Swingle, W.T., 1943. The botany of citrus and its wild relatives of orange subfamily: Group C. The true citrus fruit trees. In: Reuther, W., Webber, H.J., Batchelor, L.D. (Eds). The Citrus Industry,Vol. I. Div. Agric. Sci. Univ. Calif., Berkeley, California, pp. 192–474.

Terry, L.A., Chope, G.A., Giné Bordonaba, J., 2007. Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria* x *ananassa*) fruit quality. *J. Agric. Food Chem.* 55, 10812–10819.

Thorpe, M.R., 1974. Radiant heating of apples. J. Appl. Ecol. 11, 755–760.

Tomás-Barberán, F.A., Gil-Izquierdo, A., Ferreres, F., Gil, M.I., 2003. Analysis and putification of flavanones, chalcones and dihydrochalcones. In Methods in Polyphenol Analysis, 1st edition; Santos-Buelga, C., Williamson, G. The Royal Society of Chemistry, Cambridge, UK, pp, 359–371.

Uckoo, R.M., Jayaprakasha, G.K., Nelson, S.D., Patil, B.S., 2011. Rapid simultaneous determination of amines and organic acids in citrus fruit using high-performance liquid chromatography. Talanta 83, 948–954.

Valpuesta, V., Botella, M.A., 2004. Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. Trends in Plant Science 9, 573–577.

Xie, R.J., Zheng, L., Jing, L., He, S.L., Xi, W.P., Lv, Q., Yi, S.L., Zheng, Y.Q., Deng, L., 2013. The Effect of Cultivar and Bagging on Physicochemical Properties and Antioxidant Activity of Three Sweet Orange Cultivars (*Citrus sinensis* (L.) Osbeck). American-Eurasian J. Agric. Environ. Sci. 13, 139–147.

Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., Jiang, P., Ye, X.Q., 2008. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73, C11–C18.

Yakushiji, H., Morinaga, K., Nonami, H., 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. J. Am. Soc. Hort. Sci. 123, 719–726.

Yen, C-R., Hoch, K.E., 1990. Developmental changes in translocation and localization of ^{14}C-labbeled assimilates in grapefruit: light and dark CO_2 fixation by leaves and fruit. J. Am. Soc. Hort. Sci. 115, 815-819.

Zhan, L., Hu, J., Ai, Z., Pang, L., Li, Y., Zhu, M., 2013. Light exposure during storage preserving soluble sugar and L-ascorbic acid content of minimally processed romaine lettuce (*Lactuca sativa* L.var. *longifolia*). Food Chem. 136, 273–278.

Zhang, B., Archbold, D.D., 1993. Solute accumulation in leaves of a *Fragaria chiloensis* and a *E virginiana* selection responds to water deficit stress. J. Am. Soc. Hort. Sci. 118, 280–285.

Table 1: Pearson correlation coefficient matrix between different measured rind physico-chemical properties and the susceptibility of fruit to progressive rind breakdown in 'Nules Clementine' mandarin.

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Dry Matter (%)																	
2	*L**	-0.35**																
3	*C**	-0.03	-0.33**															
4	*h°*	-0.08	0.72**	-0.76**														
5	Mass loss (%)	0.06	-0.35**	0.73**	-0.60**													
6	Sucrose	-0.07	0.12	-0.34**	0.15	-0.36**												
7	Glucose	-0.06	-0.35**	-0.07	-0.21**	-0.06	0.59**											
8	Fructose	-0.19*	-0.47**	0.26**	-0.50**	0.24**	0.44**	0.89**										
9	Narirutin	0.06	0.22**	0.00	0.20*	0.01	-0.35**	-0.58**	-0.48**									
10	Hesperidin	0.12	0.08	0.25**	0.00	0.28**	-0.49**	-0.54**	-0.40**	0.70**								
11	Didymin	0.21*	-0.16	0.22*	-0.17*	0.10	-0.39**	-0.37**	-0.23**	0.73**	0.65**							
12	Oxalic acid	-0.01	-0.33**	0.36**	-0.40**	0.27**	0.02	0.12	0.29**	-0.06	0.08	0.16						
13	Tartaric acid	-0.04	-0.33**	0.22*	-0.22*	0.28**	-0.14	0.23*	0.32**	-0.12	0.06	-0.10	0.15					
14	Malic acid	0.02	-0.22*	0.20	-0.12	0.27**	-0.15	0.25*	0.27**	-0.03	0.11	-0.00	0.23*	0.66**				
15	Ascorbic acid	0.03	-0.23*	0.0	-0.13	-0.10	0.13	0.25*	0.15	-0.31**	-0.29**	-0.00	-0.00	-0.07	-0.16			
16	Citric acid	-0.06	0.20*	-0.03	0.14	0.03	-0.28**	-0.29**	-0.22*	0.33**	0.39**	0.17	-0.00	0.38**	0.13	-0.24*		
17	RBD	-0.21**	0.03	0.16*	-0.07	0.22**	0.02	-0.03	0.10	0.16	0.16	0.02	0.17	0.42**	0.35**	-0.42**	0.26*	1

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). *L** lightness, *C** chroma, *h°* hue angle, RBD rind breakdown disorder.

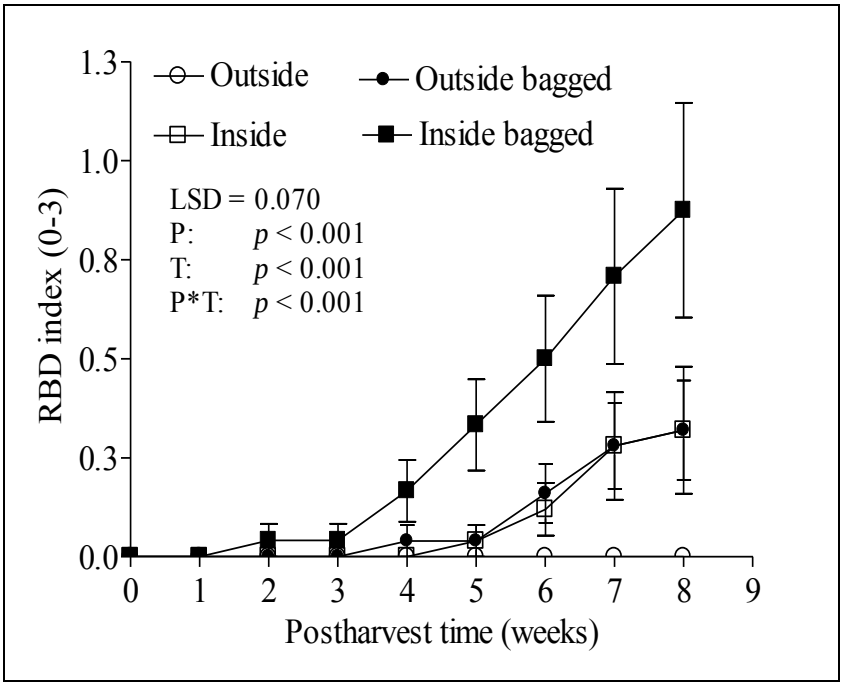

Fig. 1: Effect of preharvest canopy position and bagging treatments on the incidence of rind breakdown of 'Nules Clementine' mandarin during postharvest storage. Values are the means ± standard deviation.

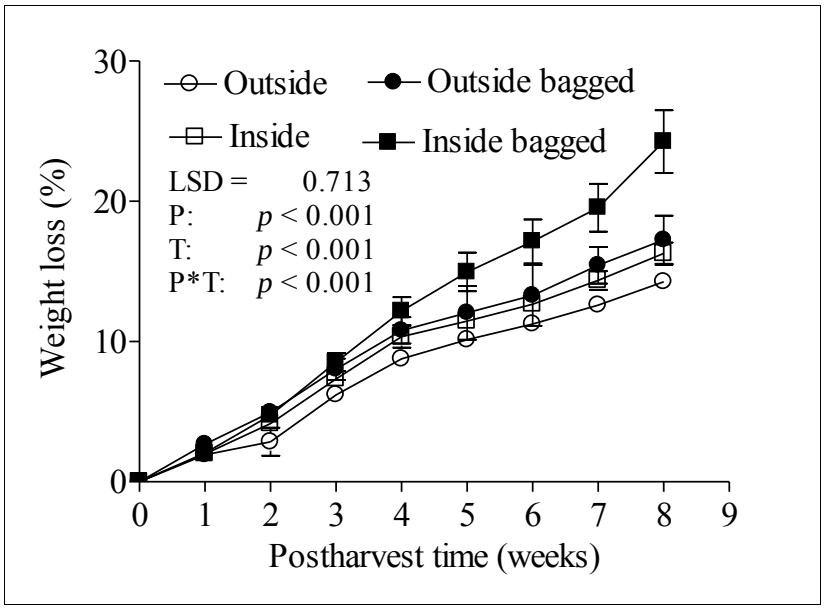

Fig. 2: Postharvest mass loss of 'Nules Clementine' mandarins harvested from different preharvest canopy position and bagging treatments. Values are the means ± standard deviation.

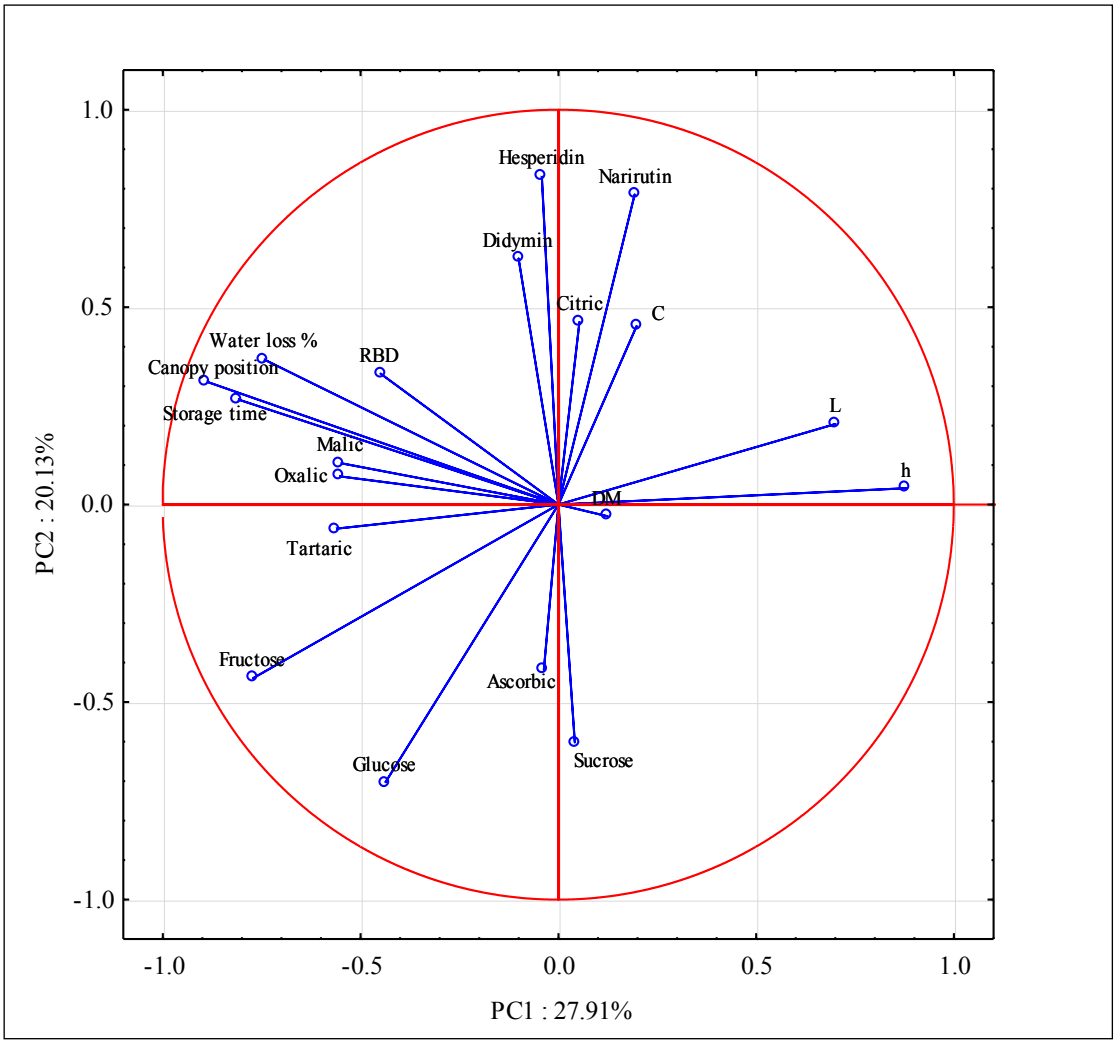

Fig. 3: Principal component analysis of the first two principal components (PC) showing correlation between preharvest canopy position, storage time, different measured rind physico-chemical properties and the susceptibility of fruit to progressive rind breakdown in 'Nules Clementine' mandarin. *RBD* rind breakdown disorder, *DM* rind dry matter content, *L* lightness. *C** chroma, *h* hue angle.

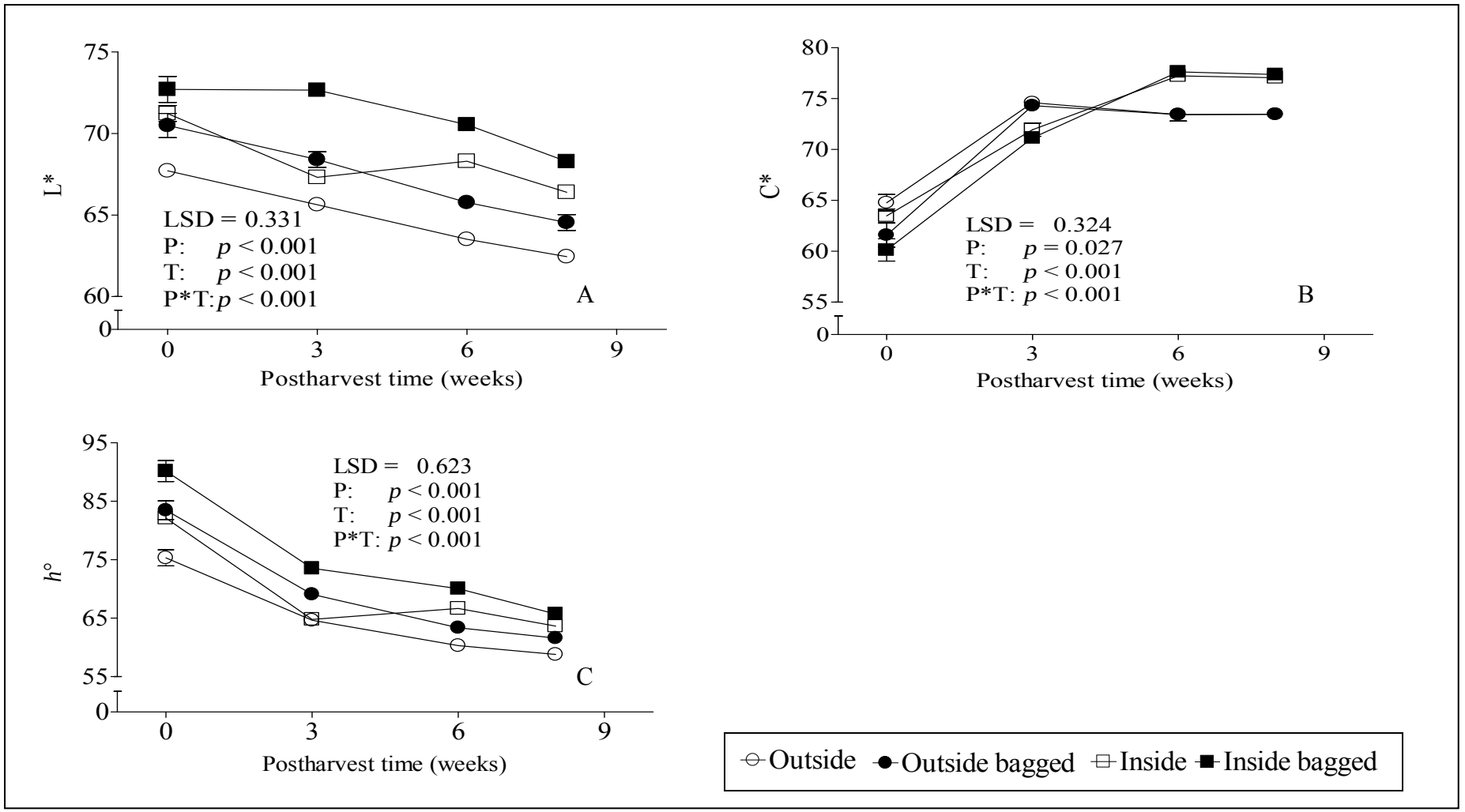

Fig. 4: Postharvest changes if rind colour parameters (A = lightness, B = chroma, C = hue angle) of 'Nules Clementine' mandarin harvested from different preharvest canopy positions and bagging treatment. Values are the means ± standard deviation. *LSD* least significant difference ($p \leq 0.001$).

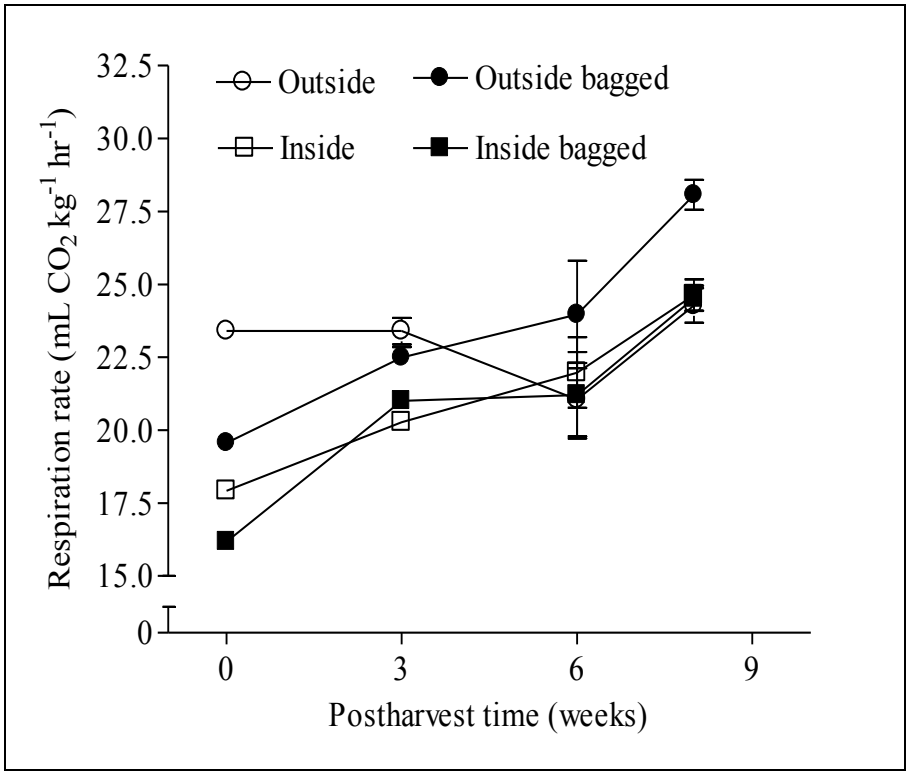

Fig. 5: Respiration rate of 'Nules Clementine' mandarins harvested from different canopy positions and bagging treatments during postharvest storage. *LSD* least significant difference ($p \leq 0.001$). Values are the means ± standard deviation. *P* canopy position, *T* postharvest storage time, *P*T* interaction between the two main effects.

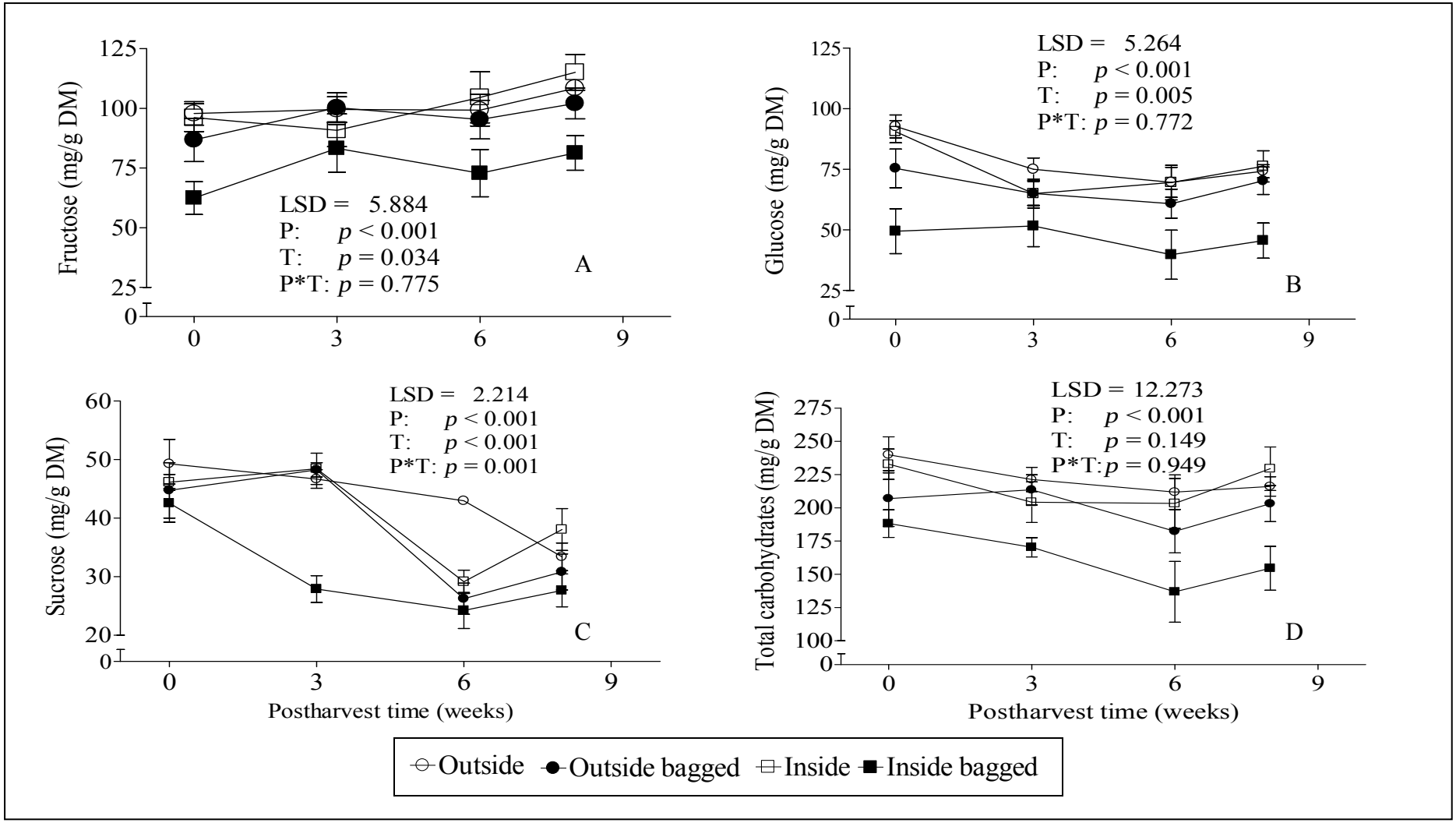

Fig. 6: Positional and bagging effects on non-structural carbohydrates composition in rinds of 'Nules Clementine' mandarins during postharvest storage. (A = fructose, B = glucose, C = sucrose, D = total carbohydrates). Values are the means ± standard deviation. *LSD* least significant difference ($p \leq 0.001$). *P* canopy position, *T* postharvest storage time, *P*T* interaction between the two main effects.

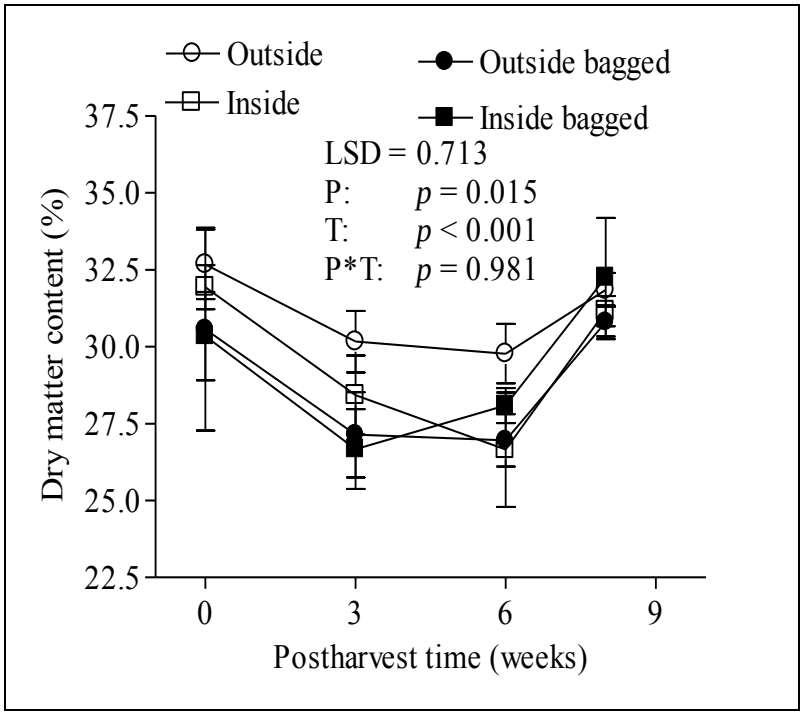

Fig. 7: Preharvest canopy position and bagging treatments effects on rind dry matter content of 'Nules Clementine' mandarins during postharvest storage. Values are the means ± standard deviation. *LSD* least significant difference ($p \leq 0.001$). *P* canopy position, *T* postharvest storage time, *P*T* interaction between the two main effects.

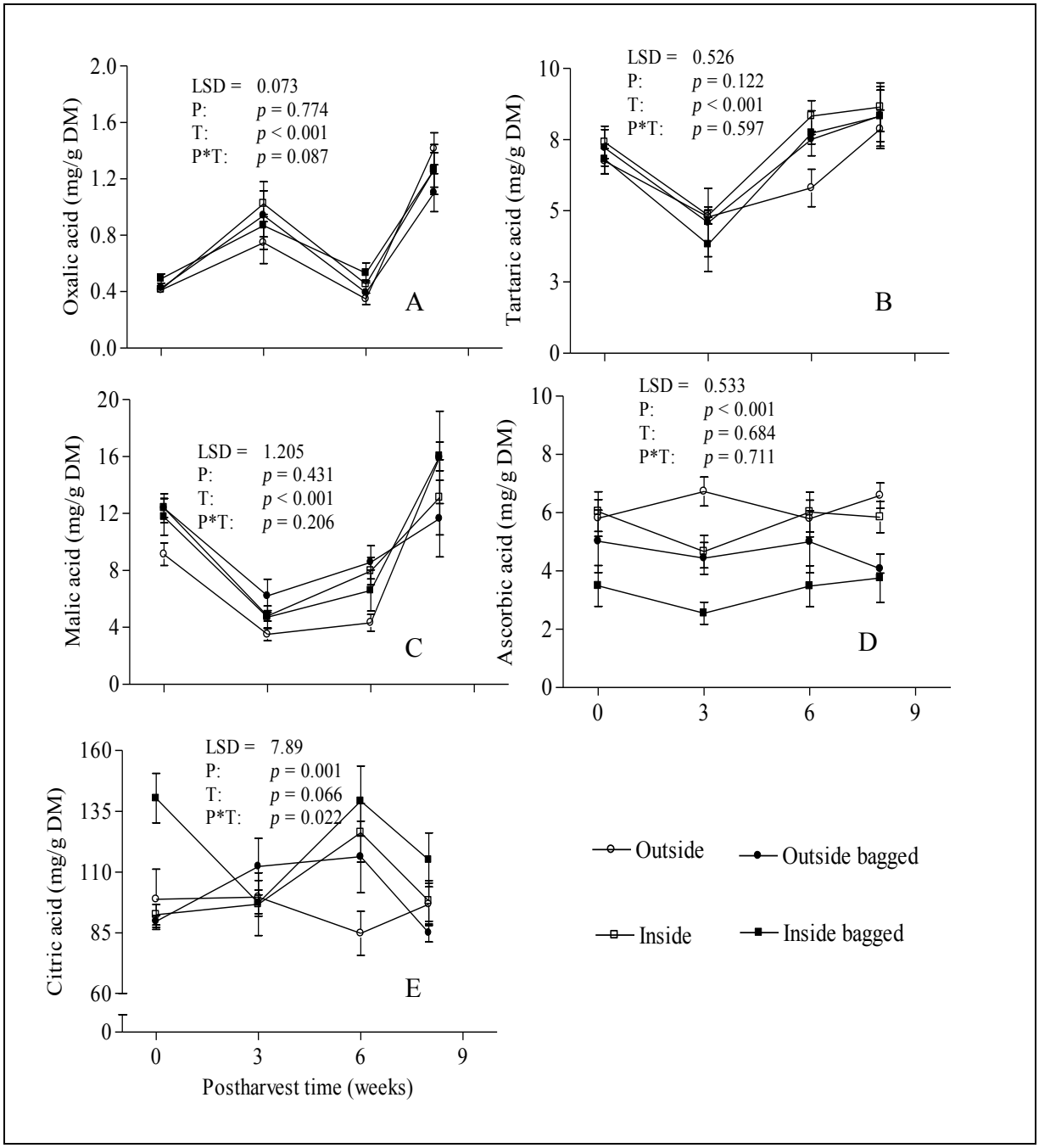

Fig. 8: Positional and bagging and storage time effects on non-volatile organic acids composition in rinds of 'Nules Clementine' mandarin fruit. (A = Oxalic, B = tartaric, C = malic, D = ascorbic, and E = citric acids). Values are the means ± standard deviation. *LSD* least significant difference ($p \leq 0.001$). *P* canopy position, *T* postharvest storage time, *P*T* interaction between the two main effects.

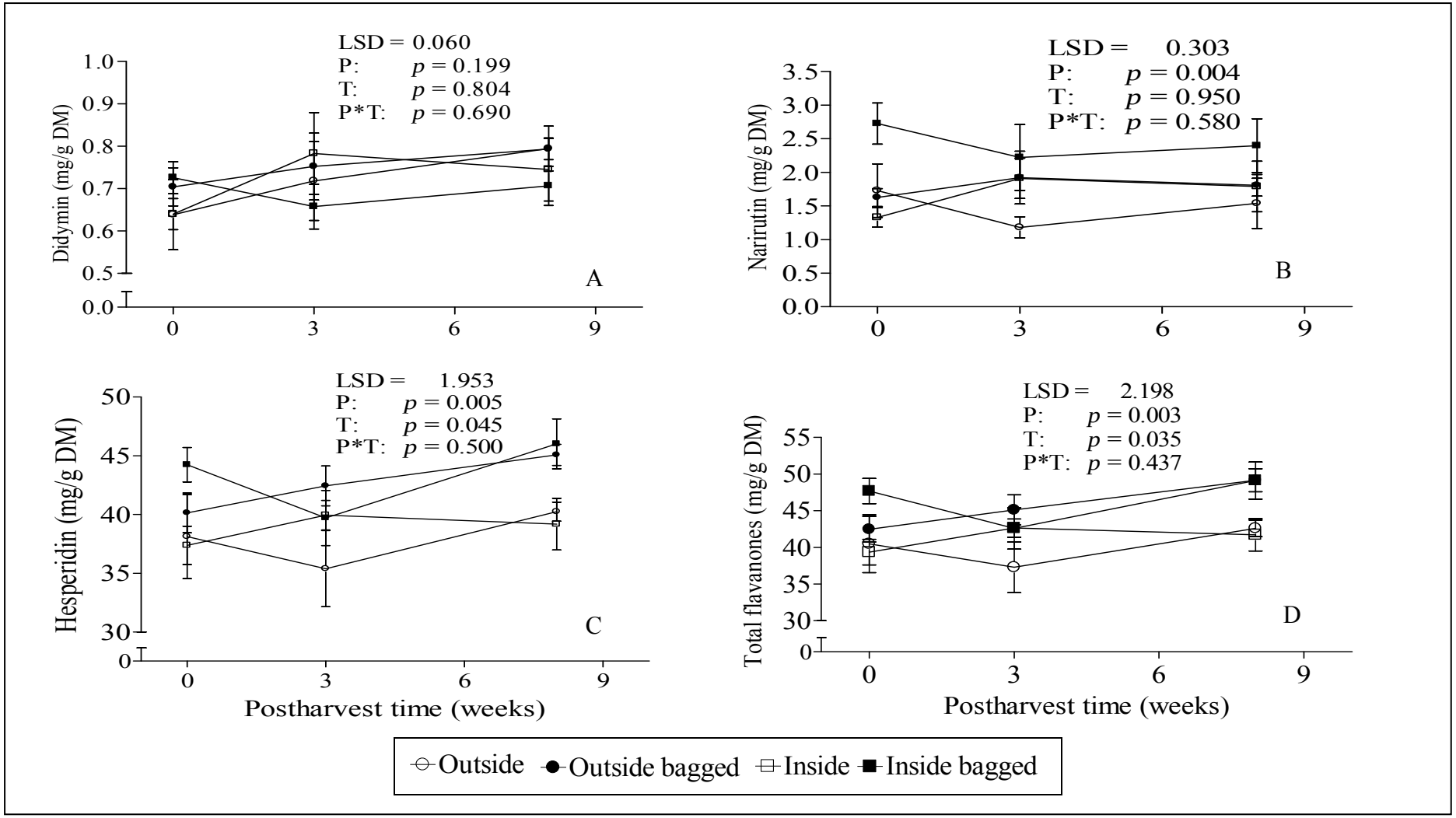

Fig. 9: Effect of fruit preharvest position and bagging treatments, postharvest storage on concentration of rind didymin (A), narirutin (B), hesperidin (C), and total flavanones (D). *LSD* least significant difference ($p \leq 0.001$). Values are the means ± standard deviation. *P* canopy position, *T* postharvest storage time, *P*T* interaction between the two main effects.

CHAPTER 11

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

1. Introduction

South Africa exports approximately 120 000 tons of 'Nules Clementine' mandarins (*Citrus reticulata* Blanco.) per year, making it the third largest producer and exporter of Clementine mandarins after Spain and Morocco (Citrus Growers' Association of Southern Africa, 2012). 'Nules Clementine' mandarin fruit are prone to develop a progressive non-chilling physiological rind disorder during postharvest storage, referred to as rind breakdown (RBD). Although RBD is a superficial rind disorder that does not compromise the edible internal portion of the fruit, it, dramatically decreases fruit market value. On some citrus sorting lines, cameras are already being used to detect the presence of rind lesions such as disorders and colour development. However, these are not suitable for detecting RBD, since the disorder characteristically does not show visual symptoms during packhouse grading and packing, but develops about 3 to 5 weeks after harvest (Cronje et al., 2011a). In the exported-oriented South African citrus industry, this delayed symptom development is extremely problematic. Previous study by Cronje (2009) reported that fruit harvested from inside the canopy had lower sugars and were more susceptible to RBD. These findings suggested that rind carbohydrates concentration could be used as potential biochemical indicators of fruit susceptibility to RBD. A major challenge facing the citrus industry and researchers alike, is to develop non-destructive technology to determine quality on the packing line. As such, the use of visible to near infrared (Vis/NIR) spectroscopy (Vis/NIRS) to evaluate fruit physiological defects and predict postharvest quality attributes of fruit are topical in postharvest research (Cozzolino et al., 2010).

Existing analytical methods for the quantification of carbohydrates in fruit rind and biomaterials are expensive, time consuming, and require specialised sample preparation. Increasing consumer demand for internal quality attributes such as sweetness and nutritional content, coupled with industry demand for innovative tools for rapid and cost-effective detection and monitoring of physiological disorders, have spurred considerable interest among researchers on the application of non-destructive methods on citrus fruit quality monitoring and evaluation. These non-destructive instrument-based methods for assessing fruit quality will assist citrus packers to segregate fruit based on their desired quality attributes and also susceptibility to rind physiological disorders. Internationally, there are

considerable research activities into the investigation of Vis/NIR-based sensors for determining various quality factors in a range of fruit and vegetables, including citrus (Nicolaï et al., 2007). As such, Vis/NIRS has become one of the mostly used technologies for the non-destructive evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Miyamoto and Kitano, 1995; Nicolaï et al., 2007; Cozzolino et al., 2010; Wedding et al., 2013). Vis/NIRS has been applied to packing house fruit sorting lines for TSS quantification of citrus and other fruit since the mid 1990s (Kawano et al., 1993). In contrast, very limited research has been conducted to evaluate Vis/NIRS to predict and monitor physiological disorders and rind physiological disorders of citrus fruit in particular (Zheng et al., 2010). Vis/NIRS offers a potential for the non-invasive assessment of the chemical composition and changes thereof, such as carbohydrate content in the rind of intact fruit that might predispose fruit to the development of rind disorders. Based on the current literature information, the overall aim of this study was to develop non-destructive methods based on Vis/NIR spectroscopy to predict external and quality of citrus fruit during postharvest storage.

2. Non-destructive evaluation of citrus fruit quality using Vis/NIR spectroscopy

A preliminary study was conducted in Chapter 4 to determine optimum conditions of near infrared (NIR) spectroscopy (NIRS) measurements and evaluate the accuracy of this technique to detect physico-chemical properties of citrus fruit. Most of the previous NIRS investigations on citrus fruit have focused on assessing specific internal quality attributes and did not integrate quantitative assessment of external and internal quality attributes in one system (Gómez et al. 2006; Lu et al., 2006; Liu et al., 2010; Jamshidi et al., 2012). This study evaluated the feasibility of three Fourier transform near infrared (FT-NIR)-based spectrophotometers namely, a fibre-optic probe for solid samples (SP), an integrating sphere (IS) and an emission head (EH), for integrated non-destructive measurements of external and internal quality attributes of 'Valencia' oranges [*Citrus sinensis* L. (Osb.)]. The results showed that calibration models developed using the EH predicted mass, colour index, and vitamin C better than other spectrometers (or acquisition modes), while the IS gave the best prediction for TSS. However, the prediction statistics for the TSS obtained using EH were also comparable (RMSEP = 0.65 °Brix) to those of IS (RMSEP = 0.58 °Brix). Considering that consumers can detect a difference in taste of about 1 °Brix, an RMSEP of 0.65 °Brix for

EH showed that the model possesses the ability to assign the oranges to specific taste requirements of consumers.

Good correlation between spectral information obtained by the EH spectrophotometer and quality attributes measured by conventional methods (mass, colour index, TSS and vitamin C) demonstrated the potential of this FT-NIR instrument as a non-destructive tool to holistically evaluate fruit external and internal quality parameters. Although mass was predicted with high accuracy, the future use of NIRS in predicting mass is questionable considering that there are cheaper and more user-friendly methods. However, if FT-NIR could be successfully developed to automatically and simultaneously measure a combination of internal and external quality attributes, as shown to be possible using EH, investing on the technology and model development could be justified. Considering that before the current study, very limited studies have sought to assess the suitability of NIRS for integrated assessment of internal and external quality parameters in intact oranges (Sánchez et al., 2013), this research made a significant contribution towards on-line non-destructive evaluation of citrus fruit.

Considering that the chances of a successful commercial implementation of non-destructive technology depends on model robustness, external validation of calibration models using samples from different orchards, growing regions and production season has become a critical issue in Vis/NIRS and an active area of research (McGlone et al., 2002; Guthrie et al., 2005b; Zude et al., 2008). In this study, sufficient variation of quality attributes was added to calibration and validation data sets. This was shown on calibration models developed using all fruit which performed better in predicting all quality parameters compared to models developed based on small sample population. The better model performance developed across a population of fruit with larger range in the quality attribute in question demonstrated the importance of having enough variability in the reference samples tested.

High predictive performance of calibration models when validated using fruit from another location demonstrated high level of model robustness. However, before EH FT-NIR spectrophotometers can be successfully implemented on a commercial sorting line, validation across different seasons would be advisable. Guthrie et al. (2005a, b) recommended that optical and reference sampling for all citrus fruit should be at any position around the equatorial position of the fruit in order to best represent the entire fruit. In the current study, the spectra taken from the equatorial position of orange fruit was used for the PLS calibration and validation. The problem with this approach is that in commercial packing lines, it is

currently not possible to align fruit in a manner that allows the NIR device to always scan equatorial cross-sectional portion of the fruit. It is recommended that semi-commercial studies be conducted where spectra is acquired at random on fruit rolling on a conveyor belt to test the predictive ability of the NIR device and model, to overcome the effect of spatial differences in quality of fruit, which may result in significant increase in the prediction error. Williams and Sobering (1993), Cléments et al. (2008) and Davey et al. (2009), reported that pigments and colour results obtained using an NIR instrument, which includes a visible range of the electromagnetic spectrum were better than those without visible range. Therefore, the modification of the lamps and detectors of the NIR instruments to incude the visible range of the spectrum (350 to 750 nm) would enable colour to be measured from principle, rather indirectly through PLS models against chromameter measured colour space. Based on this recommendation, subsequent experiments conducted to develop calibration models to predict susceptibility of 'Nules Clementine' mandarin fruit to RBD used Vis/NIRS. The use of chemometrics to analyse Vis/NIR spectra collected from intact 'Nules Clementine' mandarin fruit at harvest, was explored to predict the rind biochemical profile after eight weeks of storage.

In Chapter 5 to 7, studies were conducted to evaluate the ability of Vis/NIRS to non-destructively predict rind physico-chemical profile of 'Nules Clementine' mandarins and susceptibility to RBD. The studies further explored different chemometric analysis of spectra acquired from intact fruit at harvest to predict postharvest rind physico-chemical properties related to rind quality and susceptibility to RBD. Due to the discrete nature of RBD scores, correlating it with complex Vis/NIRS data was difficult and partial least squares (PLS) model statistics were poor, suggesting that the technique is not able to accurately predict the disorder. The complexity of biological factors involved in the development of RBD (Cronje et al., 2011a, b) may also account for the difficulty of developing a reliable prediction model for RBD.

Nevertheless, rind physico-chemical properties such as rind sugars and dry matter content, which could be used for potential on-line application of Vis/NIRS, were predicted with accuracy for up to eight weeks of fruit storage. Previous studies have shown that RBD-susceptible fruit located inside the canopy had lower rind sugar concentration and dry matter content (Cronje et al., 2011a). Taking this into account, the ability of Vis/NIRS to predict rind sugars and dry matter content as possible biochemical indicators of fruit susceptibility to RBD was explored. The good prediction statistics for these rind properties which predispose fruit to RBD revealed the ability of Vis/NIRS and chemometrics to predict postharvest

behaviour of mandarins and hence susceptibility to the disorder. The chemical composition of citrus fruit varies from stem to blossom end and from sun to shade sides of the same fruit (Peiris et al., 1999). This would limit the implementation of the Vis/NIRS for a real-time on-line sorting system, unless spectra acquisition is repeated at several positions around the fruit in order to minimise the effect of variation within fruit. However, this might not be practically compatible with a typical speed of commercial sorting lines, which may be as high as 10 fruit per second (Nicolaï et al., 2007). Therefore, parallel sorting lines with turning fruit and a number of spectrophotometers might overcome this problem, although costs of implementation would be high.

Vis/NIRS models obtained in the current study also demonstrated the importance of model robustness in predicting new population of fruit. Adding orchard and seasonal variability to the calibration models reduced prediction errors of target analytes. These observations are similar to those by Wedding et al. (2013), who reported improved model performance when more seasonal variability was included in the calibration set. Although the models developed in this study showed high level of robustness, it is important to stress that special attention should be paid when new populations of fruit from different orchards or growing regions and production or marketing seasons are evaluated. Considering the observed variation between growing locations and seasons, it is advisable to upgrade calibration models, using fruit from successive seasons and orchard locations. Spiking existing calibration models with a few samples from the target prediction orchard will potentially improve model performance and reduce calibration time and costs.

Although this study has demonstrated the considerable potential of Vis/NIRS to predict biochemical properties associated with RBD, certain aspects could be improved. The performance of calibration models for sucrose was not satisfactory and had very high prediction errors, which could be attributed to high skewness in data distribution. The skewness has recently been shown to affect interpretation of PLS model statistical parameters such as RPD, which was developed for biological data sets showing normal distribution (Bellon-Maurel et al., 2010). The use of the standard deviation (the numerator in the RPD ratio) of highly skewed data with many low values samples, such as the distribution of RBD, may not be statistically correct. In addition, there is still no definite upper or lower limit of carbohydrates concentration in which the disorder occurs or does not occur. Hence, further research still needs to be conducted to explain whether Vis/NIRS-predicted carbohydrates concentration is useful for determining fruit susceptibility to postharvest RBD.

Principal component analysis (PCA) and PLS discriminant analysis (PLS-DA) models based on spectra acquired before harvest were able to discriminate fruit based on their position within the canopy. The accuracy of the two regression methods was very high indicating that both methods could be used, individually or in combination, for screening purposes. The results in Paper 6 of this thesis indicated that fruit located inside the canopy were more susceptible to RBD than outside fruit. Therefore, the ability of Vis/NIRS to discriminate between inside and outside fruit suggests the potential of this technology to discriminate fruit based on their susceptibility to RBD. In practical terms, the capability of Vis/NIRS to non-destructively segregate fruit based on their origin within the canopy, suggested the use of this technology as an on-line deciding tool to classify individual fruit destined for either local (inside fruit) or export market and long term storage (outside fruit).

3. Biochemical profile of fruit located in different positions of the canopy

Recent results from Cronje (2009) demonstrated that outside fruit with higher sugar content developed less RBD than inside fruit. The hypothesis was that limited photosynthetically active radiation (PAR) on shaded portions of the canopy reduced fruit photosynthesis rate and rind carbohydrates, which in turn increase susceptibility to RBD. However, these studies were conducted in one citrus growing region of South Africa (Stellenbosch), therefore, the proposed hypothesis for the mechanism underlying the development of RBD may not be generalised for all growing regions.

To test the hypothesis describing the effect high or low light on the sensitivity of 'Nules Clementine' mandarin fruit to rind sugar concentration and RBD incidence, the study in Paper 6 was extended to include more orchards from other citrus growing locations. The main finding of this study was that fruit position within the canopy and exclusion of sunlight by bagging fruit during growth had a significant effect on rind physiological and biochemical properties as well as susceptibility to RBD. In all four orchard locations, 'Nules Clementine' mandarin fruit borne inside the canopy were more susceptible to RBD compared to other preharvest treatments, while bagging of inside fruit resulted in the highest incidence of the disorder. These results indicated that regardless of orchard location, reduced exposure of fruit to sunlight during growth was the major preharvest factor contributing to the disorder. The results of the current study were similar to those reported by Cronje et al. (2011a, b), who observed higher RBD on shaded fruit compared to sun-exposed outside fruit. In contrast to disorders such as rind staining in 'Fortune' mandarins (Almela et al., 1992; Duarte and

Guardiola, 1995) and rind breakdown in 'Navel' oranges (Agustí et al., 2001) where peel disorders were associated with sun/heat exposure, RBD in 'Nules Clementine' occurred on inside fruit because the disorder is associated with low mineral and carbohydrate allocation and therefore premature senescence (Cronje et al., 2011a). These findings of the current study provided further evidence of the pre-harvest conditions affecting postharvest behaviour of citrus rinds in relation to RBD. In one orchard (Citrusdal), the concentrations of sucrose, glucose and fructose in the rind of bagged fruit from inside and outside canopy positions were lower than those of unbagged fruit. Reduced radiation by bagging has previously been reported to negatively affect non-structural carbohydrates concentration of citrus fruit (Hiratsuka et al., 2012). An opposite pattern was observed in rinds of fruit harvested from two other orchards (Porterville and Paarl), which had higher concentrations of non-structural carbohydrates (fructose, sucrose and total carbohydrates) than sun-exposed fruit. For fruit grown in Stellenbosch area, sucrose of bagged inside fruit was also higher compared to outside fruit. In these three orchards, shaded fruit containing higher non-structural carbohydrates were more susceptible to RBD during storage, suggesting that the carbohydrate content was not a limiting factor in the susceptibility of 'Nules Clementine' mandarins to RBD in these regions. Different carbohydrates responses to reduced sunlight indicated that water soluble sugars might not be a universal biochemical marker or the primary cause for RBD, since it is highly dependent on orchard and location.

Although non-structural carbohydrates of fruit from different locations responded inconsistently to preharvest canopy position and light reduction treatments, rinds of sun-exposed outside had significantly higher dry matter content (30 to 35 g/100g FM) than inside fruit (27 to 30 g/100g FM), indicating a close relationship between sunlight level within the tree canopy and the levels of accumulated structural carbohydrates. The strong negative correlation between RBD and rind dry matter shown in the PCA plots in Chapters 7 and 9 confirmed that rind dry matter content could be used as a potential biochemical indicator of fruit susceptibility to the disorder. The results obtained using Vis/NIRS to predict rind dry matter was another positive breakthrough towards on-line predicting RBD. Therefore, as a possible benchmark for fruit harvested in the studied orchards, it is recommended that for fruit destined for export market or long term storage, rind dry matter should not be below 30 g/100g FM at harvest. However, detailed studies to quantify change in dry matter content as fruit develops would still be required to determine the optimum range of dry matter to minimise or eliminate incidence of RBD.

The shortcomings of the studies in Chapters 7 and 9 are that physico-chemical properties of fruit were only determined at the beginning of storage and in final evaluation date (after 8 weeks) and not throughout the storage period along with the recording of RBD incidence. Taking this limitation and inconsistent respond of rind sugars of fruit from different orchards to preharvest canopy and light reduction treatments into account, a more detailed investigation was conducted. The assessment of such biochemical markers and correlation constituted the principal framework of the research in Paper 9 of this thesis which was projected towards understanding the mechanism of RBD, which in turn could lead to a pre-symptomatic detection and/or prediction of the disorder. In this part of the study, the focus was on several rind quality parameters and composition of sugars, organic acids, and phenolic compounds in rinds of harvested inside and outside canopy, and also in bagged fruits. Another objective of this comprehensive study was to investigate physiological changes associated with senescence during postharvest storage.

Analysis of sugars concentration patterns in the rind of mandarin fruit during postharvest storage showed that sucrose levels declined which may be due to its hydrolysis to monosaccharides, glucose and fructose. Rind fructose concentration gradually increased during storage, whereas glucose declined, indicating the possibility of its use as a substrate during senescence-associated respiration. Osmotic adjustment resulting from a decline in sucrose (a solute with the lowest contribution to osmotic potential) and accumulation of fructose may have contributed to RBD development. The proposed hypothesis is that during postharvest storage, higher osmotic potential due to increased fructose resulted in cellular water loss, leading to progressive shrinkage of the vacuole, which in turn induced cell and oil gland collapse, allowing RBD to develop. Similar to results obtained in Papers 4, 6 and 8, shaded inside fruit had higher mass loss during storage and this corresponded with higher levels of RBD. In accordance with literature (Alférez et al., 2003, 2005, 2010; Alférez and Burns, 2004; Alquezar et al., 2010), changes in the water status of the rind was one of the primary postharvest factors contributing to the susceptibility of ‘Nules Clementine’ mandarin fruit to RBD. The low correlation coefficient (0.22) between the RBD and mass loss observed in this study (Paper 9), suggested that development of the disorder might be attributed to other factors, in addition to mass loss during postharvest storage. Due to the complexity of biological factors involved in the development of RBD, we have not been able to establish the upper limit or lower limit of in which the disorder occurs.

The findings discussed above, support the hypothesis that reducing PAR levels around an individual fruit reduced fruit photosynthesis rate, which in turn reduced accumulation of

solutes, osmotic potential, decreased rind condition and ultimately, increased fruit susceptibility to RBD. From a horticultural perspective, the incidence of this disorder can therefore be controlled by maintaining sufficient light penetration within the canopy by pruning, high water potential postharvest, and therefore reducing the rate of fruit senescence and water loss.

4. Identification of potential presymptomatic biochemical markers for RBD

Detailed review of the literature in Chapter 2 showed that existing knowledge of chemical changes in the rind of citrus fruit that could be used to predict fruit susceptibility to RBD is limited, with most research focusing on content and concentration of carbohydrates. It was therefore crucial to explore other potential pre-symptomatic biochemical indicators or markers descriptive of rind condition and fruit susceptibility to RBD, which could be detected by Vis/NIR spectroscopy. In the quest to identify further biochemical indicators correlated to RBD, non-volatile organic acids and flavanone glycosides were selected as possible candidates because of their high concentration in rinds of citrus fruit. In a study like this one, it is customary to first address major compounds known to have a marked response to stress. Before this was done, novel method developments to improve extraction efficiency and quantification of phenolic compounds was carried out and results presented in Appendix 1. From these experiments, a total of seven phenolic acids, including three hydroxybenzoic acids (ρ-hydroxybezoic and vanillic), and five hydroxycinnamic acids (chlorogenic, caffeic, ρ-coumaric, ferrulic, and sinapic) as well as three flavanones (narirutin, hesperidin and didymin) were identified and quantified. The method separated 10 phenolic compounds faster (52 minutes) than 120 minutes as reported by Li et al. (2006), Kelebek (2010) and Kelebek and Selli (2011).

At harvest, the rind of fruit harvested from outside the canopy had significantly lower hesperidin concentration compared to shaded fruit. Narirutin concentration in the rind of inside bagged fruit was 1.7-fold higher than outside fruit indicating the positive effect of reduced light availability on the concentration of this polyphenolic compounds. Reasons for the observed responses of hesperidin and narirutin are unclear, since preharvest canopy position effects on postharvest flavanone glycosides profile of stored citrus fruits, remain largely unknown. Manthey (2004) and Xu et al. (2008a, b) reported that phenolic compounds contribute a major proportion to the total antioxidant capacity of the citrus rind. Therefore, results in the current study strengthen the hypothesis that the elevation of FGs could possibly

be involved in the defence mechanism of the fruit due to stressful postharvest storage conditions. It can therefore be speculated that elevation of these phenolic compounds (flavanone glycosides) could possibly be involved in the defence mechanism of the fruit, due to stressful postharvest storage conditions.

Sun-exposed outside fruit had higher concentrations of ascorbic acid compared to shaded fruit and this observation was consistent in results reported in both Paper 8 and Paper 9. Ascorbic acid accounts for a great proportion of the antioxidant capacity of citrus fruit (Sdiri et al., 2012). The high concentration of ascorbic acid observed in sun-exposed fruit from the outside position of the canopy, could also explain the increased tolerance of these fruit to RBD. These observations were consistent in Chapter 9 and 10, reporting results of fruit from Stellenbosch and Citrusdal on experiments conducted during 2011 and 2012, respectively. These findings suggest the possibility that ascorbic acid concentration in the rind could be used as a potential biochemical marker of fruit position within the canopy and susceptibility to RDB. However, due to conducting the NIR study simultaneously with the biochemical study, only 5 fruit replicates per treatments were analysed for ascorbic acid. The number of latent variables used to construct vitamin C models in Chapter 4, was . Using only 20 samples (from four treatments in the study) would have violated the statistical rule of thumb, which states that the ratio of the number of samples for calibration to the number of latent variables should be equal to, or larger than 10 (Lammertyn et al., 2000). Secondly, this population of samples would not have had enough variability of ascorbic acid to obtain a robust calibration model. The ability of NIR spectroscopy to predict vitamin C in 'Valencia' oranges was demonstrated in Chapter 4, although the prediction error was high. It is safe to conclude that predicting rind concentration using Vis/NIRS will be possible in future studies with bigger sample sizes.

5. Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins

In order to gain a better insight of the mechanism of RBD, the feasibility of OCT, as a potential non-destructive technique to visualise microstructural changes associated with the development of the disorder, was investigated in Chapter 8. Immediate and non-destructive acquisition of images showing histological and microstructural features of intact rind tissues was demonstrated. The study showed that OCT is capable of acquiring immediate, non-

destructive and contactless cross-sectional images rind structures in real-time mode by measuring their optical reflections.

Comparing OCT images with conventional microscopy images would have allowed better assessment of the applicability and effectiveness of the new OCT technique. The comprehensive literature study conducted in Chapter 2 of this thesis revealed that the use of light and electron microscopic techniques on citrus rinds have been studied extensively. Therefore, OCT images obtained in this study were compared with light and electron microscopy images from the literature.

OCT images have lower resolution than conventional microscopy, but they have very high contrast for an image taken *in-situ*. The fundamental limitation of the OCT in the current study was imposed by the source bandwidth, which was about 7 µm. The dense tissues of intact flavedo and albedo are strongly scattering, resulting in the attenuation of OCT signal, which resulted in low image resolution at greater depths than 1.1 mm. Therefore, although we successfully demonstrated the potential of OCT in imaging rind microstructural features associated with RBD, the sensitivity of the technique still needs to be enhanced to increase signal-to-noise ratio, hence improving the image resolution. In future studies, this could be achieved by using a higher wavelength to reduce scattering, and/or a broader spectrum. Essentially, the use of different optical parameters and improved image processing techniques may be crucial to improve the performance of this system.

The OCT images showed that the oil glands stayed intact in unaffected fruit and gradually collapsed on RBD affected fruit. At advanced stages of the disorder, the collapsed oil glands became increasingly deformed and flattened. Other previous studies reported that oil glands are not involved in the development of non-chilling rind disorders on certain mandarin cultivars such as 'Fortune' (Almela et al., 1992), 'Encore' (Medeira et al., 1999; Vitor et al., 2000) and 'Clementine' (Assimakopoulou et al., 2009). Therefore, the disruption of the oil gland during the development of RBD in 'Nules Clementine' mandarins offers some new biological information about the mechanism of disorder. It was therefore hypothesised that, in 'Nules Clementine' mandarins, oil glands are the primary sites of damage in RBD-affected fruit and that rupture of these oil bodies, in turn, release phytotoxic oil into the surrounding cells, causing the collapse of the oil gland and damage to adjacent cellular structures.

Until the present study, there has been lack of quantitative information on oil gland volume of fruit with different degrees of non-chilling physiological disorders. The study therefore successfully demonstrated the feasibility of OCT to visualise dimensional changes

of rind microstructures and to quantify the degree of collapse of the oil glands with increased level of the disorder. The merits of this work are that it opens the future possibility for monitoring by spotting the oil gland that is in the process of collapsing (they usually become darker) and then follow it over a period until the final symptoms of RBD are clear. This information could point researchers in this field a step further towards understanding the actual mechanism of RBD and not just the symptoms. Understanding oil gland development in citrus fruit might assist in identifying some features of the oil gland and perhaps rind tissue that could provide information about the growing conditions to which the fruit was exposed. The next logical step in future studies would be targeting specific spots on fruit with potential of developing the disorder and monitor these over time to gain information regarding the time when cellular changes take place. Although OCT has been used extensively in the medical field for visualisation of internal structures of humans and animals (Kamensky et al. 1999; Gladkova et al. 2000), the application as an imaging system in plant science or plant materials is currently in its infancy (Meginsky et al., 2010; Verboven et al., 2013) and therefore available at least to researchers for experiment purposes.

6. Conclusions

This study makes a significant contribution to the potential prediction of citrus external and internal quality. It showed that FT-NIR fitted with an emission head can be used as a tool to rapidly assess rind colour, fruit mass, TSS, and vitamin C levels in 'Valencia'oranges. Results from this study also improved understanding the mechanism of RBD. The study further demonstrated the importance of dry matter content, ascorbic acid and flavanone glycosides content on 'Nules clementine' mandarin rinds and how these compounds vary across canopy position and bagging treatments. These findings showed how these quality parameters and compounds could be potentially employed as biomarkers in predicting rind quality and fruit susceptibility to RBD. Lastly, this study has successfully demonstrated the feasibility of OCT as a novel non-invasive tool to visualise dimensional changes of rind microstructures and to quantify the degree of collapse of the oil glands with increased level of RBD.

References

Agustí, M., Almela, V., Juan, M., Alférez, F., Tadeo, F.R., Zacarìas, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415–422.

Alférez, F., Burns, J., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79–87.

Alférez, F.M., Agustí, M., Zacarìas, L., 2003. Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143–152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarìas, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges. I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222–231.

Alférez, F., Alquezar, B., Burns, J.K., Zacarìas, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. Postharvest Biol. Technol. 56, 44–49.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in 'Fortune' mandarin. Origin and control. Physiol. Plant. 85, A65 (Abstract).

Alquezar, B., Mesejo, C., Alférez, A., Agustí, M., Zacarìas, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biol. Technol. 56, 163–170.

Assimakopoulou, A., Tsougrianis, C., Elena, K., Fasseas, C., Karabourniotis, G., 2009. Pre-harvest rind-spotting in 'Clementine' mandarin. J. Plant Nutr. 32, 1486–1497.

Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J-M., McBratney, A., 2010. Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy. Trends Anal. Chem. 29, 1073–1081.

Citrus Growers' Association of Southern Africa, 2012. Key industry statistics for citrus Growers 2012. http://www.cga.co.za/site/files/5438/CGA%20Stats%20Book%202012%20e(1).pdf. Last accessed 07 May 2013.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible-NIR spectroscopy. J. Agric. Food Chem. 56, 9813–9818.

Cozzolino, D., Cyankar, W.U., Shah, N., Smith, P., 2010. Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. Food Res. Int. 44, 1888–1896.

Cronje, P.J.R., 2009. Postharvest rind breakdown of 'Nules Clementines' mandarins (*Citrus reticulata* Blanco) fruit. PhD Dissertation, Department of Horticultural Science, Stellenbosch University, Stellenbosch, South Africa.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011a. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biol. Technol. 60, 192–201.

Cronje, P.J.R., Barry, G.H., Huysamer, M., 2011b. Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. Sci. Hort. 130, 829–837.

Davey, M. W., Saeys, W., Hof, E. Ramon, H., Swennen, R. L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. J. Agric. Food Chem. 57, 1742–1751.

Duarte, A.M.M., Guardiola, J.L., 1995. Factors affecting rind pitting in the mandarin hybrids 'Fortune' and 'Nova'. The influence of exogenous growth regulators. Acta Hort. 379, 59–67.

Gladkova, N.D., Petrova, G.A., Nikulin, N.K., Radenska-Lopovok, S.G., Snopova, L.B., Chumakov, Y.P., Nasonova, V.A., Gelikonov, G.V., Gelikonov, V.M., Kuranov, R.V., Sergeev, A.M., Feldchtein, F.I., 2000. In-vivo optical coherence tomography imaging of human skin: norm and pathology. Skin Res. Technol. 6, 6–11.

Gómez, A. H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis-NIR spectroscopy techniques. J. Food Eng. 77, 313–319.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Liebenberg, C.J., 2005a. Assessment of internal quality attributes of mandarin fruit. 1. NIR calibration model development. Aust. J. Agric. Res. 56, 405–416.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005b. Assessment of internal quality attributes of mandarin fruit. 2. NIR calibration model robustness. Aust. J. Agric. Res. 56, 417–426.

Hiratsuka, S., Yokoyama, Y., Nishimura, H., Miyazaki, T., Nada, K., 2012. Fruit photosynthesis and phosphoenolpyruvate carboxylase activity as affected by lightproof fruit bagging in Satsuma mandarin. J. Am. Soc. Hort. Sci. 137, 215–220.

Jamshidi, B., Minaei, S., Mohajerani, E., Ghassemian, H., 2012. Reflectance Vis/NIR spectroscopy for nondestructive taste characterization of Valencia oranges. Comp. Electron. Agric. 85, 64–69.

Lu, H., Xu, H., Ying, Y., Fu, X., Yu, H., Tian, H., 2006. Application of Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits. J. Zhejiang Univ. Sci. 7, 794–799.

Kamensky, V.A., Feldchtein, F.I., Gelikonov, V.M., Snopova, L.B., Muraviov, S.V., Malyshev, A.Y., Bityurin, N.M., Sergeev, A.M., 1999. In situ monitoring of laser modification process in human cataractous lens and porcine cornea using coherence tomography. Biomed. Opt. 4, 137–143.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Non-destructive determination of sugar content in 'Satsuma' mandarins using NIRS transmittance. J. Japan. Soc. Hort. Sci. 62, 465–470.

Kelebek, H., 2010. Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (*Citrus paradisi*) cultivars grown in Turkey. Ind. Crops Prod. 32, 269–274.

Kelebek, H., Selli, S., 2011. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus Sinesis* L. Osbeck). J. Sci. Food Agric. 91, 1855–1862.

Lammertyn, J., Peirs, J., De Baerdemaeker, J., Nicolaï, B.M., 2000. Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment. Postharvest Biol. Technol. 18, 121–132.

Li, B.B., Smith, B., Hossain, M.M., 2006. Extraction of phenolics from citrus peels II. Enzyme-assisted exctraction method. Sep. Purification Technol. 48, 189–196.

Manthey, J.A., 2004. Fractionation of orange peel phenols in ultrafilterd molasses and mass balance studies of their antioxidant levels. J. Agric. Food Chem. 52, 7586–2193.

McGlone, V.A., Jordan, R.B., Martinsen, P.J., 2002. Vis-NIR estimation at harvest of pre- and post-storage quality indices for 'Royal Gala' apple. Postharvest Biol. Technol. 25, 135–144.

Medeira, M.C, Maia, M.I., Vitor, R.F., 1999. The first stages of preharvest peel pitting development in 'Encore' mandarin: a histological and ultrastructural study. Ann. Bot. 83, 667–673.

Meglinski, I.V., Buranachai, C., Terry, L.A., 2010. Plant photonics: Application of optical coherence tomography to monitor defects and rots in onion. Laser Phys. Lett. 7, 307–310.

Miyamoto, K., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. J. Near Infrared Spectrosc. 3**,** 227–237.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I. K., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Technol. 46, 99–118.

Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J., 1999. Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. HortScience 34, 114–118.

Sánchez, M-T., Dela Haba, M-J., Serrano, I., Pérez-Marín, D., 2013. Application of NIRS for nondestructive measurement of quality parameters in intact oranges during on-tree ripening and at harvest. Food Anal. Method. 6, 826–837.

Sdiri, S., Navarro, P., Monterde, A., Benabda, J., Salvador, A., 2012. Effect of postharvest degreening followed by a cold-quarantine treatment on vitamin C, phenolic compounds and antioxidant activity of early-season citrus fruit. Postharvest Biol. Technol. 65, 13–21.

Verboven, P., Nemeth, A., Abera, M.K., Bongaers, E., Daelemans, D., Estrade, P., Herremans, E., Hertog, M., Saeys, W., Vanstreels, E., Verlinden, E., Leitner, M., Nicolaï, B., 2013. Optical coherence tomography visualizes microstructure of apple peel. Postharvest Biol. Technol. 78, 123–132.

Vitor, R.F., Lidon, M., Barreiro, M.D., Maia, M.I., Medeira, M.C., Guerreiro, A., 2000. 'Peel pitting' of 'Encore' mandarin fruits: Etiology, control and implications in fruit quality. Fruits 56, 27–35.

Wedding, B.B., Wright, C., Grau, S., White, R.D., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biol. Technol. 75, 9–16.

Williams, P.C., Sobering, D.C., 1993. Comparison of commercial near infrared transmittance and reflectance instruments for the analysis of whole grains and seeds. J. Near Infrared spectrosc. 1, 25–32.

Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., jiang, P., Ye, X.Q., 2008a. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73, C11–C18.

Xu, G.H., Ye, X.Q., Liu, D.H., Ma, Y.Q., Chen, J.C., 2008a. Composition and distribution of phenolic acids in Ponkan (*Citrus poonensis* Hort. Ex Tanaka) and Hoyou (*Citrus paradisi* Macf. Changshanhuyou) during maturity. J. Food Comp. Anal. 21, 382–389.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using Vis-NIR reflectance spectroscopy. Sci. Hort. 125, 401–405.

Zude, M., Pflanz, M., Kaprielian, C., Aivazian, B., 2008. NIRS as a tool for precision horticulture in citrus industry. Biosyst. Eng. 99, 455–459.

APPENDIX 1: DEVELOPMENT OF METHOLOGY FOR PHENOLIC COMPOUNDS EXTRACTION AND HPLC QUANTIFICATION

Dry powder samples of mandarin rind were extracted with 80:20 (v/v) aqueous ethanol compared to acidic aqueous methanol 70:29.5:0.5 (v/v/v; methanol:H_2O:HCL) and 50:50 (v/v; DMSO:methanol) to determine the efficacy of the extraction procedure for optimum phenolic acid and flavanones yield. Extraction solvent and extraction time were the two main parameters that affected the yield of phenolic compounds (Table 1). The concentration of phenolic acids increased with an increase in ultrasonic extraction time, while flavanones stayed the same. Results showed that an extraction period of 30 minutes using 70:29.5:0.5 (v/v/v; methanol:H_2O:HCL) was sufficient to extract phenolic acids. For example, the concentration of ferulic acid after extraction using acidic methanol for 10, 20 and 30 minutes, gradually increased (12.43, 13.37, 25.19 µ/g DM, respectively. The same trend was observed on the sinapic acid, where the corresponding concentrations were 41.35, 61.23 and 64.87 µ/g DM. In general, phenolic acids yield was higher on samples extracted for 30 minutes using aqueous methanol. For flavanones, the highest yield was observed on samples extracted using 50:50 (v/v; DMSO:methanol) for 10 minutes. However, phenolic acids yield was lower using this extraction combination. Therefore, acidic aqueous methanol extraction in ultrasonic bath for 30 minutes was selected to extract phenolic acids and 50:50 (v/v; DMSO:methanol) for 10 minutes was used to extract flavanones.

A typical chromatogram with phenolic compounds separation obtained using conditions described earlier is portrayed in Fig. 1. A total of seven phenolic acids, including three hydroxybenzoic acids (ρ-hydroxybezoic and vanillic), and five hydroxycinnamic acids (chlorogenic, caffeic, ρ-coumaric, ferrulic, and sinapic) as well as three flavanones (narirutin, hesperidin and didymin) were identified and quantified. The method separated 10 phenolic compounds faster (50 min) than 120 min as reported by Li et al. (2006), Kelebek (2010) and Kelebek and Selli (2011). Hesperidin was the dominant compound ranging from 31179 to 32019 µg/g DM on samples extracted using DMSO (Table 1). These results are similar to those observed by Xu et al. (2008 a, b), who reported a total of seven phenolic acids and four flavanones. The flavanones profile was similar to that reported by Ye et al. (2011) who reported hesperidin as the major flavanone in mandarin fruit.

Table 2 summarises the concentration range, retention times, regression equation (y = mx), coefficient of determination (R^2), LOD, LOQ and the relative standard deviation (RSD) for each compound. The reproducibility of the retention time of phenolic compounds under selected HPLC conditions was executed by doing repeated injections (n = 10) of the mixture of the 10 standards at the concentration of 10.0 µg/mL. The regression equation, LOD, LOQ and RSD were calculated for each identified phenolic compound using only the best extraction method, which in this case was acidic methanol. The LOD, defined as the smallest concentration that the analytical procedure can reliably distinguish from the noise levels and LOQ for all analytes were very small, ranging from 1.35 to 5.02 and 4.51-16.72 µg/mL, respectively. The RSD values for all retention times ranged from 0.45 to 1.67 indicating good stability and adequate performance of the method investigated.

References

Kelebek, H., 2010. Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (*Citrus paradisi*) cultivars grown in Turkey. Ind. Crops Prod. 32, 269–274.

Kelebek, H., Selli, S., 2011. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus Sinesis* L. Osbeck). J. Sci. Food Agric. 91, 1855–1862.

Li, B.B., Smith, B., Hossain, M.M., 2006. Extraction of phenolics from citrus peels II. Enzyme-assisted exctraction method. Sep. Purification Technol. 48, 189–196.

Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H., jiang, P., Ye, X.Q., 2008a. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73, C11–C18.

Xu, G.H., Ye, X.Q., Liu, D.H., Ma, Y.Q., Chen, J.C., 2008b. Composition and distribution of phenolic acids in Ponkan (*Citrus poonensis* Hort. Ex Tanaka) and Hoyou (*Citrus paradisi* Macf. Changshanhuyou) during maturity. J. Food Comp. Anal. 21, 382–389.

Ye, X.Q., Chen, J.C., Liu, D.H., Jiang, P., Shi, J., Xue, S., Wu, D., Xu, J.G., Kakuda, Y., 2011. Identification of bioactive composition and antioxidant activity in young mandarin fruits. Food Chem. 124, 1561–1566.

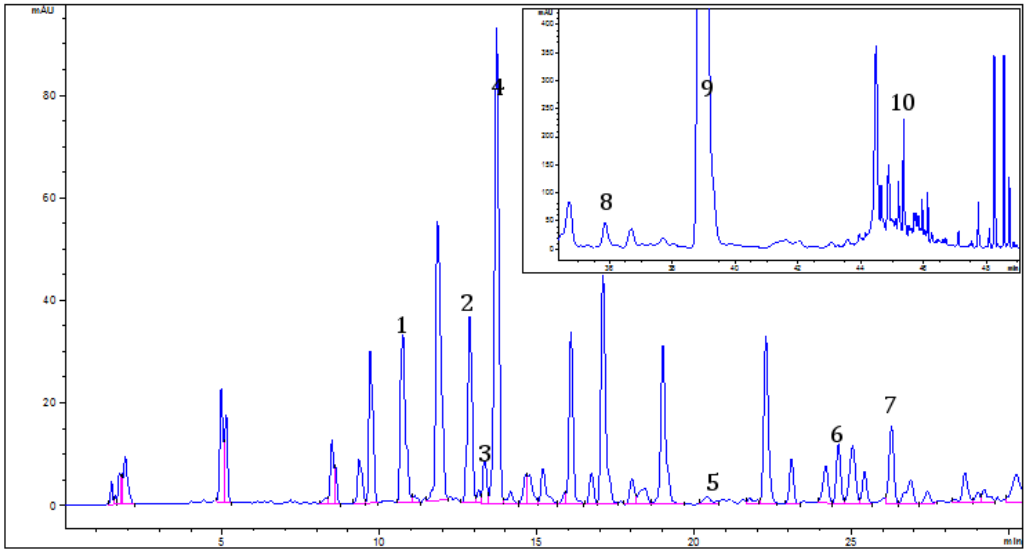

Fig. 1: Typical HPLC-DAD chromatogram at 280 nm showing separation of phenolic compounds in the rind sample (1, ρ-Hydroxybezoic acid; 2, Vanillic acid; 3, Chlorogenic acid; 4, Caffeic acid; 5, ρ-Coumaric acid; 6, Ferulic acid; 7, Sinapic acid; 8, Narirutin; 9, Hesperidin, and 10, Didymin, respectively.

Table 1: Composition of phenolic compounds in rind extracts using different extraction solvents and time combination. Means with different letter in the three rows corresponding to the same compound are significantly different ($p \leq 0.05$).

Phenolic compound	Extraction solvent	Concentration (µg/g DM)		
		10 min	20 min	30 min
Hydroxybenzoic acids				
ρ-Hydroxybezoic acid	Methanol	22.08±0.57 ^{ab*}	19.78±0.74 ^{a}	21.02±3.10 ^{ab}
	DMSO	92.85±1.53 ^{e}	87.75±5.60 ^{d}	86.66±1.87 ^{d}
	Ethanol	29.26±3.51 ^{c}	25.32±1.47 ^{bc}	29.29±0.28 ^{c}
Vanillic acid	Methanol	17.82±0.23 ^{c}	12.69±0.57 ^{b}	24.47±2.67 ^{d}
	DMSO	nd	nd	nd
	Ethanol	8.86±0.76 ^{a}	7.11±1.09 ^{a}	17.25±1.86 ^{c}
Hydroxycinnamic acids				
Chlorogenic acid	Methanol	15.37±0.36 ^{c}	25.91±0.49 ^{e}	43.25±1.21 ^{h}
	DMSO	5.98±1.06 ^{a}	11.89±0.28 ^{b}	33.89±4.39 ^{g}
	Ethanol	18.76±0.95 ^{d}	11.06±0.73 ^{b}	29.85±0.43 ^{f}
Caffeic acid	Methanol	28.21±0.67 ^{e}	23.57±0.46 ^{d}	39.81±3.94 ^{f}
	DMSO	11.95±0.89 ^{a}	12.44±0.19 ^{a}	23.33±1.89 ^{d}
	Ethanol	15.49±1.69 ^{b}	11.94±0.57 ^{a}	19.70±0.76 ^{c}
ρ-Coumaric acid	Methanol	9.63±0.09 ^{c}	14.55±1.79 ^{e}	6.94±0.08 ^{b}
	DMSO	5.63±0.42 ^{ab}	5.35±1.14 ^{a}	9.80±1.27 ^{c}
	Ethanol	4.49±0.21 ^{a}	10.43±0.05 ^{c}	12.53±0.15 ^{d}
Ferulic acid	Methanol	12.43±0.80 ^{b}	13.37±0.85 ^{b}	25.19±4.93 ^{d}
	DMSO	7.92±1.25 ^{a}	6.28±2.48 ^{a}	13.50±1.10 ^{b}
	Ethanol	17.81±0.33 ^{c}	15.81±0.12 ^{bc}	40.55±0.55 ^{e}
Sinapic acid	Methanol	41.35±0.47 ^{e}	61.23±3.78 ^{d}	64.87±2.83 ^{e}
	DMSO	15.19±1.63 ^{a}	23.52±2.42 ^{c}	23.58±2.83 ^{c}
	Ethanol	19.45±4.31 ^{b}	24.99±0.83 ^{c}	39.45±1.12 ^{d}
Flavanones				
Narirutin	Methanol	737.0±1.41 ^{b}	737.6±7.91 ^{b}	689.6±14.42 ^{b}
	DMSO	1370±29.59 ^{d}	1299±139.9 ^{d}	1151±23.05 ^{c}
	Ethanol	395.9±30.65 ^{a}	354.7±11.21 ^{a}	408.0±12.57 ^{a}
Hesperidin	Methanol	8005±529.60 ^{cd}	8628±269.18 ^{d}	7553±290.4 ^{c}
	DMSO	32008±372.9 ^{e}	31179±1181 ^{e}	32019±866.3 ^{e}
	Ethanol	5456±388.8 ^{b}	4329±438.9 ^{a}	3966±160.7 ^{a}
Didymin	Methanol	268.0±4.64 ^{d}	246.0±23.56 ^{bc}	257.4±11.14 ^{cd}
	DMSO	401.7±7.19 ^{e}	401.7±9.55 ^{e}	403.7±4.49 ^{e}
	Ethanol	238.3±5.06 ^{ab}	223.7±7.43 ^{a}	232.2±3.24 ^{ab}

nd, non detectable; *Mean ±SD of three samples.

Table 2: Response characteristics of phenolic compound standards using HPLC. The presented values LOQ, LOD and RSD were measured with repeated injections (n = 10) of standard mixture at a concentration of 10.0 µg/mL each. In the regression equation, x represents concentration of phenolic compounds and y represents the peak area.

Phenolic compound (µg/mL)	Linear range	Retention time (min)	Regression Equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	R.S.D (%)
Hydroxybenzoic acids							
ρ-Hydroxybezoic acid	5.0-150.0	11.45	y = 23.45x	0.9997	1.48	4.92	0.49
Vanillic acid	5.0-150.0	14.13	y = 30.62x	0.9995	1.39	4.62	0.46
Hydroxycinnamic acids							
Chlorogenic acid	5.0-150.0	14.44	y = 33.83x	0.9997	1.48	4.92	0.49
Caffeic acid	5.0-150.0	14.88	y = 62.73x	0.9995	1.35	4.51	0.45
ρ-Coumaric acid	5.0-150.0	20.28	y = 96.81x	0.9997	1.45	4.84	0.48
Ferulic acid	5.0-150.0	25.00	y = 60.08x	0.9997	1.42	4.74	0.47
Sinapic acid	5.0-150.0	26.87	y = 27.45x	0.999	2.32	7.73	0.77
Flavanones*							
Narirutin	5.0-150.0	17.01	y = 30.83x	0.9994	1.50	3.01	0.50
Didymin	5.0-150.0	24.77	y = 31.13x	0.9994	1.32	4.23	0.45
Hesperidin	5.0-150.0	20.95	y = 30.84x	0.9994	5.02	16.72	1.67

*Flavanones were determined on a different HPLC run.