FACTORS AFFECTING POST-STORAGE QUALITY OF 'NULES CLEMENTINE' MANDARIN FRUIT WITH SPECIAL REFERENCE TO RIND BREAKDOWN

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Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture at the University of Stellenbosch.

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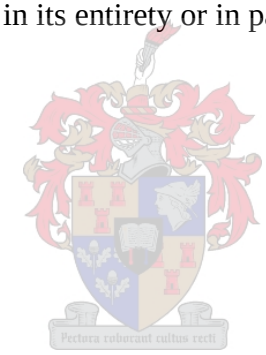
DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.



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Signature Date

DEDICATION

This thesis is dedicated to my wife, Nosipho Martha Khumalo, with all my love.

SUMMARY

Rind breakdown of ‘Nules Clementine’ mandarin (*Citrus reticulata* Blanco) is a physiological rind disorder that develops during storage. The disorder appears following leakage of essential oil from oil glands in the flavedo, which then leaks into and oxidises the albedo. Oxidised tissue appears as brown spots in the rind. Occurrence of this disorder over the years has caused high financial losses to ‘Clementine’ mandarin producers and exporting companies. Therefore, research aimed at solving this problem was identified as a priority by the citrus industry.

Several factors have been reported to be associated with rind breakdown of ‘Clementine’ mandarin fruit, and include environmental factors, fruit maturity at harvest, ethylene gas degreening, storage temperature and storage duration, canopy position where fruit are borne, plant growth regulators, and differences in susceptibility among selections. Practical information has been generated on rind breakdown, but the basic physiology of the disorder is still unresolved.

The objective of this study was, therefore, to quantify the effects of various factors on the development of rind breakdown of ‘Nules Clementine’ mandarin fruit, as well as to establish an association between rind pigments and rind antioxidant capacity on the development of this disorder. In this study a series of five experiments was conducted, and included quantifying the differences in susceptibility to rind breakdown between ‘Nules’ and ‘Oroval Clementine’ mandarin fruit, investigating the effects of fruit canopy position, harvest date, ethylene gas degreening, storage temperature and duration on the development of rind breakdown. The effect of these factors on rind pigments and antioxidant capacity was also reported.

Generally, ‘Nules’ and ‘Oroval Clementine’ mandarin fruit exhibited similar characteristics at harvest, in terms of maturity and antioxidant capacity. After storage, ‘Nules Clementine’ mandarin developed higher levels of rind breakdown than ‘Oroval Clementine’ mandarin. However, the difference in susceptibility to rind breakdown of ‘Nules’ and ‘Oroval Clementine’ mandarin fruit could not be associated with the antioxidant capacity measured at harvest.

Fruit canopy position significantly affected rind pigments and antioxidant capacity at harvest, with fruit borne on the outside of the tree canopy having a higher carotenoid content and antioxidant capacity. However, the development of rind breakdown after storage was not significantly affected by fruit canopy position. From this experiment it was shown that antioxidants and rind pigments measured only at harvest cannot be used as indicators for fruit susceptibility to rind breakdown.

Fruit harvest date significantly affected the development of rind breakdown. However, the trend was not consistent among seasons and production regions. The experiment was conducted in three different production regions in three different seasons, and in two of the regions rind breakdown levels were higher on early harvested fruit than on fruit harvested later. In the third region the levels of rind breakdown were highest in late harvested fruit. The antioxidant capacity and rind carotenoid concentration at harvest could not be used as indicators for fruit susceptibility to rind breakdown.

Ethylene gas degreening significantly affected carotenoid and chlorophyll contents, as fruit degreened with ethylene had better colour and higher carotenoid concentration than non-degreened fruit. Antioxidant capacity was, however, not significantly affected by the degreening treatments. After storage ethylene gas degreening did not accentuate rind breakdown of 'Nules Clementine' mandarin fruit.

Storage temperature and storage duration significantly affected the development of rind breakdown of 'Nules Clementine' mandarin fruit. The levels of this disorder were markedly and significantly higher in fruit stored at 7.5 °C and increased over time compared to lower levels of rind breakdown which occurred in fruit stored at the other temperatures, -0.5, 4.5 and 11 °C. Optimum quality was achieved in fruit stored at 4.5 °C for <60 days. Rind antioxidant capacity and carotenoid concentration, although significantly affected by storage temperature and storage duration, did not seem to be directly associated with the development of rind breakdown.

This study quantified the factors affecting fruit quality of 'Clementine' mandarins, particularly rind breakdown. Undoubtedly progress has been made in understanding rind breakdown and some of the factors associated with the disorder. This progress should, however, not be allowed to hide the need for further fundamental research on rind breakdown

due to the fact that the cause of rind breakdown is still unknown and no commercial treatment is available for the control of the disorder. In this study an association between rind pigments and antioxidant capacity on the development of rind breakdown could not be established. Future research on antioxidants, incorporating multiple sampling times and using more than one assay, has been suggested. At this stage optimum quality of 'Nules Clementine' mandarin fruit will be achieved in fruit degreened and stored at 4.5 °C for <60 days.

OPSOMMING

Skilafbraak van ‘Nules Clementine’ mandaryn (*Citrus reticulata* Blanco) is ‘n fisiologiese skildefek wat geassosieer word met platgevalde olieselle in die vrugskil. Olie lek van hierdie selle na die albedo waar oksidasie van die weefsel plaasvind. Geoksideerde weefsel word dan as bruin kolle sigbaar op die flavedo. Die voorkoms van die defek het in die verlede groot finansieële verliese aan produsente en uitvoermaatskappye veroorsaak. Gevolglik is navorsing gemik op die oplossing van die probleem as prioritiët in die sitrusbedryf geidentifiseer.

Verskeie faktore soos vrugrypheid tydens oes, etileen-gas ontgroening, opbergingstemperature en -periode, posisie waar die vrug geset het op die boom, plantgroeireguleerders, omgewingsfaktore en sensitiwiteit tussen cultivars word geassosieer met skilafbraak van ‘Clementine’ mandaryn vrugte. Praktiese inligting rondom die verskynsel is bekend, maar die basiese fisiologie rakende die defek is egter steeds onverklaard.

Die doel van hierdie studie was gemik om die effek van verskillende faktore op die ontwikkeling van skil afbraak van ‘Nules Clementine’ vrugte te kwantifiseer, sowel as om die verband tussen skilafbraak, pigmente en antioksidant kapasiteit op die voorkoms van die defek te bepaal. In hierdie navorsing is ‘n reeks van vyf eksperimente uitgevoer om die verskil tussen ‘Nules’ en ‘Oroval Clementine’ mandaryn vrugte te kwantifiseer. Die navorsing het gefokus op die effek van oesdatum, opbergingstemperatuur en-periode, vrugposisie en etileen-gas ontgroening op die ontwikkeling van skilafbraak.

Oor die algemeen het ‘Nules’ en ‘Oroval Clementine’ mandaryn vrugte soortgelyke kenmerke tydens oes in terme van oesrypheid en antioksidant kapasiteit getoon. Na opberging het ‘Nules Clementine’ mandaryne egter meer skilafbraak ontwikkel as ‘Oroval Clementine’ mandaryn. Die verskil in die vatbaarheid van ‘Nules’ en ‘Oroval Clementine’ mandaryne vir skilafbraak, kon egter nie met die antioksidant kapasiteit, soos gemeet tydens oes, geassosieer word nie.

Vrugposisie op die boom het ‘n betekenisvolle effek op skilpigment en antioksidant kapasiteit gehad tydens oes. Die vrugte aan die buitekant van die boom het ‘n hoër karotenoied en

antioksidant kapasiteit as die vrugte aan die binnekant van die boom getoon, maar dit het nie skilafbraak tydens opberging beinvloed nie. Dit dui daarop dat antioksidante en skilpigmente tydens oes nie as aanduiding kan dien van die vrug se vatbaarheid vir skilafbraak nie.

Die oesdatum het die ontwikkeling van skilafbraak betekenisvol beïnvloed. Die tendens was egter nie konstant tussen seisoene en areas nie. Die eksperiment het gestrek oor drie seisoene en in twee van areas was skilafbraak hoër in vroeër geoeste vrugte as later geoeste vrugte, terwyl die derde area hoër die skilafbraak getoon het by later geoeste vrugte. Die antioksidant kapasiteit en skil karotenoied konsentrasie tydens oes kon nie gebruik word as aanduiding van vatbaarheid ten opsigte van skilafbraak nie.

Karotenoied- en chlorofilinhoud is betekenisvol deur etileen-gas ontgroening beïnvloed, maar ontgroening het nie skilafbraak by ‘Nules Clementine’ mandaryn vrugte verhoog nie. Vrugte wat met etileen ontgroen is, het ’n beter kleur en hoër karotenoied konsentrasie getoon as nie-ontgroende vrugte. Antioksidant kapasiteit is egter nie betekenisvol deur ontgroenings behandelings beinvloed nie.

Opbergingstemperatuur en -periode het ‘n betekenisvolle effek gehad op die ontwikkeling van skilafbraak by ‘Nules Clementine’ mandaryn vrugte. Die defek was betekenisvol hoër in vrugte wat by 7.5°C gestoor was en het toegeneem met verlengde opbergingsperiodes, vergeleke met laer vlakke van skilafbraak wat voorgekom het by –0.5, 4.5 en 11°C. Optimum vrugkwaliteit is waargeneem in vrugte wat opgeberg is teen 4.5°C vir korter as 60 dae. Alhoewel die skil antioksidant kapasiteit en karotenoied konsentrasie beïnvloed was deur opbergingstemperatuur en –periode, kon dit nie direk geassosieer word met die ontwikkeling van skilafbraak nie.

Faktore wat vrugkwaliteit, veral skilafbraak, beinvloed is deur hierdie navorsing gekwantifiseer. Daar is ongetwyfeld vordering gemaak in die kennis omtrent skilafbraak en sommige faktore wat daarmee geassosieer word is geidentifiseer. Alhoewel daar vordering gemaak is, is daar steeds ‘n behoefte vir basiese navorsing, aangesien die oorsaak van skilafbraak steeds onbekend is en daar nie kommersiele behandelings vir die defek beskikbaar is nie. Toekomstige navorsing op antioksidante word voorgestel waar gefokus moet word op herhaalde oestye en meer as een analise. Met die inligting wat tans beskikbaar is, moet

‘Nules Clementine’ mandaryne ontgroen word en by 4.5°C gestoor word vir minder as 60 dae om optimum kwalteit te verseker.

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TABLE OF CONTENTS

Language and style used in this thesis are in accordance with the requirements of the scientific journal *Postharvest Biology and Technology*. This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

The South African citrus industry comprises ~52 407 ha, of which mandarins are grown on ~10% of this area (Citrus Growers' Association, 2005). 'Clementine' mandarin (*Citrus reticulata* Blanco) was introduced into South Africa in about 1970. Over time different selections of this cultivar were planted, with 'Nules', 'Oroval', 'Marisol', 'SRA 63' and 'SRA 92' forming the main commercial selections planted (Barry and Rabe, 2004).

The majority of citrus fruit produced in South Africa is destined for the export market (Von Broembsen, 1986; Stanbury, 1996), which demands a high quality product. However, due to the distance between South Africa and the various overseas markets, and hence the time it takes to transport fruit to these markets, a loss in product quality during transport is inevitable. Among postharvest problems, various physiological disorders can develop in citrus fruit during storage, as described by Murata (1997), including physiological rind disorders, deterioration of internal quality and pathological disorders.

Rind breakdown of 'Nules Clementine' mandarin fruit is a commercial problem due to the high financial losses experienced by 'Clementine' mandarin producers and exporters, and has received scientific attention in South Africa for more than 10 years (Van Rensburg et al., 1995). Furthermore, the sporadic occurrence of rind breakdown on 'Nules Clementine' mandarin slowed down the understanding of this disorder thereby allowing rind breakdown to persist to date. Therefore, research aimed at better understanding the disorder and ultimately solving it was identified as a priority by the South African citrus industry.

Rind breakdown of 'Nules Clementine' mandarin is a physiological rind disorder that develops during storage. The disorder appears following leakage of essential oil from oil glands in the flavedo, which then leaks into and oxidises the albedo. Oxidised tissue appears as brown spots on the flavedo (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Other postharvest rind disorders that may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin and which affect other citrus types have been reported, and include rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Petracek et al., 1995), postharvest pitting of 'Temple' tangor [*C. reticulata* Blanco x *C. sinensis* (L.) Osbeck]

(Petracek et al., 1997) and 'Fallglo' mandarin (*C. reticulata* Blanco x *C. reticulata* Blanco x *C. paradisi* Macf.) (Petracek et al., 1998a), superficial flavedo necrosis (noxan) of 'Shamouti' orange [*C. sinensis* (L.) Osbeck] (Ben Yehoshua et al., 2001), and rind staining of 'Navelina Navel' orange (Agusti et al., 2001; Alférez et al., 2003).

Rind breakdown is predominantly a disorder of 'Nules Clementine' mandarin and several factors have been reported to be associated with the development of this disorder (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). These include environmental factors, fruit maturity at harvest, ethylene gas degreening, storage temperature and storage duration, canopy position where fruit are borne, and plant growth regulators (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). The primary cause of rind breakdown of 'Nules Clementine' mandarin is thought to be weather-related as this disorder generally occurs following a relatively warm winter and when the fruit matures during a relatively warm autumn (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). In addition, it was found that late-harvested fruit were more susceptible to rind breakdown than fruit harvested earlier. Fruit originating from the inside of a tree's canopy were identified as being more susceptible to rind breakdown than fruit borne on the outside canopy position. High storage temperature was found to increase the risk of rind breakdown of 'Nules Clementine' mandarin compared with low temperature storage (Van Rensburg and Bruwer 2000; Van Rensburg et al., 2004). Storage temperature was also found to have a similar effect on rind pitting of 'Marsh' grapefruit (Petracek et al., 1995) and 'Fallglo' mandarin (Petracek et al., 1998a), superficial flavedo necrosis (noxan) of 'Shamouti' orange (Ben Yehoshua et al., 2001), and rind staining of 'Navelina Navel' orange (Agusti et al., 2001; Alférez et al., 2003). Van Rensburg et al. (2004) reported that long exposure to ethylene increased the risk of rind breakdown. However, ethylene pre-treatment was found to reduce the incidence of rind staining of 'Navelina Navel' orange (Lafuente and Sala, 2002; Sala and Lafuente, 2004). A synthetic auxin, 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA), applied as a fruit thinning and fruit size enhancement treatment reduced rind breakdown of 'Nules Clementine' mandarin fruit (Van Rensburg et al., 2004).

There is increasing information linking the occurrence of rind disorders in other citrus types to oxidative stress occurring during storage. Research conducted on 'Navelina Navel' orange showed that fruit susceptible to rind staining had a lower activity of certain antioxidant enzymes, superoxide dismutase, catalase and glutathione reductase (Sala and Lafuente, 2004).

However, this concept has not been tested on rind breakdown of ‘Nules Clementine’ mandarin.

Research conducted over several years on rind breakdown has led to the development of a damage control strategy to manage and prevent the occurrence of rind breakdown of ‘Nules Clementine’ mandarin fruit from South Africa (Van Rensburg et al., 2004). Therefore, practical information on the development of rind breakdown is available. However, the basic physiology of this disorder still remains unresolved, and the cause of rind breakdown is unknown. Consequently, rind breakdown still occurs unpredictably. The objective of this study was to quantify the effects of various factors on rind breakdown of ‘Nules Clementine’ mandarin. It was also an objective of this study to demonstrate whether there is an association between rind antioxidant capacity and the development of rind breakdown of ‘Nules Clementine’ mandarin fruit. The study further gives suggestions on how to better maintain fruit quality through optimum postharvest handling and storage of ‘Nules Clementine’ mandarin fruit.

CHAPTER 2

REVIEW OF LITERATURE

Published information and unpublished data on rind breakdown and other rind disorders of *Citrus spp.* that may be morphologically similar to rind breakdown are reviewed. The discussion provides an overview of rind anatomy, the symptoms of rind breakdown and other related disorders, factors affecting fruit quality with particular reference to rind breakdown, and a brief review of antioxidants and their effects on the development of rind breakdown. From this discussion a research hypothesis was formulated.

2.1 Citrus rind anatomy

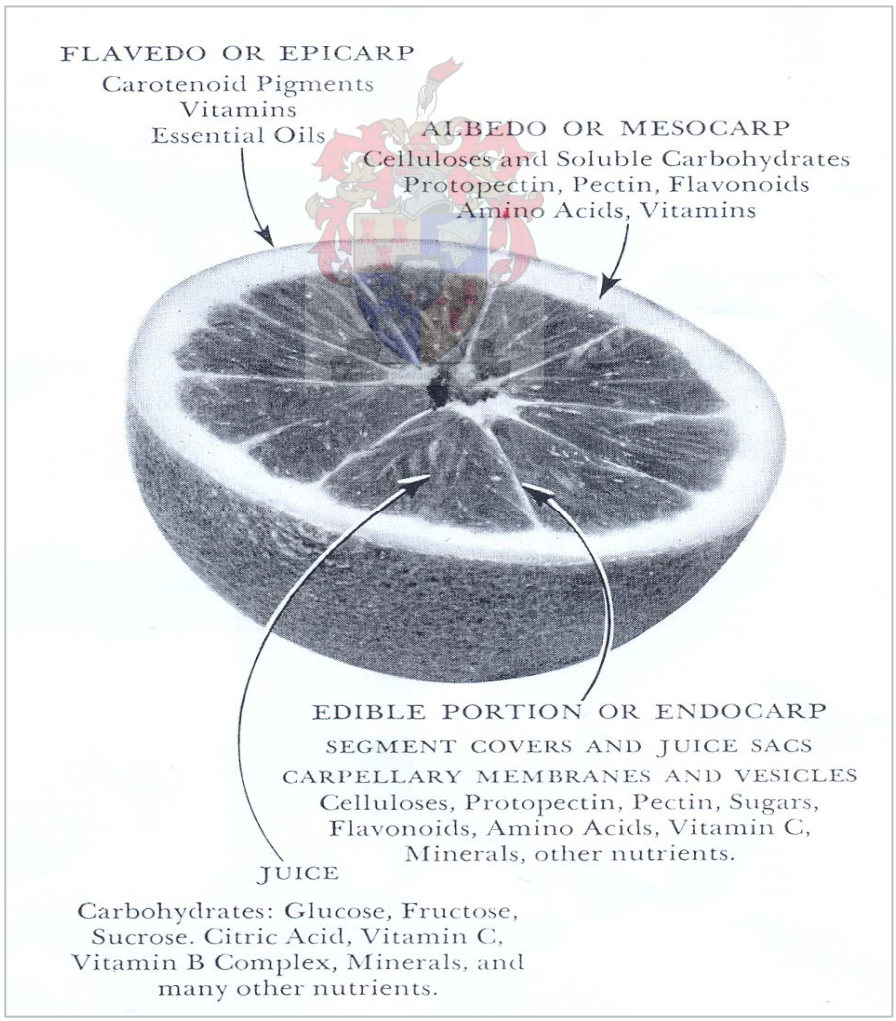

Figure 2.1. Cross-sectional view of the citrus fruit showing the epicarp, mesocarp and endocarp (Sinclair, 1961).

The citrus fruit consists of two distinct layers, the pericarp and the endocarp (Figure 2.1) [Spiegel Roy and Golschmidt, 1996]. The pericarp refers to the outer layers of the citrus fruit, which are also collectively referred too as the rind or peel. The rind is divided into the flavedo, which is the outermost coloured portion, and the albedo, which is the white spongy mesophyl layer immediately below the flavedo (Soule and Grierson, 1986). In some cases the albedo is also referred to as the measocarp (Sinclair, 1961).

The flavedo is multi-layered, consisting of a single layer epidermis covered with a cuticle (Spiegel Roy and Goldschmidt, 1996). The epidermis is made up of four types of cells: epidermal cells, guard cells, accessory cells and oil gland cover cells. The epidermal cells are relatively unspecialised and have a living protoplast with plastids. During early stages of fruit development the epidermal cells contain chloroplasts that are converted to chromoplasts as the fruit changes colour during maturation (Soule and Grierson, 1986).

Below the epidermis lies the hypodermis, which consists of several layers of compactly arranged, colourless parenchyma cells (Soule and Grierson, 1986; Spiegel Roy and Goldschmidt, 1996). Imbedded in these cells are oil glands, which range in size from 10 to 100 μm or larger (Figure 2.2) [Soule and Grierson, 1986; Spiegel Roy and Goldschmidt, 1996]. The theory of oil gland formation was reviewed by Turner (1999), and Storey and Treeby (2002) studied rind morphology of a lemon fruit and defined the oil glands as subepidermal structures with many layers of epithelial cells. The oil gland had a lysigenous cavity, which in most cases contained essential oils. Knight et al., (2001) investigated the process of oil gland formation in 'Washington Navel' orange [*C. sinensis* (L.) Osbeck]. The research showed that oil glands were present in the pre-anthesis floral ovary. Their anatomical development was outlined in a series of six stages. In the early stages, the cluster of cells, situated adjacent to the epidermis, giving rise to oil glands were clearly distinguishable from the surrounding parenchyma cells, in that they were radially elongated and lacked starch (Knight et al., 2001). In subsequent stages the cells increased in size, and differentiated into flattened boundary cells enclosing inner polyhedral cells. The walls of the inner polyhedral cells separate to form a cavity. This cavity is expanded in mature glands, and the glands may mature while the fruit is still immature but continue to enlarge with fruit growth (Knight et al., 2001).

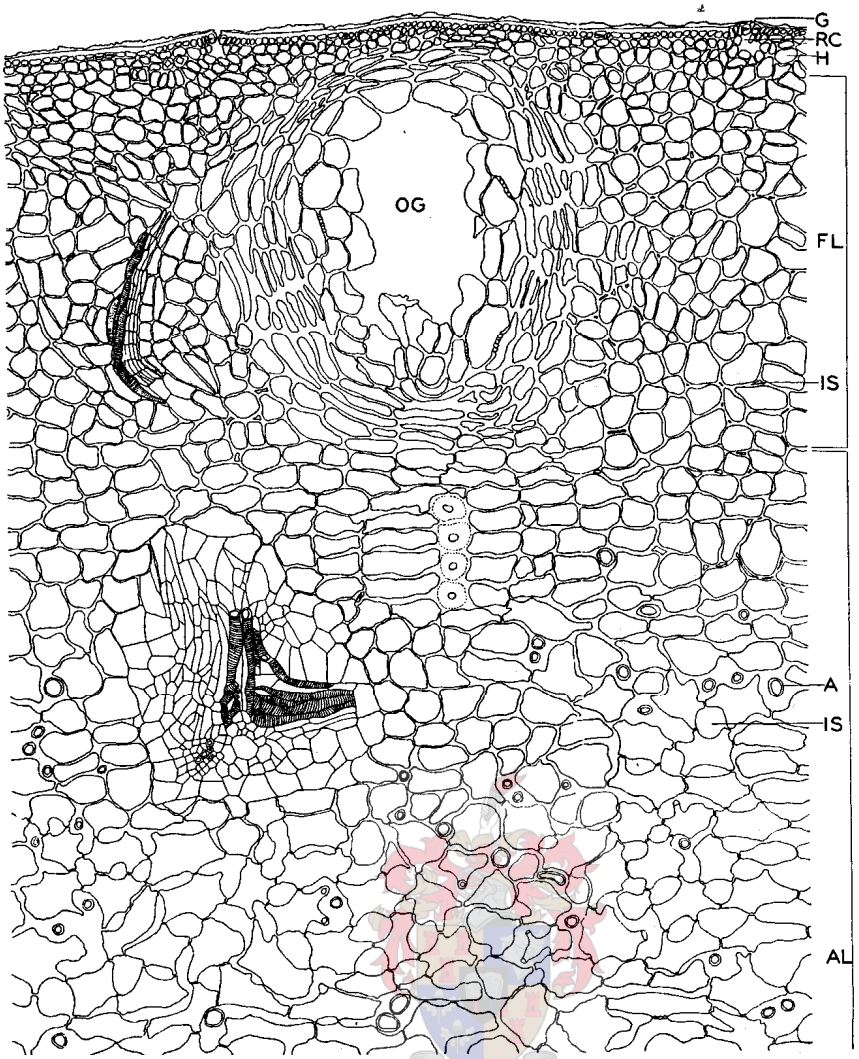

Figure 2.2. Transection of rind of a mature citrus fruit showing the flavedo (FL) and oil gland (OG) as well as the albedo (AL) (Scott and Baker, 1947).

The deep layers of the flavedo merge with the spongy albedo, which consists of a loose network of parenchyma cells with numerous large air spaces (Soule and Grierson, 1986).

2.2 Rind disorders of the flavedo

2.2.1 Rind breakdown

Rind breakdown of 'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit manifests as sunken brown spots on the rind (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Anatomical studies showed that rind breakdown was always associated with one or more collapsed oil glands (Van Rensburg et al., 1995). The collapsed oil glands contained little or no oil. It was therefore suggested that an unknown factor caused the degradation of

membranes in cells surrounding the oil glands. Oil then leaks from these glands into the surrounding cells of the albedo and oxidises this tissue. The oxidised albedo tissue appears as sunken brown spots on the fruit surface. The gland oil consists largely of terpenes and sesquiterpenes, which are highly phytotoxic (Soule and Grierson, 1986), and may therefore directly contribute to the brown discolouration.

Figure 2.3. Rind breakdown symptom in 'Nules Clementine' mandarin fruit

Olleocellosis is another disorder, also associated with collapsed oil glands and may therefore be morphologically similar to rind breakdown. However, the mechanism of oil damage is the main difference between the two disorders. In the case of olleocellosis, oil gland rupture was observed to occur at the junction of the epidermis and the gland stalk, and this rupturing of the oil gland results in essential oil leakage up towards the adjacent epidermis causing necrosis of the surrounding epidermal cells (Soule and Grierson, 1986; Knight et al., 2001). By contrast rupture of the oil gland in rind breakdown results in oil leakage downwards into the albedo (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Another difference between rind breakdown and olleocellosis is that, ollecellosis is in most cases the result of rough handling of fruit especially during harvesting (Soule and Grierson, 1986), whereas rind breakdown is a progressive disorder that occurs during postharvest storage (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

2.2.2 Rind pitting

Rind pitting of grapefruit (*C. paradisi* Macf.) and sweet oranges manifests as discrete areas of the rind with sunken lesions which become bronze or reddish-brown in colour and tend to coalesce with time producing larger affected areas (Grierson, 1986; Vercher et al., 1994; Petracek et al., 1995). This disorder is also associated with collapsed oil glands scattered over the fruit surface with slight depressions in regions directly above the oil gland (Petracek et al., 1995). Epidermal, subepidermal and the large albedo cells underlying the oil gland appeared collapsed and disoriented in fruit with rind pitting (Vercher et al., 1994; Petraceck et al., 1995). A study on the cuticle of fruit with rind pitting showed that the occurrence of this disorder was also associated with high water permeability of the cuticle (Vercher et al., 1994).

Rind pitting has been reported as a preharvest disorder as well as one that occurs post-harvest (Grieson, 1986; Vercher et al., 1994). The postharvest occurrence of this disorder has largely been attributed to low temperature storage (Grierson, 1986; Vercher et al., 1994). However, Petracek et al. (1995) showed that rind pitting could occur in waxed fruit stored at non-chilling temperatures, and the disorder developed within 1 week in fruit stored at high temperatures. It was therefore concluded that rind pitting was distinguishable from chilling injury, which may also show similar symptoms (Petracek, et al., 1995).

2.2.3 Rind staining

Rind staining, also referred to as non-chilling peel pitting, of 'Navelate Navel' orange is characterised by irregular colourless depressed areas on the rind, directly above and among oil glands (Agusti et al., 2001; Lafuente and Sala, 2002; Alferez et al., 2003). The affected areas on the rind develop into reddish-brown dry areas with time (Agusti et al., 2001; Lafuente and Sala, 2002). Histological characterisation of rind staining of 'Navelate Navel' orange showed that the disorder initially appeared in the transitional zone of the albedo and flavedo (Agusti et al., 2001). The affected cells in that region of the rind had reduced cytoplasm and the walls were twisted and squashed, forming a layer of collapsed cells between healthy intact cells of the albedo and flavedo (Agusti et al., 2001). Wax morphology and cuticular permeability of fruit affected by rind staining were normal showing no sign of disruption or difference in comparison with healthy fruit (Agusti et al., 2001).

Rind staining can occur on the tree and under postharvest conditions in 'Navelate Navel' orange (Agusti et al., 2001; Alferez et al., 2003), whereas the same disorder only occurs

during postharvest storage in 'Navelina Navel' orange (Lafuente and Sala, 2002; Alferez et al., 2003).

2.2.4 Superficial flavedo necrosis (noxan)

Superficial flavedo necrosis (noxan) of 'Shamouti' orange appears as superficial pits on the flavedo, and in time the few pits grow in both number and size to form a necrotic area (Ben Yehoshua et al., 2001; Peretz et al., 2001). Morphological studies showed that noxan was associated with collapsed hypodermis tissue and destroyed oil glands, oil leaks from these glands into the surrounding tissue, which was suggested to be the reason for the blemish (Ben Yehoshua et al., 2001).

Noxan develops during the postharvest life of the fruit and may be attributed to moisture loss from the fruit rind causing cracks in the oil gland. These cracks may lead to the leakage of essential oils from the gland, which results in the development of noxan (Ben Yehoshua et al., 2001).

2.2.5 Peteca spot

Peteca spot is a rind disorder of lemons [*C. limon* (L) Burm f.] and manifests as deep depressions on the rind, these depressions turn brown or appear dehydrated (Wild, 1991; Leguizamon et al., 2001). Storey and Treeby (2002) defined the disorder as faint discolouration of the internal rind and subepidermal oil glands. Morphological studies of the rind of fruit with peteca spot showed that the browning, while seen on the surface, actually occurred in the lower albedo tissue (Storey and Treeby, 2002). It was further shown that while there was deposition of oil in the intercellular spaces between albedo cells in fruit with peteca spot, no catastrophic rupture of oil glands was observed (Storey and Treeby, 2002).

Several factors, both pre-harvest and post-harvest, have been shown to affect the development of this disorder (Wild, 1991). Storey and Treeby (2002) suggest that the brown discolouration in the albedo may be indicative of non-enzymatic browning caused by oxygen depletion due to the accumulation of oil in this tissue. Pottasium and Na concentrations were significantly lower in peteca-affected regions of the rind than unaffected regions, whereas Mg, Mn, and Ca were significantly higher, indicating the role of nutional imbalance in the development of peteca sopt (Storey and Treeby, 2002). Peteca spot has been reported on mature fruit on the tree and also as a disorder developing after the fruit has been harvested (Wild, 1991).

2.2.6 Stem-end rind breakdown

Stem-end rind breakdown symptoms (SERB) involve the collapse and subsequent darkening of epidermal tissue around the stem end of citrus fruit (Grierson, 1986). The affected area is sunken and darkens with time (Grierson, 1986). Characteristic of stem-end rind breakdown is a small ring of undamaged tissue around the calyx due to the fact that this tissue contains no stomata and has a thick layer of cuticular wax (Albrigo 1972; Grierson, 1986).

This disorder develops during storage of fruit and is common on smaller, more mature fruit (Grieson 1986). The disorder may be due to excessive dehydration of the rind around the stem end of detached fruit (Lafuente and Zacarias, 2006). Another breakdown occurring on the stylar-end of 'Tahiti' limes (*C. aurantifolia* Sering) also occurs. This breakdown, known as stylar-end rind breakdown is classically different from stem-end rind breakdown due to the translucent or water-soaked appearance it presents (Davenport et al., 1976; Grieson 1986). Furthermore, stylar-end rind breakdown appears following rupture of juice vesicles resulting in the release of juice which then invades rind tissue at the stylar-end and on occasion at the stem-end resulting in the symptom seen (Davenport et al., 1976; Grierson 1986).

2.3 Factors affecting fruit quality, with special reference to rind breakdown

The occurrence of rind breakdown is principally determined by genetic variability, environmental factors, fruit maturity at harvest, plant growth regulators, antioxidants, and the storage environment. A discussion is presented on how these factors influence fruit quality with special reference to the development of rind breakdown.

2.3.1 Genetic

Due to natural bud mutation and selection, several 'Clementine' mandarin selections exist and some of these were introduced into South Africa since 1970 (Barry and Rabe, 2004). Plantings of the different 'Clementine' mandarin selections increased from the time of introduction and reached a peak of ~3.5 million trees in 2000 and plantings stabilised thereafter. 'Nules', 'Marisol' and 'Oroval', comprise the bulk of the total 'Clementine' plantings in South Africa (Barry and Rabe, 2004).

The different 'Clementine' mandarin selections present some diversity in morphological and horticultural characteristics. Saunt (2000) mentioned that 'Nules Clemntine' mandarin fruit

are able to retain good quality on the tree. Hence they can be harvested over extended periods. By contrast, 'Oroval Clementine' mandarin fruit, hereafter referred to as 'Oroval', have a poor hanging ability on the tree, a pebbly rind and become excessively puffy with delayed harvest (Saunt, 2000). 'Oroval Clementine' mandarin fruit are reported to develop a dark orange colour when mature, which is a more intense orange colour than that developed by mature 'Nules Clementine' mandarin fruit (Wahl and Le Grange, personal communication). Beyond the differences in morphological and horticultural characteristics, genetic differences have also been reported between some of the 'Clementine' selections. Russo et al. (2000) reported that different 'Clementine' selections from Apulia (Southern Italy) exhibited different fruit sizes, fruit weight, rind thickness and differences in internal quality. It was further reported that the germplasm tested showed large variability indicating that the 'Clementine' selections were not genetically similar. However, results demonstrating similarities between mandarin cultivars have also been reported. It was reported that 'Clementine' mandarins derive from a single plant, and therefore, genetic variation between selections should be relatively narrow (Bretó et al., 2001). In another study it was shown that the enantiomeric distribution of selected essential oils of 'Cai' and 'Montenegrina' mandarins (*C. deliciosa* Tenore) was not significantly different (Frizzo et al., 2004), again illustrating the narrow genetic variation among mandarins.

Different citrus cultivars, or selections of the same cultivar, are known to respond differently to the same storage temperature and also have different susceptibilities to the development of disorders. Underhill et al. (1999) showed that 'Lisbon' lemon stored for 14, 28 or 42 days at 1 °C followed by 7 days at 20 °C developed higher levels of chilling injury than 'Eureka' lemon stored at identical conditions. 'Nules Clementine' mandarin was identified as the 'Clementine' mandarin selection most susceptible to rind breakdown, whereas 'Oroval' was identified as the least susceptible (Van Rensburg and Bruwer, 2000).

2.3.2 Environmental

2.3.2.1 Macroclimate

The incidence of rind breakdown and other rind disorders varies between seasons and between orchards, suggesting that climatic factors may influence the susceptibility of individual fruit to this disorder (Van Rensburg and Bruwer, 2000; Agusti et al., 2001; Lafuente and Sala, 2002; Alferez et al., 2003; Van Rensburg et al., 2004). The precise conditions leading to the development of rind breakdown of 'Nules Clementine' mandarin

fruit have not been identified. However, it has been suggested that rind breakdown occurs following a warm winter and when the fruit matures during a warm autumn (Van Rensburg et al., 2004). Agusti et al. (2001) reported that rind breakdown of 'Navelate Navel' orange appeared following sudden climatic change from days with low temperature and high relative humidity to high temperature and low relative humidity resulting in high evapotranspiration.

2.3.2.2 Canopy microclimate

The influence of environmental factors on fruit quality has been related to canopy position in which fruit is borne (Arpaia, 1994). The modifications in the microclimate of different sectors of a tree and how these affect fruit quality have been reported (Cohen, 1988; Barry et al., 2000; Morales et al., 2000). The influence of fruit canopy position on fruit quality at harvest, measured in terms of rind colour, total soluble solids and titratable acid content showed a particular trend, which was not, however, always significant. 'Orlando' tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) fruit originating from the bottom inside of the tree canopy were found to be greener than fruit originating from the top of the tree canopy (Morales et al., 2000). Verreynne et al. (2004) reported no significant difference in rind colour development between inside and outside fruit harvested from different sectors in 'Satsuma' mandarin (*C. unshiu*), 'Clementine' mandarin and 'Temple' tangor trees. Total soluble solids or soluble solids concentration was found to be higher in outside citrus fruit (exposed) than in inside fruit (partially shaded) (Barry et al., 2000; Morales et al.,, 2000; Barry et al., 2004a; Verreynne et al., 2004). Generally, higher titratable acidity was reported in fruit originating from the inside of the tree canopy (Cohen, 1988; Verreynne et al., 2004) than fruit from the outside. However, marginal and non-significant differences between fruit canopy positions in titratable acidity of citrus fruit have been also reported (Barry et al., 2000; Morales et al., 2000; Barry et al., 2004a).

Since canopy position in which fruit are borne affects fruit quality at harvest, it may then as a consequence affect the shelf-life of fruit as an association between post-storage fruit quality and "orchard quality" has been reported (Crisosto et al., 1997). It has been suggested that 'Nules Clementine' mandarin fruit originating from the inside of a tree's canopy have low carotenoid content at harvest and consequently are more susceptible to rind breakdown after storage than fruit originating from the outside of the tree canopy (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). The reverse was reported for chilling injury, where grapefruit harvested from the exterior canopy of the tree were more susceptible to the disorder

than fruit harvested from the interior canopy position (Purvis, 1980). In another fruit kind, Songold plums (*Prunus salicina*), shaded fruit from the bottom sector of the tree canopy were reported to develop significantly higher levels of the internal disorder, gel breakdown, after cold storage than exposed fruit from the top sector of the tree canopy (Taylor et al., 1993).

Canopy position has also been reported to have an effect on the antioxidant capacity of fruit. Studies conducted on apple fruit originating from a low light environment showed that this fruit had lower phenol content and lower activity of some antioxidant enzymes at harvest than fruit originating from a high light environment (Ju, 1998; Ma and Cheng, 2003).

2.3.2.3 Storage environment

Citrus fruit are non-climacteric with low respiration rates during maturation and senescence, suggesting that they can be stored for relatively long periods (Davies and Albrigo, 1994). However, differences exist among species and cultivars (Chalutz et al., 1985) and, as a result, no single storage protocol is applicable to all citrus cultivars. Davies and Albrigo (1994) suggested that citrus types not sensitive to chilling can be stored at temperatures below 4 °C, whereas cultivars sensitive to chilling injury should be stored at temperatures above 10 °C. The humidity in the storage environment should be as high as possible, to reduce moisture loss (Grierson and Ben-Yehoshua, 1986).

Harvested fruit are living organs, as they continue to respire and lose moisture in storage (Burdon, 1997). These and other ongoing metabolic processes in a fruit during storage can result in changes, detrimental to fruit quality, which may be pathological or physiological (Burdon, 1997). These detrimental changes can be exacerbated by fruit storage at sub-optimal condition, in terms of storage environment (temperature and relative humidity) and storage duration (Burdon, 1997). Van Rensburg et al. (2004) mentioned that rind breakdown of 'Nules Clementine' mandarin fruit is aggravated by high storage temperature and long storage duration, although specific temperatures and durations were not provided.

Other rind disorders, associated with collapsed oil glands, that may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin and which affect other citrus types have also been reported. The incidence of these disorders is higher at non-chilling temperatures (>15 °C) compared to lower temperatures, and they include rind pitting of 'Marsh' grapefruit (Petracek et al., 1995) and 'Fallglo' mandarin (Petracek et al., 1998),

superficial flavedo necrosis (noxan) of 'Shamouti' orange (Ben Yehoshua et al., 2001), and rind staining of 'Navelina Navel' orange (Agusti et al., 2001; Alférez et al., 2003).

Low storage temperature generally suppresses fungal decay development (Eckert and Brown, 1986; Shellie and Skiria, 1998). However, in citrus fruit, due to the presence of chilling injury or other rind disorders at low temperatures, opportunistic infection can occur, resulting in higher decay levels in fruit stored at low than at high temperatures (Cohen and Schiffmann-Nadel, 1978; Chalutz et al., 1985).

Apart from physiological and pathological disorders, storage temperature and storage duration can also affect biochemical properties of citrus fruit. Rind colour development may be influenced by storage temperature, with better rind colour (more orange) development reported in fruit stored at high temperature than at lower or subzero temperatures (Cohen and Schiffmann-Nadel, 1978; Van Wyk, 2004). Prolonged storage at chilling temperatures can induce oxidative stress on fruit. Oxidative stress occurs when the production of active oxygen species exceeds the capacity of the cell to remove them and maintain a cellular redox homeostasis (Hodges et al., 2004; Toivonen, 2004). Symptoms of oxidative stress can include the development of postharvest disorders (Hodges at al., 2004). In citrus fruit, the postharvest disorders chilling injury and rind staining of 'Navelina Navel' orange have been associated with oxidative stress (Sala, 1998; Sala and Lafuente, 2004).

The relative humidity in the storage environment was found to be a significant factor in the occurrence of some of rind disorders. It was reported that post harvest rind staining of 'Navel', oranges and rind pitting of 'Marsh' grapefruit and 'Fallglo' mandarine was higher in fruit stored at a low relative humidity then transferred to a high relative humidity storage environment (Alferez et al., 2003; Alferez and Burns 2004; Alferz et al., 2005b). Upon transfer of fruit from low to high RH environment there is a difference in water potential recovery between flavedo and albedo tissue, with flavedo tissue recovering faster than albedo tissue. It is this differential recovery in water potential between the rind tissue that is postulated to cause collapse of internal albedo layers resulting in rind staining or pitting (Alferez et al., 2003; Alferez et al., 2005b). 'Shamouti' orange showed a different response to RH in the storage environment. In this citrus type noxan was markedly reduced by fruit storage at high RH (96%) compared to low RH (75-80%) (Ben Yehoshua et al., 2001 Peretz et al., 2001).

Modifying the gas composition in the fruit storage environment has also been reported to affect the development of rind disorders (Petracek et al., 1997; Petracek et al., 1998b; Ben Yehoshua et al., 2001; Porat et al., 2004). Porat et al. (2004) demonstrated that attaining the CO_2 and O_2 concentrations at 2-3% and 17-18%, respectively, around the fruit through modified atmosphere packaging (MAP) reduced chilling injury and other rind disorders. However, results to the contrary have also been reported, where inappropriate MAP was found to enhance the development of rind disorders (Petracek et al., 1997; Petracek et al., 1998b; Ben Yohashua et al., 2001).

2.3.3 Nutritional

Plant growth requires the incorporation of mineral nutrients into the materials from which plants are made, therefore required by plants to survive (Salisbury and Ross 1991). Mineral nutrients can be classified as essential or nonessential. A mineral nutrient is classified as essential when a plant cannot complete its life cycle without that element or when the element forms part of any molecule or constituent of the plant that is itself essential in the plant (Salisbury and Ross 1991). The concentration of essential elements in plant tissue appears critical for plant growth and fruit quality, over supply results in toxicity whereas under supply results in deficiency symptoms (Salisbury and Ross, 1991; Story and Treeby, 2000; Kruger et al., 2003).

Specific patterns of seasonal mineral nutrient distribution have been reported on citrus. Two patterns for the seasonal distribution of mineral nutrients in citrus have been identified. In the first pattern, some nutrients gradually decrease from fruit set and then plateau, at a minimum concentration during fruit maturation (Story and Treeby, 2000; Kruger et al., 2005). In the second pattern some nutrients initially increase during the early stages of fruit development reaching a peak at this phase and then decrease gradually thereafter, reaching a minimum during fruit maturation (Story and Treeby, 2000; Kruger et al., 2005). Other seasonal mineral nutrient distribution patterns different to these have also been reported (Story and Treeby, 2000 and references therein).

Mineral nutrients are not uniformly distributed between structural parts of the fruit. In 'Bellamy Navel' orange, S and Mg occurred at similar concentrations in whole fruit, pulp and rind but K and P concentrations were higher in the pulp than in the rind (Story and Treeby 2000). However, Kruger et al. (2003) mentioned that the concentration of N and most other

mineral nutrients is higher in the rind than the pulp. In other fruit kinds, the distribution of mineral nutrients has also been reported. Ca concentration was found to be highest in skin and core and lowest in the cortex of apple fruit (Ferguson and Watkins, 1992 and references therein). Differences in nutrient concentrations between fruit structural parts were determined by xylem flow and the proximity of an evaporative surface (Ferguson and Watkins, 1992). However, Story and Treeby (2000) reported that it was both xylem and phloem transport, as some nutrient are phloem-mobile, that determined the distribution of mineral nutrients to fruit parts.

The concentration of mineral nutrients in parts of the fruit has been found to have an effect on fruit quality at harvest and after storage. Investigations into the poor post-storage quality of avocado (*Persea americana* Mill) from South Africa led to the conclusion that harvest maturity and mineral nutrient composition were significant factors leading to poor fruit storage potential (Kruger et al., 2003). High N levels late in the season are antagonistic to orange colour development in citrus (Reitz and Embleton, 1986). Creasing, a physiological disorder that develops predominantly on over mature fruit on the tree has also been associated with nutritional imbalances, particularly K, Ca and Mg nutrition, among other factors (Grierson, 1986; Reitz and Embleton, 1986; Storey and Treeby, 2000). However, Ca has been shown to have a significant effect on the development of postharvest disorders. Different Ca treatments resulted in reduced chilling injury of different fruit kinds, lower incidence of bitter pit in apples and delayed senescence of tomatoes (*Lycopersicon esculentum* L.) and mandarins (Wang, 1990; Ferguson and Watkins, 1992).

Rind disorders of citrus fruit have also been associated with nutritional imbalances. Initially the development of rind breakdown of 'Nules Clementine' mandarin fruit was thought to be associated with B deficiency (Van Rensburg et al., 1995). However, B application to citrus trees did not affect the development of rind breakdown. Therefore, it was concluded that B does not play a role in the occurrence of rind breakdown (Van Rensburg et al., 1995; Van Rensburg and Bruwer 2000; Van Rensburg et al., 2004). Extra N application to 'Marsh' grapefruit and late N application to 'Valencia' orange resulted in higher rind pitting after storage (Kruger et al., 2005). Late season fluctuations in mobile nutrient content were found to have an effect on the development of rind pitting in 'Valencia' orange (Kruger et al., 2006). Other rind disorders that may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin have also been associated with nutritional imbalances. Zaragoza et al.

(1996) demonstrated that application of Ca $(NO_3)_2$ to 'Fortune' mandarin fruit at colour-break reduced rind pitting incidence at harvest. Superficial rind pitting of 'Shamouti' orange was reduced with the application of a K-spray fertilizer (Tamim et al., 2001).

2.3.4 Hormonal

Plant growth regulators have been used to improve postharvest quality of fresh citrus since the 1920s (Davies, 1986). The effects of ethylene, gibberellins, abscisic acid, auxins and cytokinins on rind breakdown are discussed.

2.3.4.1 Ethylene

Ethylene is a naturally occurring plant hormone that is largely associated with fruit ripening. However, differences exist between climacteric and non-climacteric fruit in response to exogenously applied ethylene and the ethylene production trends (Salisbury and Ross, 1991).

Citrus fruit is classified as non-climacteric and thus produces relatively low levels of ethylene during maturation and senescence (Kader, 1992). Although endogenous levels of ethylene are low, citrus fruit are known to respond to exogenously applied ethylene (Cohen, 1978; Porat et al., 1999). The exogenous application of ethylene is a common practise in early-maturing citrus cultivars, which usually meet commercial internal maturity before their rinds attain the desired colour for harvest. Ethylene is then used to enhance rind degreening, to make the fruit more appealing to the consumer (Cohen, 1978; Yamauchi at al., 1997; Porat et al., 1999). The degreening process of citrus fruit involves postharvest application of ethylene, at a specific concentration, air temperature and RH (Saltveit, 1999). It has been reported that the observed improvement in rind colour of citrus from green to yellow following exposure to ethylene is largely due to accelerated breakdown of chlorophyll (Mizutani et al., 1992; Yamauchi et al., 1997). Goldschmidt et al. (1993) showed that during the degreening process there is promotion of carotenoid biosynthesis, which also contributes to the improvement of citrus rind colour from green to yellow.

When not applied correctly ethylene degreening can have adverse effects on fruit quality. Variable fruit colour, green spotting, brown calyx, green rings and wilting are some of the damaging effects of ethylene degreening (Krajewski and Pittaway, 2002). Other unwanted responses of citrus fruit to ethylene degreening include development of physiological disorders and postharvest diseases (Kader, 1985). The influence of ethylene gas degreening

on post-storage decay development varies according to the host-pathogen association (Palou et al., 2003). Ethylene degreening can enhance decay development on citrus fruit, particularly stem-end rot caused by *Diplodia natalensis* (Brown and Burns, 1998; Porat et al., 1999). However, Porat et al. (1999) also reported that degreening reduced the appearance of mould rots on citrus caused by *Penicillium digitatum* and *P. italicum*. Other researchers demonstrated that decay development on stone fruit, peaches [*Prunus persica* (L.)], plums (*P. salicina* Lindel), nectarines [*P. persica* (L.) Batsch. var. *nucipersica* (Suckow) Schneid], apricots (*P. armeniaca* L.) and sweet cherries (*P. avium* L.), as well as on table grapes (*Vitis vinifera* L.) was not affected by continuous exposure to ethylene (Palou et al., 2003).

The effect of ethylene degreening on physiological disorders of citrus is variable. Van Rensburg et al. (2004) reported that long ethylene exposure increased the risk of rind breakdown in 'Nules Clementine' mandarin fruit. However, ethylene pre-treatment was found to reduce the incidence of rind staining in 'Navelina Navel' orange, a physiological rind disorder associated with collapsed oil glands, which may be morphologically similar to rind breakdown of 'Nules' (Lafuente and Sala, 2002; Sala and Lafuente, 2004). Ethylene exposure was also found to enhance chilling injury symptoms to citrus fruit (Yuen et al., 1995). However, Bower et al. (1999) reported results to the contrary, their work showed that degreening reduced chilling injury of lemon, 'Marsh' grapefruit and 'Navel' orange.

2.3.4.2 Gibberellins

Gibberellins are naturally occurring plant hormones that are largely associated with growth of intact plant among other functions. These hormones are synthesized in young leaves and are transported by diffusion through the xylem and phloem. Due to their effects on plants, gibberellins are used commercially to enhance fruit quality (Salisbury and Ross, 1991).

Gibberellins have a pronounced effect on citrus rind quality, as they are known to retard rind senescence (Davies, 1986; Salisbury and Ross, 1991). The application of gibberellic acid to 'Clementine' mandarin and 'Washington Navel' orange delayed colour development from green to orange on the tree and in storage (Davies, 1986; El-Otmani and Coggins, 1991; Miller and McDonald, 1996). Davies (1986) mentioned that gibberellic acid delayed the chloroplast to chromoplast conversion when applied to the orange peel. It was also shown

that gibberellic acid had antagonistic effects on chlorophyll degradation in senescing 'Shamouti' orange rind (Davies, 1986).

The application of gibberellic acid is known to increase and maintain rind firmness in citrus (El-Otmani and Coggins, 1991). Monselise et al. (1976) reported that the firmer rind in fruit treated with gibberellic acid was because of higher protein content and lower incorporation of amino acids into enzymes important for pectin degradation.

Singh and Singh (1981) showed that gibberellic acid application reduced the percentage of rind and increased the pulp and juice fraction of 'Kaula' mandarin fruit. However, in cultivars with a naturally well-developed albedo, gibberellic acid application increased rind thickness (Goldschmidt, 1983).

Gibberellic acid was also found to have an effect on the internal quality of citrus fruit. However, this effect was variable and inconsistent (Davies, 1986). Some studies reported an increase in TSS for fruit treated with gibberellic acid (Singh and Singh, 1981). Others have shown no significant differences in the internal quality of fruit treated with gibberellic acid (El-Otmani and Coggins, 1991; Miller and McDonald, 1996). Pre-harvest gibberellic acid application resulted in a sudden change in GA-like substances in the flavedo, a reduction in abscisic acid and sugar levels in the rind of 'Satsuma' mandarin fruit and consequently reduced puffiness (Kuraoko et al., 1977; Luis et al., 1985). Rind pitting in 'Marsh' grapefruit irradiated (0.3 or 0.6 kGy) and stored at 10°C was reduced by pre-harvest application of GA_3 (Miller and McDonald, 1996). Rind staining of 'Navel' orange is another disorder that was reduced by GA_3 application (Davies, 1986).

2.3.4.3 Auxins

Auxins are naturally occurring plant hormones that promote elongation of root sections at extremely low concentrations, among other functions. However, at higher concentrations they were found to inhibit elongation (Salisbury and Ross, 1991).

Synthetic auxins have been used to delay natural pre-harvest abscission of 'Temple' tangor fruit (Zur and Goren, 1977; Salisbury and Ross, 1991) and in some cases induce abscission (Salisbury and Ross, 1991). Naphthalene acetic acid successfully reduced granulation in 'Kaula' mandarin fruit (Singh and Singh, 1981). Van Rensburg et al. (2004) reported that the

use of a synthetic auxin, 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA), significantly reduced the incidence of rind breakdown in 'Nules Clementine' mandarin fruit.

2.3.4.4 Cytokinins

Cytokinins are substituted adenine compounds, naturally occurring in plants, which promote cell division in tissue systems (Salisbury and Ross 1991). This function of cytokinins has been used commercially to enhance fruit size. The application of a synthetic cytokinin, (N-2-chloro-pyridyl)-N-phenylurea (CPPU), resulted in an appreciable (>50%) increased fruit size of apples (Stern et al., 2006a). The application of CPPU to green or slightly red litchi (*Litchi chinensis)* fruitlets on the tree, delayed harvesting by 2 to 3 weeks compared to the control (Stern et al., 2006b). Furthermore, storage life of the litchi fruit was extended by the application of CPPU (Stern et al., 2006b).

2.3.4.5 Abscisic acid

Abscisic acid (ABA) is a 15-carbon sesquiterpenoid synthesised partly in chloroplasts and other plastids in plants (Salisbury and Ross, 1991). ABA is regarded as a stress or senescence signal and may also cause responses that protect plants against stress (Goldschmidt et al., 1973; Salisbury and Ross, 1991).

ABA reduced chilling injury in a wide range of plants. Nayyar et al. (2005) demonstrated that cold acclimation of chickpea (*Cicer arietinum* L.) seedlings increased endogenous ABA content and consequently reduced cold damage to the seedlings. Furthermore, seedlings treated with 0.1μM abscisic acid showed a cold acclimation-like response (Nayyar et al., 2005). However, it was concluded that the beneficial effects of acclimation could not be substituted by ABA treatment (Nayyar et al., 2005). A similar response was reported on maize (*Zea mays* L.) seedlings pre-treated with 1 mM ABA (Prasad et al., 1994).

However, Alferez et al. (2005a) mentioned that the role of ABA as a protective stress signal in citrus fruit is still controversial. It was shown in 'Fortune' mandarin fruit that changes in ABA content with fruit maturity and in storage did not indicate an association between ABA and the development of chilling injury (Lafuente et al., 1997). Investigation of ABA as a protective stress signal was further extended to the non-chilling disorder, rind staining of 'Navelina Navel' orange. Changes in ABA concentration as a result of ethylene treatment and the development of rind breakdown were inconsistent, therefore it was concluded that

ethylene and not ABA was the important factor in protecting 'Navelina Navel' orange fruit from developing rind staining (Lafuente and Sala, 2002). To further understand the role of ABA in the development of chilling injury and non-chilling rind damage in citrus fruit, an ABA-deficient mutant was used in several experiments. ABA does not appear to play a protective role against rind damage induced by low temperature storage (Alferez et al., 2005a).

2.3.3 Fruit maturity at harvest

Harvest date is an important factor in 'Nules Clementine' mandarin fruit because this cultivar has a protracted flowering period, which results in up to three fruit set periods (Saunt, 2000). This flowering pattern and subsequent fruit set results in an extended harvest period. In the Western Cape province of South Africa, 'Nules Clementine' mandarin is harvested over a lengthy period, with the harvest window starting in May and extending into June (Barry and Rabe, 2004).

As a result of the long harvest window of 'Nules Clementine' mandarin, fruit quality from the early harvest may be different to those from the later harvest. Saunt (2000) reported that 'Nules Clementine' mandarin fruit from the first set are smaller and have a smooth rind, becoming larger and coarser with each subsequent fruit set. Other researchers working on different citrus cultivars reported that fruit harvested periodically showed physiological changes, over the harvest period, associated with maturity (Holland et al., 1999; Kato et al., 2004).

Harvest maturity of fruit influences storage life and post-storage quality (Reid, 1992). Van Rensburg et al. (2004) reported that 'Nules Clementine' mandarin fruit harvested late in the harvest window were more susceptible to rind breakdown than fruit harvested earlier.

Several factors affecting other rind disorders that may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin have been investigated. However, little attention has been given to the effect of harvest date on their occurrence. Sala and Lafuente (2004) mentioned that more mature fruit had a lower activity of antioxidant enzymes and were more susceptible to rind damage than less mature fruit. Nevertheless, substantial literature exists suggesting that occurrence of these rind disorders may be due to changes in relative humidity (Lafuente and Sala, 2002; Alferez et al., 2003), modification of internal gas composition as

influenced by waxing (Petracek et al. 1997; Petracek et al., 1998b), and storage temperature and water stress (Ben Yehoshua et al., 2001). The influence of harvest date on the occurrence of rind breakdown is a subject that needs to be better explored.

Harvest maturity has also been reported to effect the antioxidant capacity of different fruit kinds. In apples late harvested fruit contained higher levels of antioxidants compared to early harvested fruit and were found to be resistant to scald (Vasilakasis and Manseka, 1995). Generally the hydrophilic and lipophilic antioxidant fraction of pepper fruit increased with increasing maturity (Navarro et al., 2006).

2.4 Antioxidants

In foods, antioxidants have been defined as substances that in small quantities, are able to prevent or significantly retard the oxidation of easily oxidisable materials (Frankel and Meyer, 2000). In biological systems the definition is modified to any substance that when present in lower concentrations than an oxidisable substrate, significantly delays or prevents oxidation of the substrate (Frankel and Mayer, 2000).

Plant cells have antioxidants for protection against the harmful effects of reactive oxygen species (ROS) (Scandalios, 1993; Chandru et al., 2003; Purvis, 2004). These reactive oxygen species are partially reduced forms of atmospheric oxygen (Elstner, 1982; Mittler, 2002). The term ROS also includes molecules such as hydrogen peroxide, singlet oxygen and ozone (Blokhina et al., 2003). These ROS are generated as by-products of natural aerobic metabolism, e.g. photosynthesis and respiration (Mittler, 2002). Their production can also be enhanced when the cell perceives a server stress (Mittler, 2002; Toivenen, 2004). In recent years other sources of ROS have been identified, and include NADPH oxidases, amine oxidases, and cell-wall-bound peroxidases (Mittler, 2002). These new sources of ROS are tightly regulated and are triggered by processes such as programmed cell death and pathogen defence (Mittler, 2002). Within a plant cell there are three sites for the generation of ROS: 1) the apoplastic region, 2) the cytoplasm, and 3) cellular organelles, including chloroplast, mitochondria and peroxisomes/glysosomes. The nucleus and vacuole have not been well investigated as sites for ROS generation (Mittler, 2002; Toivonen, 2004).

The enhanced production of ROS poses a threat to cells but it is also thought that the ROS act as signals for the activation of stress and defence mechanisms by the cell (Mittler, 2002).

However, when the generation of ROS exceeds the capacity of the cell to remove them and maintain cellular redox homeostasis, oxidative stress occurs (Hodges, 2004; Toivonen, 2004). This stress is associated with lipid peroxidation and membrane degradation (Fu and Huang, 2001; Toivonen, 2004), and consequently postharvest disorders in different fruit types (Hodges et al., 2004).

2.4.1 Metabolism of selected antioxidants and their biological role

To control the levels of ROS and consequently avert cellular damage, plant tissues have numerous antioxidant systems (Scandalios, 1993; Blokhina et al., 2003). These antioxidant systems can deactivate free radicals by two major mechanisms (Prior et al., 2005). Firstly the antioxidant systems are able to break radical chains by donating hydrogen atoms to the chain carrier (Frankel and Meyer, 2000; Prior et al., 2005). In the second mechanism, antioxidants can transfer an electron to reduce any compound or radical (Prior et al., 2005). These antioxidant systems can be enzymatic or non-enzymatic (Scadalios, 1993; Toivonen, 2004).

Some of the enzymatic antioxidants include glutathione reductase (GR), the peroxidases (PR), superoxide dismutase (SOD) and catalase (CAT) (Scadalios, 1993; Blokhina et al., 2003). The different enzymatic antioxidant systems may be located in different cellular compartments (Table 2.1) and may vary in their affinity for a particular free radical (Scandalios, 1993; Mittler, 2002). Mittler (2002) reported differences in the affinities of ascorbate peroxidase (APX) and CAT for hydrogen peroxide, and suggested that these enzymes belong to different classes of hydrogen peroxide scavenging enzymes with APX being responsible for the fine modulation of ROS for signalling, whereas CAT might be responsible for removing ROS during oxidative stress.

Superoxide dismutase exists in multiple forms within plant cells, tissue or organelles and is found in almost all cellular compartments (Table 2.1) (Scandalios, 1993; Mittler, 2002). All forms of SOD are nuclear encoded, therefore multiple genes for SOD exist in most plants (Scandalios, 1993; Blokhina, 2002). The main function of this enzyme is to catalyse the dismutation of superoxide to hydrogen peroxide and hence protect cells against oxidative stress (Scandalios, 1993; Blokhina, 2003; Toivonen, 2004). However, Blokhina (2003) reported that activation of oxygen can proceed through other means not necessarily producing substrate for SOD. Therefore the ability of plants to overcome oxidative stress only partly relies on the induction of SOD activity.

Catalase is present mainly in the peroxisomes and functions to regulate intercellular levels of hydrogen peroxide (Mittler, 2002; Blokhina, 2003). Mittler (2002) reported that CAT does not require a supply of reducing equivalents for its function, therefore this antioxidant enzyme might be insensitive to the redox status of the cell and its function might not be affected during stress.

Table 2.1. Antioxidant enzymes systems in higher plants (Scandalios, 1993).

Subcellular location	Type of active O_2 species	Source of active O_2 species	Enzymic scavenging system	Products	Nonenzymic scavenging system
Chloroplast	Superoxide H_2O_2	PSII Enzymic	SOD Ascorbate peroxidase	H_2O_2 Dihydroascorbate GSH $NADP^+$	Fd Carotenoids Xanthophylls
Mitochondria	Superoxide H_2O_2	Electron transport and enzymic	SOD Peroxidase CAT (CAT-3, maize)	H_2O_2 H_2O H_2O, O_2	
Cytosol	Superoxide H_2O_2	Enzymic	SOD CAT Peroxidase	H_2O_2 H_2O, O_2 H_2O	
Glyoxysome and peroxisome	Superoxide H_2O_2	β-Oxidation Photorespiration	CAT	H_2O, O_2	

Some of the non-enzymatic antioxidants include glutathione, ascorbic acid, carotenoids, plant phenolics and α-tocopherol. The tripeptide glutathione is an abundant low-molecular weight thiol found in most plants (Hausladen and Alscher, 1993; Blokhina et al., 2003). This non-enzymatic antioxidant is synthesized from its constituent amino acids, glutamate and cystein (Hausladen and Alscher, 1993). The subcellular distribution of glutathione is variable as different researchers report differences in the distribution of this antioxidant. Hausladen and Alscher (1993) reported that glutathione was largely distributed between the chloroplast and cytosol. However, a wider subcellular distribution of glutathione has also been reported where the antioxidant was found in most cell compartments, including the cytosol, endoplasmic reticulum, vacuole and mitochondria (Blokhina et al., 2003). Glutathione scavenges cytotoxic hydrogen peroxide and reacts non-enzymatically with other ROS to

protect cells against oxidative stress, and it functions in the regeneration of ascorbic acid, another antioxidant, via the ascorbate-gluthathione cycle (Blokhina et al., 2003).

Ascorbic acid is a product of hexose metabolism and it can be detected in a majority of plant cell types (Foyer, 1993; Blokhina et al., 2003). This antioxidant can be found in various plant tissues and within a cell occurs in the apoplast and also in chloroplasts (Foyer, 1993; Blokhina et al., 2003). Ascorbic acid can directly scavenge ROS and can reduce hydrogen peroxide to water via ascorbate peroxidase reduction (Sies and Stahl, 1995; Blokhina et al., 2003). Therefore this antioxidant combats deleterious events occurring to the photosynthetic membranes and thus prevents metabolic disruption and cellular damage (Foyer, 1993).

Carotenoids are C_{40} isoprenoids or tetrapenes with lipophilic properties and occur in the chloroplast (Pallet and Young, 1993; Sies and Stahl, 1995). Carotenoids are responsible for the yellow to red pigmentation of many plant tissues, where they are located in other plastids e.g. chromoplasts (Pallet and Young, 1993). Sies and Stahl (1995) mentioned that >500 different carotenoids have been identified and β-carotene is the most prominent. Carotenoids can scavenge singlet oxygen and other free radicals thus preventing deleterious event such as lipid peroxidation (Pallet and Young, 1993; Sies and Stahl, 1995). This antioxidant activity of carotenoids is imparted by the extended system of conjugated double bonds in their structure (Sies and Stahl, 1995).

α-tocopherol, also referred to as vitamin E occurs in all photosynthetic organisms, and because the compound is hydrophobic, it is always located in cell membranes (Hess, 1993). Vitamin E belongs to a family of antioxidants that includes four methylated tocols, substituted with a phytyl chain and tocotrienols, substituted with a geranylgeranyl chain (Hess, 1993). In higher plants, vitamin E is synthesized in chloroplasts and protoplastids (Hess, 1993). The major biochemical function of α-tocopherol is antioxidant activity (Sies and Stahl, 1995). Hess (1993) mentioned that α-tocopherol is an effective quenching agent for singlet oxygen and other free radicals. This antioxidant activity of vitamin E is associated with the redox properties of the chromane ring in its structure (Sies and Stahl 1995). In addition to the antioxidant activity, vitamin E also carries out non-antioxidant functions, such as stabilization of membranes through hydrogen bonding between the chromane ring of vitamin E and the carboxyl group of free fatty acids in the membrane (Hess, 1993).

2.4.2 Measurement of antioxidant activity

Although there are numerous methods used to measure antioxidant activity there are no approved and standardised methods that can accurately and quantitatively measure antioxidant activity (Frankel and Meyer, 2000; Prior et al., 2005). The reason for the dilemma in the assay of antioxidant activity is that there are different components of the antioxidant protection system and these components may not respond in the same manner in all cases of oxidative stress or to different radicals (Frankel and Meyer, 2000; Toivonen, 2004; Prior et al., 2005). It is therefore appreciated that the influence of these different antioxidant system components cannot be measured in a one-dimensional assay (Frankel and Meyer, 2000; Prior et al., 2005).

The method of determining antioxidant activity can consist of oxidising a lipid or lipoprotein substrate and determining how much oxidation is inhibited by the various antioxidants (Frankel and Meyer, 2000). Other methods directly measure the consumption of a free radical by the antioxidants, and these are known as free radical trapping methods (Frankel and Meyer, 2000). Some of the commonly used antioxidant capacity assays include, Oxygen radical absorbance capacity (ORAC) assay, total radical-trapping antioxidant parameter (TRAP), total oxidant scavenging capacity (TOSC) assay, chemiluminescence (CL), photochemiluminescence (PCL), low density lipoprotein (LDL) oxidation, Ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) assay, 2,2,-diphenyl-1-picrylhydrazyl (DPPH) assay, and the super anion scavenging assay. These methods have been recently reviewed (Frankel and Meyer, 2000; Prior et al., 2005) where they are described, and the advantages and disadvantages of each method stated.

2.4.3 Influence of antioxidants on the development of rind disorders

Symptoms of oxidative stress vary greatly among different fruit types and even within the same fruit type. They do, however, share similar features (Toivonen, 2004). The biochemical oxidative stress indicators are common for most oxidative stresses induced by postharvest practises (Toivonen, 2004).

Sala (1998) demonstrated that antioxidant enzymes, catalase, superoxide dismutase, ascorbate peroxidase and glutathione reductase, were consistently lower in chilling sensitive citrus cultivars than in more chilling tolerant ones after storage for 8 weeks at 2.5 °C. Sala (1998)

concluded that oxidative stress may be involved in the development of chilling injury and that chilling resistant cultivars have a more efficient antioxidant system. In 'Fortune' mandarin fruit catalase activity was increased by hot water treatment prior to storage at 2 °C and the higher levels of catalase persisted even during storage and consequently resulted in lower chilling injury in preconditioned fruit than in untreated fruit (Sala and Lafuente, 2000). However, El-Hilali et al. (2004) showed that peroxidase activity in the rind of 'Fortune' mandarin stored at 4 °C increased continuously and this increase in peroxidase activity was correlated with development of chilling injury on fruit. It was therefore concluded that the development of chilling injury induces peroxidase activity (El-Hilali et al., 2004).

The knowledge generated from the role of antioxidants in the development of chilling injury was also extended to rind disorders developing at non-chilling temperatures. Sala and Lafuente (2004) demonstrated that 'Navelina Navel' orange stored at 22 °C and low RH environment had a higher ability to metabolise toxic oxygen forms. This result was indicated by a high activity of ROS detoxifying enzymes, compared with fruit stored at the same temperature but at a high RH environment. Consequently, fruit with higher antioxidant activity developed lower levels of rind staining (Sala and Lafuente, 2004). Catalase activity was reported to decrease in 'Navelina Navel' and 'Pinalate Navel' oranges stored at 2 or 12 °C (Sala et al., 2005). However, the levels of this antioxidant enzyme, even though decreasing, remained higher in 'Pinalate Navel' orange fruit stored at 2 °C than in 'Navelina Navel' orange. Consequently, this cultivar developed less chilling injury than 'Navelina Navel' orange stored at the same temperature (Sala et al., 2005). The reverse occurred in fruit stored at 12 °C, with 'Navelina Navel' orange having a higher catalase activity and consequently developing less non-chilling rind pitting than 'Pinalate Navel' orange (Sala et al., 2005). It was concluded that the occurrence of physiological disorders may depend on the ability of harvested fruit to maintain high CAT activity during storage at different temperatures (Sala et al., 2005). The other antioxidant enzymes, ascorbate peroxidase and glutathione reductase, were found not to play a role in protecting 'Pinalate Navel' and 'Navelina Navel' orange against chilling and non-chilling rind disorders (Sala et al., 2005).

2.4 Overall research hypothesis and objectives

There are several rind disorders with similar morphological descriptions, which may, therefore, present similar visual symptoms. These rind disorders appear during storage but seem to be due to sub-optimal cultural practices or environmental factors, as their occurrence

varies between seasons and between orchards. Another similarity between the disorders is that low temperature storage seems to suppress their development.

However, differences also exist between rind disorders, e.g. rind staining of 'Navelina Navel' orange is aggravated by high relative humidity in the storage environment but the reverse was true for noxan on 'Shamouti' orange, which is reduced by high relative humidity storage of fruit. Because of these differences, recommendations to reduce the incidence of a rind disorder on a particular citrus type may not be applicable to other rind disorders occurring on other cultivars.

Rind pitting of different citrus cultivars, rind staining of 'Navelina Navel' orange, and noxan of 'Shamouti' orange have been extensively studied and scientific information on these disorders is available. However, little published information is available on rind breakdown of 'Nules Clementine' mandarin.

There is increasing information linking the occurrence of some rind disorders to oxidative stress or showing an association between certain antioxidants and the development of rind disorders. This study was therefore set up to test whether low antioxidant capacity results in the development of rind breakdown on 'Nules Clementine' mandarin fruit after storage. There were two main objectives to this study: (1) to establish the factors contributing to the occurrence of rind breakdown during storage. The factors that will be studied are harvest date, storage temperature and duration, ethylene gas degreening, and fruit canopy position. In addition the effect of these above mentioned factors on the antioxidant capacity will be measured as well as comparing the antioxidant capacities of different 'Clementine' mandarin selections and their susceptibilities to rind breakdown after storage; and (2) to provide suggestions on how to optimise postharvest handling and storage of 'Nules Clementine' mandarin fruit.

CHAPTER 3

VARIATION IN HARVEST AND POST-STORAGE QUALITY OF 'NULES CLEMENTINE' MANDARIN AND 'OROVAL CLEMENTINE' MANDARIN FRUIT (*CITRUS RETICULATA* BLANCO) WITH SPECIAL REFERENCE TO RIND BREAKDOWN INCIDENCE

Abstract

Rind breakdown has been reported on various citrus types, but mainly occurs on 'Clementine' mandarins (*Citrus reticulata* Blanco). Differences in susceptibility to rind breakdown also exist between the different 'Clementine' mandarin selections with 'Nules' being most susceptible and 'Oroval' being more tolerant to the disorder. The objectives of this study were to quantify the difference in sensitivity to rind breakdown between 'Nules' and 'Oroval Clementine' mandarin fruit and to establish whether differences could be associated with their carotenoid contents and antioxidant capacities at harvest. The two 'Clementine' mandarin selections, 'Nules' and 'Oroval, were harvested from orchards in Paarl, Saron, and Robertson. Fruit were degreened, packed and stored at 7.5 °C for 10 weeks before evaluations were conducted. Generally, the maturity of 'Nules' and 'Oroval' harvested from Paarl was similar. However, 'Nules' fruit harvested from Saron and Robertson tended to have higher SSC and lower titratable acidity than 'Oroval'. Significant variations in mineral nutrient concentration between 'Nules' and 'Oroval' were reported but these differences were not consistent in fruit sampled from the three areas. No difference in mineral nutrient concentration was observed in fruit sampled from Paarl, whereas, some difference occurred in fruit from Saron and Robertson. The tendency was for 'Oroval' to contain higher N whereas 'Nules' contained higher Ca and B. The antioxidant capacity and rind pigments did not differ significantly between the two 'Clementine' mandarin selections from the three areas. However, 'Oroval' sampled from Robertson was less green. After storage plus shelf-life, 'Nules' fruit developed significantly higher levels of rind breakdown than 'Oroval'. However, 'Oroval' tended to be more susceptible to decay and puffiness after the storage period. Differences in rind breakdown incidence could not be associated with the antioxidant capacity and rind pigment concentration at harvest, since these biochemical properties were similar between the two selections at harvest, but the levels of rind breakdown after storage were different. It was concluded that 'Nules' was more susceptible to rind breakdown than 'Oroval' and rind

pigments and antioxidant capacity may not be associated with the development of the disorder.

Introduction

Rind disorders have been reported on various citrus types, including mandarins (*Citrus reticulata* Blanco), oranges (*Citrus sinensis* (L.) Osbeck), grapefruit (*Citrus paradisi* Macf.), tangelo (*Citrus reticulata* Blanco x *Citrus paradisi* Macf.) and lemons [*Citrus limon* (L.) Burm f.] (Petracek et al., 1995; Van Rensburg and Bruwer, 2000; Ben Yehoshua, et al., 2001; Alferez et al., 2003; Van Rensburg et al., 2004). 'Clementine' mandarin is affected by rind breakdown, and 'Nules Clementine' mandarin, hereafter referred to as 'Nules', was identified as the selection most susceptible to this disorder (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

Due to natural bud mutation and selection, several 'Clementine' mandarin selections exist and some of these were introduced into South Africa since 1970 (Barry and Rabe, 2004). Plantings of the different 'Clementine' mandarin selections increased from the time of introduction and reached a peak of ~3.5 million trees in 2000 and plantings stabilized thereafter. 'Nules', 'Oroval', 'Marisol', 'SRA 63' and 'SRA 92' form the main commercial 'Clementine' selections in the South Africa (Barry and Rabe, 2004).

The different 'Clementine' mandarin selections present some diversity in morphological and horticultural characteristics. Saunt (2000) mentioned that 'Nules' fruit are able to retain good quality on the tree. Hence they can be harvested over extended periods. By contrast, 'Oroval Clementine' mandarin, hereafter referred to as 'Oroval', fruit have a poor hanging ability on the tree, a pebbly rind and become excessively puffy with delayed harvest (Saunt, 2000). 'Oroval' fruit are reported to develop a dark orange colour when mature, which is a more intense than that developed by mature 'Nules' fruit (Wahl and Le Grange, personal communication). Beyond the differences in morphological and horticultural characteristics, genetic differences have also been reported between some of the 'Clementine' selections. Russo et al. (2000) reported that different 'Clementine' selections from Apulia (Southern Italy) exhibited different fruit sizes, fruit weight, rind thickness and differences in internal quality. It was further reported that the germplasm tested showed large variability indicating that the 'Clementine' selections were not genetically similar. However, results demonstrating

similarities between mandarin cultivars have also been reported. It has been reported that 'Clementine' mandarins derive from a single plant, and therefore, genetic variation between selections should be very narrow (Bretó et al., 2001). In another study it was shown that the enantiomeric distribution of selected essential oils of 'Cai' and 'Montenegrina' mandarins (*C. deliciosa* Tenore) was not significantly different (Frizzo et al., 2004), again illustrating the narrow genetic variation among mandarins.

Different citrus cultivars, or selections of the same cultivar, are known to respond differently to the same storage temperature and also have different susceptibilities to the development of disorders (Chalutz et al., 1985). Underhill et al. (1999) showed that 'Lisbon' lemon stored for 14, 28 or 42 days at 1 °C followed by 7 days at 20 °C developed higher levels of chilling injury than 'Eureka' lemon stored at identical conditions.

Available information on the susceptibility of different mandarins, from South Africa, to rind breakdown has been generated largely through commercial data obtained from exporting companies on their claims system. The objective of this experiment was therefore, to quantify the difference in susceptible to rind breakdown between 'Nules' and 'Oroval' fruit. Additional data on rind pigments and the antioxidant capacity at harvest were also generated, to establish whether these biochemical properties could be associated with the development of rind breakdown.

Materials and methods

Plant material and sampling

Healthy 'Nules' and 'Oroval' trees budded on Troyer citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] rootstock were used. Three areas, Paarl, Saron and Robertson (Western Cape province, South Africa), were selected for the experiment and site details are summarised in Table 3.1. About 60 kg of fruit were harvested from the inside canopy position of ~50 randomly selected trees within each orchard. Fruit were harvested into 20 kg plastic lug boxes and immediately transported to Stellenpack in Simondium for degreening and packing.

Fruit degreening and packing

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 $g \cdot L^{-1}$), Deccomone® (2.4-D sodium salt) (5 $mL \cdot L^{-1}$) and Citricure® (guazatine)

(2.5 mL·L^{-1}). The fungicides were mixed in water with a wetting agent, Citowet®, added at a concentration of 0.1 mL· L^{-1}. After drenching the fruit was held at ambient temperature (~20 °C) for 8 to 12 hours before being moved to a degreening chamber. Degreening was conducted for 5 days using 1 to 2 ppm ethylene at 20 °C and at 90 to 95% RH. After degreening, fruit were again held at ambient temperature (~20 °C) for 12 to 24 hours before packing.

During packing, fruit were moved through a warm water (~38 °C) bath containing Imazalil® (chloramizol) at 500 ppm. The fruit were then dried in a hot (~42 °C) tunnel, after which a light commercial polyethylene wax (Decowax®) was applied. After waxing the fruit were again dried in a hot (~48 °C) air tunnel and then sorted and packed into the MO5I open display 5 kg plum cartons each containing ~50 fruit. A total of eight cartons (replicates) of fruit were packed per cultivar per growing area. After packing fruit were transported to Stellenbosch for storage.

Fruit storage

The eight cartons of fruit from each 'Clementine' mandarin selection per area were stored at 7.5 °C for 10 weeks. After initial cold storage, the fruit were then subjected to a shelf life period of 1 week at 20 °C before evaluations were conducted.

Data collection

Internal fruit quality and rind variables at harvest

Rind colour of 10 fruit, from each of the eight replicates, was rated using the CRI rind colour chart set number 36, where 8 = poorly coloured, dark green with no colour break and 1 = deep orange and fully coloured fruit (CRI, 2004; Appendix 1). Hue angle of the rind was measured on the same 10 fruit per replicate used for colour rating using a colorimeter (Minolta CR10, Japan), where 0° = red, 90° = yellow, and 180° = green (McGuire, 1992). Equatorial fruit diameter (mm) was measured on 10 fruit per replicate using a digital calliper (Mitutoyo CD-15c, Japan). Soluble solids content (SSC) (°Brix) was determined on a pooled juice sample from 10 fruit per replicate using a digital refractometer (ATAGO DBX-55, Japan). Titratable acid content (%) was determined on a pooled juice sample from 10 fruit per replicate. 25 mL of juice was titrated with 0.1 N NaOH to an end-point of pH 8.2. The result was converted to citric acid equivalents by the equation: tiratable acid content = (mL NaOH/ 25 mL) x (0.1 N

NaOH/0.1562). Juice content (% w/w) was determined from a sample of 10 fruit per replicate. Fresh mass (g) was determined by taking the average mass of 10 fruit per replicate. Mineral nutrient status and rind moisture of fruit was determined by obtaining rind samples from the equatorial region of five fruit per replicate. Mineral analysis was conducted by BemLab in Somerset West, South Africa.

Selected biochemical rind properties determined at harvest

The antioxidant capacity of the flavedo was determined from the rinds of five fruit per replicate per cultivar using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Preparation of standard curve: A 2 mM solution of Trolox (6- 2,5,7,8- tetramethychroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical Co.) was prepared in methanol and used as an antioxidant standard. From the Trolox stock standard, a series of dilutions (in methanol) were prepared viz 0, 0.2, 0.6, 1.0, 1.4, 1.8 and 2 mM.

ABTS (2,2'-Azino-bis3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich Chemical Co. was dissolved in distilled deionized water to a concentration of 8 mM. The ABTS radical cation, $ABTS^{\bullet+}$, was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (~20 °C) for 12 hours. After this period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2.9 mL of the diluted ABTS radical cation to 0.1 mL of each of the serial Trolox dilutions. The mixture was shaken, allowed to react for 6 minutes and an absorbance reading measured at 734 nm using a spectrophotometer (Cary 50 conc UV-visible, Varian, Musgrave, Australia). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Rind sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The flavedo strips were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Before analysis, the frozen strips were ground to a fine powder in a coffee grinder (Molinix 648, France). Liquid nitrogen was added periodically to prevent the sample from

thawing during grinding. A sub-sample of 0.2 g was extracted using 1% HCl in 95% methanol. The extraction of antioxidants was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged (Sorvall RC-58 refrigerated centrifuge, Wilmington, USA) for 5 minutes at 121 *g* and 4.5 °C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 0.1 mL of the antioxidant extract from the citrus rind was sampled and 2.9 mL of the ABTS radical cation was added to this sample. The mixture was allowed to react for 6 minutes, after which absorbance was measured at 734 nm.

Antioxidant capacity (mM Trolox equivalents·g^{-1}sample) was calculated as

(slope x $abs_{734\ nm}$ + C)/(g sample used in analysis)

Where: slope = slope of the standard curve

$abs_{734\ nm}$ = absorbance at 734 nm

C = y-intercept on the standard curve.

Total chlorophyll and carotenoid contents were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Lichtenthaler (1987).

Rind sample preparation and pigment extraction: Rind samples were prepared into a fine powder as for antioxidant extraction. The fine powder was then freeze-dried and again stored at -80 °C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL of cold 95% ethanol + butylated hydroxytoluene (BHT) (100 mg·L^{-1}) + diethyldithiocarbamate (DDC) (200 mg·L^{-1}). The sample was then vigorously stirred twice, for 1 minute in each case, on a vortex (G-560E, Bohemia, N.Y.). Thereafter, the sample was placed in the dark at 4 °C and allowed to extract for 90 minutes. The sample was then filtered through an ashless filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649 and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and total carotenoids (C_{x+c}) were calculated using the following equations:

$$C_a = 13.36A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = 5.24A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000A_{470} - 2.13\,C_a - 97.64\,C_b}{209}$$

Internal fruit quality and rind variables after cold storage plus a shelf-life

Rind colour, SSC, and tiratable acid content were again determined after each cold storage period on 10 fruit per replicate as previously described. Fruit were classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the spots regardless of size. Fruit was classified as having chilling injury when there was superficial brown discolouration on the rind (Murata, 1997). The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the blemishes regardless of size. Fungal decay, green mould caused by *Penicillium digitatum*, incidence was measured on ~40 fruit per replicate. Fruit was classified as puffy when there was disintegration of the albedo resulting in separation of the flavedo from the pulp (Kuraoka et al., 1977). The disorder was measured using 10 fruit per replicate. Rind moisture of fruit was determined by removing ten fresh rind discs from the five fruit per replicate (two discs per fruit) using a size 12 cork borer. These discs were weighed fresh then oven-dried at 70 °C for 72 hours and re-weighed after drying. The difference between the fresh weight and dry weight expressed as a percentage indicated the rind moisture content.

Statistical design and analysis

Conducting the research in a commercial orchard imposed a limitation on the experimental design as 'Clementine' selection treatments could not be randomised in the experimental plot. To allow for comparison between the 'Clementine' selections it was assumed that orchards in which each selection was planted were similar enough. Data collected at harvest and after storage plus shelf-life were analysed using a t-test on STATISTICA® (Tulsa, OK).

Results

Fruit characteristics at harvest

Paarl

'Nules' and 'Oroval' fruit sampled from Paarl had similar rind colour ratings at harvest (Table 3.2). However, the rind of 'Oroval' fruit had a lower hue angle than that of 'Nules'. 'Nules' had significantly lower SSC and titratable acidity compared to 'Oroval', but the absolute differences were small, namely 0.1% for titratable acidity and 0.9 °Brix for SSC. The fruit harvested from both selections were of similar size and mass and also had similar juice contents. All the mineral nutrients (N, P, K, Ca and B) measured and rind moisture content did not differ significantly between the two 'Clementine' selections. The antioxidant capacity was slightly higher in 'Oroval' than in 'Nules' ($P = 0.055$). The rind pigments, chlorophylls and carotenoids, were similar for the two 'Clementine' selections.

Saron

The rind colour, determined with the CRI rind colour chart or the Minolta colour reader, was similar for both the 'Clementine' mandarin selections harvested in Saron (Table 3.3). 'Nules' had a significantly higher SSC and lower titratable acidity than 'Oroval'. The fruit size and mass were similar in both cultivars, however, the juice content was significantly higher in 'Oroval' than in 'Nules'. The concentrations of P, K and Ca were similar in both 'Clementine' selections. However, the concentrations of N and B were significantly different between the two selections, with 'Oroval' having a higher N concentration and 'Nules' having a higher B concentration. Rind moisture content was significantly higher for 'Oroval' than for 'Nules'. The antioxidant capacity and rind pigments were not significantly different between the 'Clementine' mandarin selections.

Robertson

'Oroval' fruit had a significantly lower rind colour rating and hue angle of the rind than 'Nules' (Table 3.4). 'Nules' fruit had a higher SSC and lower acidity compared to 'Oroval'. 'Nules' fruit were significantly larger, heavier and had a higher juice content than 'Oroval'. The N concentration was higher in 'Oroval' than in 'Nules', whereas Ca and B were higher in 'Nules' than in 'Oroval'. The other mineral nutrients, P and K were of a similar concentration in both 'Clementine' selections. 'Oroval' had higher rind moisture content than 'Nules'. The antioxidant capacity and total carotenoid content were not significantly different between the

two ‘Clementine’ selections, but the total chlorophyll content tended to be higher in ‘Nules’ than in ‘Oroval’ (P = 0.075).

Fruit quality after cold storage plus shelf-life

Paarl

Generally, low levels (<4%) of rind breakdown were recorded in fruit from Paarl and there was no significant difference in the occurrence of rind breakdown between fruit from the two ‘Clementine’ mandarin selections (Table 3.5). However, the tendency was for ‘Nules’ to develop higher levels of rind breakdown than ‘Oroval’ (3.7% vs. 1.4%; P = 0.160). The levels of decay and puffiness did not differ significantly between the two ‘Clementine’ selections. ‘Oroval’ had a significantly higher SSC content whereas the titratable acidity of the two selections was similar after the storage period. Rind colour, rated using the CRI rind colour chart or the measured with the colorimeter, and rind moisture content were similar for the two ‘Clementine’ mandarin selections after the storage period.

Saron

‘Nules’ had significantly higher levels of rind breakdown than ‘Oroval’, which did not develop any rind breakdown (Table 3.6). However, ‘Oroval’ fruit from Saron were more susceptible to decay and puffiness as the levels of these disorders were significantly higher than those recorded in ‘Nules’ fruit. The SSC of the two selections was similar after storage but the acidity of ‘Oroval remained significantly higher than that of ‘Nules’. The rind colour of ‘Oroval’ and ‘Nules’ fruit was similar after storage. Rind moisture content of ‘Oroval’ was significantly higher than that of ‘Nules’.

Robertson

The incidence of rind breakdown was significantly higher in ‘Nules’ fruit than ‘Oroval’ (Table 3.7). ‘Oroval’ fruit developed significantly higher decay levels after cold storage plus shelf-life than ‘Nules’ fruit. The SSC was significantly higher in ‘Nules’ fruit while acidity of this fruit was significantly lower than of ‘Oroval’ after the storage period. Rind colour, measured with the CRI rind colour chart or colorimeter, was similar for both ‘Clementine’ selections. Rind moisture content was significantly higher for ‘Oroval’ than for ‘Nules’.

Discussion

To understand some of the underlying factors associated with rind breakdown, selected physiological and biochemical rind properties were measured at harvest on a susceptible 'Clementine' mandarin selection, 'Nules', and compared to those of a more tolerant selection 'Oroval'. Rind breakdown incidence was assessed after a 10-week storage period. Apart from some variation in mineral nutrients and rind moisture content, the two 'Clementine' selections generally showed similar rind characteristics at harvest. The rind colour was similar in fruit from Paarl and Saron whereas, in fruit sampled from Robertson, 'Oroval' was less green than 'Nules'. The colour trend observed in fruit sampled from the three areas could be explained by the rind chlorophyll and carotenoid contents at harvest. Fruit sampled from Saron and Paarl had similar chlorophyll and carotenoid contents at harvest, which was the reason why the fruit from these areas had similar rind colours. On the other hand, 'Oroval' from Robertson had lower chlorophyll content than 'Nules' (2.6 $\mu g \cdot g^{-1}$DW vs. 0.8 $\mu g \cdot g^{-1}$ DW), even though the carotenoid content was similar. This lower chlorophyll content was probably the reason why 'Oroval' fruit were better coloured than 'Nules' in Robertson. 'Oroval' had higher rind moisture content than 'Nules', the reason for this difference could not be established in this experiment. No previous results could be found where rind moisture differences in these 'Clementine' selections were reported. Generally, 'Oroval' had higher N levels in the rind than 'Nules'. By contrast, 'Nules' had higher B and Ca levels in the rind than 'Oroval'. These nutrient differences could possibly be a cultivar attribute with one selection having a higher demand and uptake of a particular nutrient than the other 'Clementine' selection. Another possible explanation for the differences in nutrient contents of the rind could be the different soil type and cultural practises employed in the orchards where the 'Clementine' selections were planted, as these selection were sampled from different orchards.

The study further showed that the two 'Clementine' mandarin selections had different sensitivities to rind breakdown: 'Nules' was found to be more susceptible to the disorder than 'Oroval', confirming earlier reports by Van Rensburg and Bruwer (2000) and Van Rensburg et al. (2004). It has been suggested that the difference in rind breakdown susceptibility between 'Nules' and 'Oroval' was due to differences in antioxidant capacity and rind carotenoid content. However, from this experiment there was not enough evidence to suggest that differences in rind breakdown levels recorded after the storage period were due to differences in rind pigments concentration or the antioxidant capacity. Both 'Clementine'

mandarin selections sampled from the three areas had similar levels of rind pigments and antioxidant capacities at harvest yet developed different levels of rind breakdown. Rind pigments and the antioxidant capacity were not measured during and after storage, therefore it could not be determined whether any changes and differences between the two 'Clementine' mandarin selections in antioxidant capacity occurred during the storage period. The result of the present study indicated that measuring antioxidant capacity at a single point in time, in this case at harvest, cannot be related to rind breakdown because the antioxidant capacity changes over time in storage, and this change is not addressed by a single measurement of the antioxidant capacity (Toivonen, 2004). Research conducted on apple (*Malus domestica* Bork) fruit showed that 'Delicious' had twice the antioxidant content of 'Empire', at harvest but that 'Delicious' was the cultivar more susceptible to internal browning disorders (Toivonen, 2004). This example illustrates that single point measurement of antioxidants may be misleading. Future research on antioxidants and rind pigments association with rind breakdown should involve multiple sampling to reflect the changes in these biochemical properties that occur during storage.

Puffiness (seperation of pulp from rind and segments from each other) is a disorder that has been largely attributed to advanced maturity, tree vigour, and also to high humidity in the cold room (Murata, 1997). Results in the present study have further shown that the 'Clementine' mandarin selections have different susceptibilities to this disorder with 'Oroval' being more susceptible to puffiness than 'Nules'.

After storage, 'Oroval' fruit had consistently higher rind moisture content than 'Nules' fruit and rind moisture content decreased by a greater proportion for 'Nules' than 'Oroval', ±7.1% vs ±5.5%, respectively. Research conducted on chilling injury (Purvis, 1984) and postharvest peel pitting of citrus (Alferez, et al., 2005) showed that the moisture loss could be a contributing factor to the development of these disorders. It therefore remains possible that the rind moisture content at harvest and moisture loss during storage of 'Oroval' and 'Nules' could have possibly contributed to the differences in rind breakdown levels reported between the two 'Clementine' selections. No previous research results could be found showing the association between rind breakdown development in 'Nules' and rind moisture loss.

Conclusions

In conclusion, ‘Nules’ was more susceptible to rind breakdown than ‘Oroval’. However, the difference in susceptibility to rind breakdown between the two ‘Clementine’ mandarin selections did not appear to be directly associated with rind pigments and antioxidant capacity at harvest. Other factors may be involved in the development of the disorder. Therefore, additional research on other rind properties, such as volatiles and rind moisture content, may give clarity on the reasons for differences in susceptibility to rind breakdown between the two ‘Clementine’ mandarin selections. Furthermore, these two ‘Clementine’ mandarin selections may present a good model to study gene regulation of rind breakdown. Due to the decay susceptibility and the development of puffiness in ‘Oroval’ this selection does not have as long a shelf-life as does ‘Nules’, and therefore, should not be stored for up to 10 weeks.

Table 3.1. Summary of trial sites, plant material and harvest dates.

	Site		
	Saron	**Paarl**	**Robertson**
Tree age	14 yrs	12 yrs	19 yrs
Ridging	Yes	Yes	No
Spacing (m)	5 x 3	5 x 3	5 x 2
Harvest date[1]	18 May	13 May	31 May
Degreening	Yes	Yes	Yes

1 At each harvest date fruit were selectively harvested based on colour. Only fruit that had reached colour break or better were sampled.

Table 3.2. Characteristics at harvest of 'Nules' and 'Oroval' sampled from Paarl.

Response variable	**Cultivar**		**P-value**
	'Nules'	**'Oroval'**	
Rind colour rating[1]	5.2 (0.2)[2]	5.3 (0.2)	0.744
Hue angle of the rind	90.8 (2.7)	84.1 (3.0)	0.006
SSC (°Brix)	11.0 (0.1)	11.9 (0.2)	0.001
Tiratable acid (%)	1.0 (0.02)	1.1 (0.06)	0.016
Equatorial diameter (mm)	61.9 (1.3)	62.4 (1.6)	0.633
Mass (g)	109.9 (7.1)	111.6 (9.2)	0.754
Juice content (%)	51.9 (3.3)	50.9 (4.1)	0.691
N (mg/100 g fresh mass)	266 (27.1)	255.8 (22.4)	0.535
P (mg/100 g fresh mass)	31.0 (1.7)	28.5 (2.7)	0.131
K (mg/100 g fresh mass)	323.4 (20.6)	305.6 (36.2)	0.368
Ca (mg/100 g fresh mass)	217.7 (31.8)	207.4 (33.4)	0.631
B (mg/kg fresh mass)	8.2 (0.6)	8.3 (0.7)	0.779
Rind moisture content (%)	75.4 (1.1)	73.7 (1.5)	0.079
Antioxidant capacity (mM Trolox equivalents/g sample)	3.2 (1.0)	4.2 (0.3)	0.055
Total chlorophylls (µg/g dry weight)	3.4 (1.3)	2.0 (1.7)	0.174
Total carotenoids (µg/g dry weight)	13.0 (2.5)	14.0 (1.1)	0.441

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1)

2 Value in parenthesis indicates standard deviation.

Table 3.3. Characteristics at harvest of 'Nules' and 'Oroval' sampled from Saron.

Response variable	Cultivar		P-value
	'Nules'	'Oroval'	
Rind colour rating[1]	5.4 (0.3)[2]	5.4 (0.2)	0.526
Hue angle of the rind	87.6 (0.8)	88.1 (2.1)	0.624
SSC (°Brix)	12.2 (0.4)	11.0 (0.1)	0.001
Tiratable acid (%)	1.05 (0.03)	1.27 (0.05)	0.001
Equatorial diameter (mm)	61.4 (1.8)	60.6 (1.7)	0.530
Mass (g)	101.2 (7.3)	101.2 (7.4)	0.997
Juice content (%)	52.4 (1.6)	56.8 (0.9)	0.001
N (mg/100 g fresh mass)	230.8 (29.1)	264.8 (21.8)	0.034
P (mg/100 g fresh mass)	29.9 (3.8)	30.0 (2.7)	0.983
K (mg/100 g fresh mass)	356.0 (32.0)	318.4 (44.8)	0.165
Ca (mg/100 g fresh mass)	140.4 (26.6)	128.4 (26.1)	0.492
B (mg/kg fresh mass)	5.92 (0.3)	4.84 (0.2)	0.001
Rind moisture content (%)	78.2 (1.0)	80.3 (1.2)	0.017
Antioxidant capacity (mM Trolox equivalents/g sample)	2.0 (0.4)	1.8 (0.6)	0.577
Total chlorophylls (µg/g dry weight)	0.8 (0.6)	0.2 (0.5)	0.131
Total carotenoids (µg/g dry weight)	15.2 (0.9)	17.0 (2.5)	0.181

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1)

2 Value in parenthesis indicates standard deviation.

Table 3.4. Characteristics at harvest of 'Nules' and 'Oroval' harvested from Robertson.

Response variable	Cultivar		P-value
	'Nules'	'Oroval'	
Rind colour rating[1]	5.2 (0.1)[2]	4.8 (0.2)	0.015
Hue angle of the rind	80.3 (3.9)	75.2 (1.9)	0.032
SSC (°Brix)	12.2 (0.1)	10.3 (0.2)	0.001
Tiratable acid (%)	1.05 (0.05)	1.41 (0.09)	0.001
Equatorial diameter (mm)	64.3 (1.2)	61.8 (1.6)	0.022
Mass (g)	120.5 (9.4)	99.8 (6.9)	0.004
Juice content (%)	57.1 (2.7)	51.7 (1.6)	0.005
N (mg/100 g fresh mass)	230.8 (6.22)	264.8 (29.1)	0.033
P (mg/100 g fresh mass)	29.9 (3.9)	29.9 (2.66)	0.983
K (mg/100 g fresh mass)	288.0 (41.3)	316.2 (41.0)	0.310
Ca (mg/100 g fresh mass)	195.0 (31.4)	128.9 (26.4)	0.007
B (mg/kg fresh mass)	6.7 (0.4)	4.8 (0.5)	0.001
Rind moisture content (%)	74.7 (1.9)	79.5 (1.4)	0.002
Antioxidant capacity (mM Trolox equivalents/g sample)	2.7 (0.5)	2.1 (0.4)	0.092
Total chlorophylls (µg/g dry weight)	2.6 (1.9)	0.8 (0.5)	0.075
Total carotenoids (µg/g dry weight)	19.8 (1.7)	17.9 (2.4)	0.185

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break and 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1)
2 Value in parenthesis indicates standard deviation.

Table 3.5. Quality of 'Nules' and 'Oroval' sampled from Paarl and stored for 10 weeks at 7.5 °C plus 1 week at 20 °C.

Response variable	Cultivar		P-value
	'Nules'	'Oroval'	
Rind breakdown (%)	3.7 (4.0)[1]	1.4 (2.0)	0.160
Decay (%)	6.1 (3.9)	3.4 (1.3)	0.077
Puffiness(%)	0.2 (0.6)	2.0 (2.5)	0.073
SSC (°Brix)	10.9 (0.2)	11.8 (0.3)	0.001
Tiratable acid (%)	0.72 (0.09)	0.81 (0.06)	0.127
Rind colour rating[2]	1.2 (0.1)	1.0 (0.1)	0.067
Hue angle of the rind	53.9 (1.5)	54.7 (0.7)	0.337
Rind moisture (%)	68.2 (1.9)	69.2 (2.1)	0.489

1 Value in parenthesis indicates standard deviation
2 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 3.6. Quality of 'Nules' and 'Oroval' harvested from Saron and stored for 10 weeks at 7.5 °C plus 1 week at 20 °C.

Response variable	Cultivar		P-value
	'Nules'	'Oroval'	
Rind breakdown (%)	14.9 (5.1)[1]	0.0 (0.0)	0.001
Decay (%)	21.1 (6.3)	34.0 (7.9)	0.003
Puffiness(%)	0.0 (0.0)	7.1 (3.7)	0.001
SSC (°Brix)	11.5 (0.5)	11.2 (0.3)	0.154
Tiratable acid (%)	0.72 (0.06)	1.01 (0.05)	0.001
Rind colour rating[2]	1.1 (0.2)	1.1 (0.1)	0.681
Hue angle of the rind	54.8 (0.9)	53.7 (1.2)	0.127
Rind moisture (%)	74.0 (0.4)	77.8 (0.4)	0.001

1 Value in parenthesis indicates standard deviation
2 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 3.7. Quality of 'Nules' and 'Oroval' harvested from Robertson and stored for 10 weeks at 7.5 °C plus 1 week at 20 °C.

Response variable	Cultivar		P-value
	'Nules'	'Oroval'	
Rind breakdown (%)	17.6 (3.9)[1]	0.5 (0.8)	0.001
Decay (%)	11.1 (5.4)	36.1 (5.3)	0.001
SSC (°Brix)	11.5 (0.3)	10.0 (0.3)	0.001
Tiratable acid (%)	0.74 (0.03)	0.96 (0.08)	0.001
Rind colour rating[2]	1.2 (0.1)	1.2 (0.2)	0.693
Hue angle of the rind	55.6 (0.7)	55.0 (1.7)	0.475
Rind moisture (%)	69.9 (1.2)	73.8 (1.1)	0.001

1 Value in parenthesis indicates standard deviation
2 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

CHAPTER 4

EFFECT OF CANOPY POSITION ON HARVEST AND POST-STORAGE QUALITY OF 'NULES CLEMENTINE' MANDARIN (*CITRUS RETICULATA* BLANCO) FRUIT

Abstract

Rind breakdown of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) has caused severe losses to the South African citrus industry. It is hypothesized that fruit borne on the inside of a tree canopy is most susceptible to this disorder. The objective of this study was to test this hypothesis and generate additional data on rind antioxidant capacity and rind pigment content at harvest of fruit borne in different canopy positions. It was also an objective of this experiment to establish whether an association exists between rind antioxidant capacity at harvest and the occurrence of rind breakdown. 'Nules Clementine' mandarin fruit were harvested from the inside and outside canopy position of trees and stored at -0.5 °C or 7.5 °C in the 2004 season. The storage temperatures were modified in 2005 to 7.5 °C or 10 °C and the fruit was stored for a total of 12 weeks. Evaluations were conducted at harvest and again after the storage period. Generally, fruit borne on the inside of the tree canopy had lower soluble solids content and a high titratable acid content than fruit sampled from the outside canopy position. Fruit borne on the outside of the tree canopy were significantly larger and heavier than fruit from the inside canopy position. Outside fruit were more orange in colour than inside fruit due to significantly higher rind carotenoid content. Further significant differences at harvest in fruit from the different canopy positions were reported in most of the rind mineral nutrients, as well as in the antioxidant capacity. However, these differences did not indicate the fruit's potential to develop rind breakdown or chilling injury, as fruit from different canopy positions showed similar levels of rind breakdown and chilling injury after storage plus shelf-life. Therefore, the occurrence of rind breakdown or chilling injury, in this experiment, was not associated with where fruit were borne in a tree's canopy. Furthermore, the antioxidant capacity and rind pigments measured at harvest could not be used as indicators for a fruit's potential to develop rind breakdown or chilling injury after storage. Only storage temperature influenced the occurrence of rind disorders in the 2004 season, with rind breakdown being prevalent at higher temperatures but absent in fruit stored at -0.5 °C, whereas chilling injury occurred only at -0.5 °C and not at the higher temperatures.

Introduction

Rind breakdown of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) is a physiological rind disorder that has caused severe losses to the South Africa citrus industry (Van Rensburg and Bruwer, 2000). Rind breakdown appears following leakage of essential oils from oil glands in the flavedo. The rind oil leaks into and oxidizes the albedo. Oxidized tissue appears as brown spots on the flavedo (Van Rensburg et al., 2004). The occurrence of rind breakdown is reported to be associated with canopy position in which fruit are borne (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

The influence of fruit canopy position on fruit quality at harvest, measured in terms of rind colour, total soluble solids (TSS) and titratable acid content shows a trend, which was not always significant. 'Orlando' tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) fruit originating from the bottom inside of the tree canopy were found to be greener than fruit originating from the top of the tree canopy (Morales et al., 2000). Verreynne et al. (2004) reported no significant difference in rind colour development between inside and outside fruit harvested from different sectors in 'Satsuma' mandarin (*C. unshiu*), 'Clementine' mandarin and 'Temple' tangor (*C. sinensis* x *C. paradisi*) trees. The effect of canopy position on internal fruit quality has also been reported. Total soluble solids or soluble solids concentration (SSC) was found to be higher in outside citrus fruit (exposed) than in inside fruit (partially shaded) (Barry et al., 2000; Morales et al., 2000; Barry et al., 2004; Verreynne et al., 2004). Generally, higher titratable acidity was reported in fruit originating from the inside of the tree canopy (Cohen, 1988; Verreynne et al., 2004) than fruit from the outside. However, marginal and non-significant differences between fruit canopy positions in titratable acidity of citrus fruit have been also reported (Barry et al., 2000; Morales et al., 2000; Barry et al., 2004). The effect of canopy position on fruit quality at harvest has been largely attributed to modifications in the tree microclimate as affected by pruning or shading (Cohen, 1988; Barry et al., 2000; Morales et al., 2000).

To optimise post-storage fruit quality, it is important to first maximise "orchard quality" (Crisosto et al., 1997). The canopy position in which fruit are borne has been found to affect "orchard quality" and consequently may affect the shelf-life of fruit. It has been reported that 'Nules Clementine' mandarin fruit originating from the inside of a tree's canopy have low carotenoid content at harvest and consequently are more susceptible to rind breakdown after storage than fruit originating from the outside of the tree canopy (Van Rensburg and Bruwer,

2000; Van Rensburg et al., 2004). In another fruit kind, Songold plums (*Prunus salicina*), shaded fruit from the bottom sector of the tree canopy were reported to develop significantly higher levels of the internal disorder, gel breakdown, after cold storage than exposed fruit from the top sector of the tree canopy (Taylor et al., 1993).

Studies conducted on apple (*Malus domestica* Borkh) fruit originating from a low light environment showed that this fruit had lower phenol content and lower activity of some antioxidant enzymes at harvest than fruit originating from a high light environment (Ju, 1998; Ma and Cheng, 2003). A low antioxidant activity at harvest, in apples, suggests that the fruit may not have the capacity to control the oxidation of α-farnesene and prevent the development of superficial scald in storage (Barden and Bramlage, 1994). Antioxidants have been reported to be involved in the development of rind staining of 'Navelina Navel' orange (C. *sinensis* L. Osbeck) (Sala and Lafuente, 2004), a rind disorder that is also associated with collapsed oil glands and therefore may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin.

The effect of canopy position on rind breakdown of 'Nules Clementine' mandarin fruit has been reported. However, the effect of canopy position on antioxidant capacity at harvest, in the rind of 'Nules Clementine' mandarin fruit has not been investigated. The objective of this study was to test the hypothesis that fruit borne on the inside of a tree's canopy have low carotenoid content and low antioxidant capacity, thereby possibly predisposing these fruit to greater susceptibility to rind breakdown.

Materials and methods

Plant material and sampling

This experiment was conducted on 'Nules Clementine' mandarin budded on Troyer citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliate* (L.) Raf.] rootstock over two seasons at two different sites. Site details are summarised in Table 4.1. At harvest, ~80 kg 'Nules Clementine' mandarin fruit originating from inside (shaded fruit) the tree's canopy, <1 m from the tree trunk, were harvested from ~20 trees in the orchard. From the same 20 trees, another ~80 kg of fruit originating from the periphery of the canopy (exposed fruit) were harvested at shoulder height from both the east and west side of the tree. Fruit were harvested into 15-kg plastic crates. Fruit harvested from Paarl was immediately transported to a

commercial packhouse in Simondium for degreening and packing, whereas, fruit harvested from Robertson were packed in a packhouse on the farm without degreening.

Fruit degreening and packing

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 $g \cdot L^{-1}$), Deccomone® (2.4 sodium salt) (5 $mL \cdot L^{-1}$) and Citricure® (guazatine) (2.5 $mL \cdot L^{-1}$). The fungicides were mixed in water with a wetting agent, Citowet®, added at a concentration of 0.1 $mL \cdot L^{-1}$. After drenching, the fruit was held at ambient temperature (~20 °C) for 8 to 12 hours before being moved to a degreening chamber. Degreening was conducted, for 5 days, using 1 to 2 ppm ethylene at 20 °C and at an RH of 90 to 95%. After degreening fruit were again held at ambient temperature (~20 °C) for 12 to 24 hours before packing.

During packing, fruit were moved through a warm water (~38 °C) bath containing Imazalil® (chloramizol) at 500 ppm. The fruit were then dried in a hot (~42 °C) air tunnel after which a light polyethylene wax (Decowax®) was applied. After waxing, the fruit were again dried in a hot (~48 °C) air tunnel and then sorted and packed into MO5I open display 5-kg plum cartons, containing ~50 fruit. A total of 14 cartons were packed from fruit sampled at each canopy position. After packing fruit were transported to Stellenbosch for storage.

Fruit storage

The 14 cartons of fruit from each canopy position were divided into two groups of seven cartons (replicates) each and stored at -0.5 °C or 7.5 °C in the 2004 season. The storage temperatures were modified in the 2005 season to only focus on rind breakdown therefore fruit were stored at 7.5 °C or 10 °C for 12 weeks. After initial cold storage, the fruit were subjected to a shelf-life period of 1 week at 20 °C before evaluations were conducted.

Data collection

Internal quality and rind variables at harvest

Rind colour was rated using 10 fruit from each of the seven replicates. The CRI rind colour chart set number 36 was used for the rating, where 8 = poorly coloured, dark green with no colour break and 1 = deep orange and fully coloured fruit (CRI, 2004; Appendix 1). Hue angle of the rind was measured on the same 10 fruit per replicate used for colour rating, using

a colorimeter (Minolta CR10, Japan), where 0° = red, 90° = yellow, and 180° = green (McGuire, 1992). Equatorial fruit diameter (mm) was measured on 10 fruit per replicate using a digital calliper (Mitutoyo CD-15c, Japan). Soluble solids content (°Brix) was determined on a pooled juice sample from 10 fruit per replicate using a digital refractometer (ATAGO DBX-55, Japan). Titratable acid content (%) was determined on a pooled juice sample from 10 fruit per replicate. 25 mL of juice was titrated with 0.1 N NaOH to an end-point of pH 8.2. The result was converted to citric acid equivalents by the equation: tiratable acid content = (mL NaOH/ 25 mL) x (0.1 N NaOH/0.1562). Juice content (% w/w) was determined from a sample of 10 fruit per replicate. Fresh mass (g) was determined by taking the average mass of 10 fruit per replicate. Mineral nutrient status and rind moisture content were determined by obtaining rind samples from the equatorial region of five fruit per replicate. Mineral analysis was conducted by BemLab in Somerset West, South Africa.

Selected biochemical rind properties determined at harvest

Antioxidant capacity of the flavedo was determined from five fruit per replicate at each canopy position using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Preparation of standard curve: A 2 mM solution of Trolox (6- 2,5,7,8- tetramethychroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical Co.) was prepared in methanol and used as an antioxidant standard. From the Trolox stock standard, a series of dilutions (in methanol) were prepared viz. 0, 0.2, 0.6, 1.0, 1.4, 1.8 and 2 mM.

ABTS (2,2'-azino-bis3-ethylbenz-thiazoline-6-sulfonic acid obtained from Sigma Aldrich Chemical Co). was dissolved in distilled deionized water to a concentration of 8 mM. The ABTS radical cation, $ABTS^{\bullet+}$, was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (~20 °C) for 12 hours. After this period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2.9 mL of the diluted ABTS radical cation to 0.1 mL of each of the serial Trolox dilutions. The mixture was shaken, allowed to react for 6 minutes and an absorbance reading measured at 734 nm using a spectrophotometer (Cary 50

conc UV-visible, Varian, Musgrave, Australia). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Rind sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The flavedo strips were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Before analysis, the frozen strips were ground to a fine powder in a coffee grinder (Molinix 648, France). Liquid nitrogen was added periodically to prevent the sample from thawing during grinding. A sub-sample of 0.2 g was extracted using 1% HCl in 95% methanol. The extraction of antioxidants, in this experiment, was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged (Sorvall RC-58 refrigerated centrifuge, Wilmington, USA) for 5 minutes at 121 *g* and 4.5 °C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded. To determine the antioxidant capacity, 0.1 mL of the antioxidant extract from the citrus rind was sampled and 2.9 mL of the ABTS radical cation was added to this sample. The mixture was allowed to react for 6 minutes, after which absorbance was measured at 734 nm.

Antioxidant capacity (mM Trolox equivalents·g^{-1}sample) was calculated as

(slope x $abs_{734\ nm}$ + C)/(g sample used in analysis)

Where: slope = slope of the standard curve

$abs_{734\ nm}$ = absorbance at 734 nm

C = y intercept on standard curve.

Rind pigments: Total chlorophyll and carotenoid contents were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Lichtenthaler (1987).

Rind sample preparation and pigment extraction: Rind samples were prepared into a fine powder as for antioxidant extraction. The fine powder was then freeze-dried and again stored at -80 °C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL

of cold 95% ethanol + butylated hydroxytoluene (BHT) (100 $mg \cdot L^{-1}$) + diethyldithiocarbamate (DDC) (200 $mg \cdot L^{-1}$). The sample was then vigorously stirred twice, for 1 minute in each case, on a vortex (G-560E, Bohemia, N.Y). Thereafter, the sample was placed in the dark at 4 °C and allowed to extract for 90 minutes. The sample was then filtered through an ashless filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649 and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and total carotenoids (C_{x+c}) were calculated using the following equations:

$$C_a = 13.36A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = 5.24A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000A_{470} - 2.13\,C_a - 97.64\,C_b}{209}$$

Internal quality and rind variables after cold storage plus a shelf-life

Rind colour, SSC, and titratable acid were again determined after each cold storage period on 10 fruit per replicate as previously described. Fruit was classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the spots regardless of size. Fruit was classified as having chilling injury when there was superficial brown discolouration on the rind (Murata, 1997). The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the blemishes regardless of size. Decay, green mould caused by *Penicillium digitatum*, incidence was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated juice vesicles resulting in decreased extractable juice (Murata, 1997).

Statistical analysis

The experiment was laid out as a completely randomised design. Data collected at harvest were analysed using a student t-test whereas data collected after cold storage and shelf-life were analysed using a two-way ANOVA on STATISTICA® (Tulsa, OK), a 2 x 2= 4 factorial treatment set was used with Factor A being the fruit canopy position and Factor B being the storage temperature. Treatment means were compared using the LSD method.

Results

Fruit characteristics at harvest

In fruit harvested from Paarl in the 2004 season the rind colour rating was similar for fruit sampled from the inside and outside of the tree (Table 4.2). However, colour measured with the colorimeter showed that the hue angle of the rind was significantly lower in fruit originating from the outside of the tree canopy than fruit sampled from the inside of the tree canopy. In the 2005 season, the rind colour rating was significantly lower in fruit sampled from the outside of the tree canopy than in fruit from the inside canopy position (Table 4.3). Internal quality measured in terms of SSC and titratable acid was similar between fruit originating from the different canopy positions in the 2004 season (Table 4.2). However, in the 2005 season, inside fruit had a significantly lower SSC and higher titratable acid content than outside fruit (Table 4.3). Fruit from the outside of a tree's canopy were significantly larger and heavier than fruit from the inside of the tree canopy (Table 4.2). There was no significant difference in juice content between fruit from the different canopy positions. Most of the mineral nutrients, in both fruit populations, were significantly higher in fruit originating from the inside of a tree's canopy than in fruit from the outside canopy position. Only the Ca and B contents in fruit were not significantly affected by canopy position. The rind moisture content and total chlorophylls in the rind were similar for fruit originating from different canopy positions. The total carotenoid content was higher in fruit from the outside of a tree's canopy than in fruit originating from the inside canopy position. The antioxidant capacity was also higher in outside fruit than in inside fruit, however, this trend was significant only in fruit sampled from Paarl in the 2004 season.

Fruit quality after cold storage plus shelf-life

Rind breakdown

In both seasons, the occurrence of rind breakdown was not significantly affected by fruit canopy position (Tables 4.4 and 4.5). Storage temperature significantly affected the occurrence of rind breakdown in the 2004 season, with this disorder only observed in fruit stored at 7.5 °C and not in fruit stored at -0.5 °C (Table 4.4). However, in the 2005 season, rind breakdown levels were similar in fruit stored at 7.5 °C and 10 °C (Table 4.5).

Chilling injury

Chilling injury was only recorded in the 2004 season and the occurrence of this disorder was not significantly affected by fruit canopy position (Table 4.4). Storage temperature significantly affected the occurrence of chilling injury, with this disorder only observed in fruit stored at -0.5 °C and not in fruit stored at 7.5 °C.

Decay

The occurrence of decay caused by *P. digitatum* (green mould) was not significantly influenced by canopy position or storage temperature in the 2004 season (Table 4.4). However, in the 2005 season fruit storage at 10 °C resulted in higher decay levels than storage at 7.5 °C (Table 4.5).

Internal quality

A significant interaction was observed between canopy position and storage temperature on the SSC and titratable acid content of fruit in the 2004 season (Table 4.4). At the storage temperature of -0.5 °C, inside fruit had lower SSC and titratable acid contents than outside fruit. However, in fruit stored at 7.5 °C the reverse occurred, inside fruit had a higher SSC and titratable acid content than outside fruit. In the 2005 season, inside fruit had a significantly lower SSC and higher titratable acidity than fruit harvested from the outside canopy position (Table 4.5). Fruit storage at 7.5 °C or at 10 °C did not significantly affect the internal quality of fruit.

Core drying

Core drying was only observed in the 2005 season where fruit sampled from the inside canopy position developed significantly higher levels of this disorder than fruit sampled from the outside position (Table 4.5). Storage temperature did not significantly affect the development of core drying.

Rind colour

Only storage temperature, in the 2004 season, was a significant factor on colour development of fruit from the different canopy positions (Table 4.4). Fruit stored at 7.5 °C had a lower hue angle and rind colour rating than fruit stored at -0.5°C. In the 2005 season, fruit sampled from the different canopy positions had similar rind colour ratings after storage at either 7.5 °C or 10 °C.

Discussion

The objective of this study was to establish whether 'Nules Clementine' mandarin fruit harvested from the inside or outside canopy positions of a tree showed differences in quality, e.g. rind colour, pigment concentration and antioxidant capacity, at harvest, and whether these differences could be used as indicators for fruit storage potential, in terms of rind breakdown development in particular. Fruit harvested from the different canopy positions exhibited different characteristics at harvest. The lower hue angle recorded and lower rind colour rating in fruit sampled from the outside canopy position than in fruit from the inside canopy position indicated that fruit borne on exposed canopy positions were more orange in colour. Similar colour development trends between citrus fruit harvested from shaded and exposed canopy positions have been reported (Morales et al., 2000).

In the present study, the reason for better colour in fruit sampled from the outside canopy position was attributed to higher carotenoids in fruit from this canopy position than fruit from the inside canopy position. Fruit sampled from the outside canopy position had 40% more total carotenoids than fruit sampled from the inside canopy position. The reason for differences in total carotenoids between the different canopy positions can be attributed to differences in canopy microclimate. The microclimate and direct light exposure of fruit in the periphery of the tree were favourable for carotenoid synthesis, and consequently the better colour developed by fruit sampled from this canopy position.

The higher antioxidant capacity observed in fruit sampled from the outside canopy position compared to fruit sampled from the inside canopy position may also be attributed to a favourable canopy microclimate in this sector of the tree canopy for the synthesis of antioxidants and also to fruit acclimation to light exposure. Plants or fruit acclimate to light exposure to meet the needs for dissipating excess absorbed photon flux density and to detoxify active oxygen species (Ma and Cheng, 2003). The microclimate at different canopy positions was not monitored in this study. However, the effects of citrus tree microclimate on fruit quality have been studied and are well documented (Syvertsen and Albrigo, 1980; Barry et al., 2000; Morales et al., 2000).

The tendency for higher SSC and lower titratable acidity in fruit from the outside canopy position has also been reported in Valencia sweet orange (Barry et al., 2000; Barry et al.,

2004). Other researchers reported a higher Brix/acid ratio in fruit originating from the outside canopy position (Syvertsen and Albrigo, 1980; Verreynne et al., 2004), which can also be attributed to a higher SSC or lower titratable acidity in this fruit.

The higher mineral nutrients recorded in fruit sampled from the inside canopy position compared to fruit sampled from the outside position may be due to vegetative shoot growth and active photosynthesis of leaves in the latter canopy position resulting in competition for nutrients between these shoots and fruit. Generally, the variation in mineral nutrient concentration between fruit sampled from the inside and outside canopy positions, reported in the present study concurs with earlier reports on different citrus cultivars (Kruger et al., 2005).

Evidently, fruit sampled from different canopy positions on the tree showed different characteristics at harvest. However, these differences did not necessarily lead to significant differences in fruit quality after storage. Hence, fruit canopy position did not significantly affect the development of rind breakdown and chilling injury, suggesting that rind breakdown and chilling injury are post storage disorders that develop independently of fruit canopy position. The findings of this study, on rind breakdown, do not support earlier reports where fruit from the inside canopy position, which had low carotenoids at harvest, were found to have higher levels of rind breakdown than fruit from the outside canopy position (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). The contradiction in results may be due to the fluctuation in rind breakdown incidence between seasons and the sporadic appearance of the disorder. The low levels of rind breakdown recorded in some seasons may confound treatment differences. In the current study only storage temperature significantly affected the occurrence of rind breakdown and chilling injury. The occurrence of rind breakdown at 7.5 °C and not at -0.5 °C indicated that a higher storage temperature accentuated rind breakdown than a lower storage temperature. In chapter 7 it was shown that 7.5°C was the storage temperature at which rind breakdown was most readily expressed. The similar levels of rind breakdown in fruit stored at 7.5 and 10 °C in the 2005 season indicated that in some fruit populations rind breakdown levels may be high even at 10 °C.

The influence of rind antioxidant capacity on the occurrence of rind breakdown and chilling injury could not be established in this study, as differences in the antioxidant capacity reported at harvest were not associated with the levels rind breakdown and chilling injury after storage. It is, therefore, apparent that the rind antioxidant capacity measured only at

harvest cannot be used as an indicator for 'Nules Clementine' mandarin fruit susceptibility to rind breakdown or chilling injury after storage. The reason for this is that oxidative homeostasis requires a balance between the production of ROS and the capacity to scavenge them (Mittler, 2000). Therefore, single point in time measurements do not give the full reflection of oxidative balance. Future experiments should be designed with multiple sampling times to reflect the dynamic nature of oxidative balance (Toivonen, 2004).

Decay susceptibility of fruit harvested from different canopy positions was similar, suggesting that natural fruit resistance to infection was not affected by canopy position. It has been reported that low storage temperature suppresses decay development (Eckert and Brown, 1986; Shellie and Skaria, 1998). This was confirmed in the decay results obtained in the 2005 season, where fruit stored at 10 °C developed higher decay levels than fruit stored at 7.5 °C, irrespective of canopy position. However, similar levels of decay were recorded at -0.5 °C and 7.5 °C in the 2004 season. The possible reason for this observation was that fruit stored at -0.5 °C developed chilling injury, making it possible for opportunistic infection to occur during the shelf-life period. Hence, decay levels were similar to those of fruit stored at 7.5 °C.

Fruit harvested from the inside canopy position had lower SSC and titratable acid when stored at -0.5°C than fruit harvested from the outside canopy position, but the reverse occurred in fruit stored at 7.5 °C. From this study the reason for this trend could not be fully established, therefore, additional work is required to establish the reason for this trend. In the 2005 season the trend observed at harvest between inside and outside fruit was maintained even after storage where inside fruit had a higher SSC and lower titratable acidity.

Previous work, in which core drying was reported, indicated that the disorder was a result of advanced maturity (Murata, 1997) and climate effect, particularly late summer rains (Grierson, 1986). However, our work has shown that fruit shading may enhance the expression of core drying.

Canopy position did not significantly influence colour development after storage. Even though fruit harvested from the inside canopy position, which was shaded, were greener with lower carotenoids at harvest than fruit sampled from the outside canopy position, inner fruit still had the potential to synthesize carotenoids when exposed to a favourable environment.

Ju (1998) showed that bagging apple fruit resulted in poor colour development and low anthocyanin content, but when fruit was exposed to light, anthocyanin accumulated very rapidly and fruit attained colour. This indicated that even though shaded fruit may have been poorly coloured (green) as a result of the shading, it had the potential to develop better colour (red) when exposed to the correct environmental conditions. This is the likely explanation for the findings on 'Nules Clementine' mandarin fruit.

Conclusions

Fruit harvested from the inside canopy position had a lower carotenoid content and a lower antioxidant capacity than fruit harvested from the periphery of the tree canopy. However, these characteristics did not accentuate rind breakdown and chilling injury after storage for 12 weeks at the various temperatures. Fruit exposure to light is important for acceptable quality at harvest in terms of rind colour, SSC and titratable acid. Therefore, techniques to ensure adequate light penetration in the tree are important for harvest quality, but not necessarily for reducing rind breakdown after storage. Postharvest fruit quality of 'Nules Clementine' mandarin can best be managed through the use of an optimum storage temperature and storage duration regime.

Table 4.1. Summary of trial sites, plant material and harvest dates.

	2004	2005
Site	**Paarl**	**Robertson**
Tree age	12 yrs	19 yrs
Ridging	Yes	No
Spacing (m)	5 x 3	5 x 2
Harvest date[1]	13 May	15 June
Degreening	Yes	No[2]

1 At each harvest date fruit were selectively harvested based on colour. Only fruit that had reached colour break or better were sampled.

2 Fruit from Robertson were harvested very late in the season and had attained good colour on the tree so did not require degreening.

Table 4.2. Characteristic at harvest of 'Nules Clementine' mandarin fruit sampled from the inside (shaded) and outside (non-shaded) canopy positions of trees in the Paarl area in 2004.

Response variable	**Canopy position**		**P-value**
	Inside fruit	**Outside fruit**	
Rind colour rating[1]	5.3	5.4	0.426
Hue angle of the rind	89.5	83.5	0.009
SSC (°Brix)	11.3	11.4	0.369
Titratable acid (%)	1.12	1.02	0.048
Equatorial diameter (mm)	59.9	63.7	0.009
Mass (g)	98.7	115.5	0.009
Juice content (%)	53.0	54.5	0.091
N (mg/100 g fresh mass)	272.8	238.8	0.141
P (mg/100 g fresh mass)	31.2	25.1	0.034
K (mg/100 g fresh mass)	338.6	285.2	0.043
Ca (mg/100 g fresh mass)	199.7	241.0	0.148
B ($mg \cdot kg^{-1}$ fresh mass)	7.9	7.6	0.506
Rind moisture content (%)	76.1	76.8	0.304
Antioxidant capacity (mM Trolox equivalents$\cdot g^{-1}$ FW)	5.4	6.3	0.001
Total chlorophylls ($\mu g \cdot g^{-1}$ DW)	1.1	1.4	0.616
Total carotenoids ($\mu g \cdot g^{-1}$ DW)	11.3	18.7	0.001

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 4.3. Characteristic at harvest of 'Nules Clementine' mandarin fruit sampled from the inside (shaded) and outside (non-shaded) canopy positions of trees in the Robertson area in 2005.

Response variable	Canopy position		P-value
	Inside fruit	Outside fruit	
Rind colour rating[1]	3.6	2.4	0.001
SSC (°Brix)	9.8	10.8	0.001
Titratable acid (%)	0.93	0.82	0.006
N (mg/100 g fresh mass)	285.6	250.0	0.021
P (mg/100 g fresh mass)	34.7	24.5	0.001
K (mg/100 g fresh mass)	345.0	246.6	0.001
Ca (mg/100 g fresh mass)	90.0	89.9	0.973
B ($mg \cdot kg^{-1}$ fresh mass)	11.1	10.3	0.159
Rind water content (%)	74.0	74.4	0.564
Antioxidant capacity (mM Trolox equivalents$\cdot g^{-1}$ FW)	1.4	2.0	0.255
Total chlorophylls ($\mu g \cdot g^{-1}$ DW)	0.9	0.0	0.062
Total carotenoids ($\mu g \cdot g^{-1}$ DW)	17.0	26.3	0.007

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 4.4. Quality of 'Nules Clementine' mandarin fruit sampled from inside (shaded) and outside (non-shaded) canopy positions in the Paarl area in 2004, and stored for 10 weeks at -0.5 °C or 7.5 °C plus one week at 20 °C.

Response variable	Storage temperature (°C)	Canopy position (Factor A)		Storage temperature (°C) (Factor B)		Prob>F[1]		
		Inside	Outside	-0.5	7.5	A	B	AxB
Rind breakdown (%)		3.2	2.0	0.0a[2]	5.2b	0.256	0.001	0.256
Chilling injury (%)		10.4	14.5	24.9b	0.0a	0.268	0.001	0.268
Decay (%)		12.9	12.7	14.3	11.4	0.945	0.317	0.388
SSC (°Brix)	-0.5	10.8a	11.9c			0.055	0.233	0.001
	7.5	11.4b	10.9a					
Titratable acid (%)	-0.5	0.62a	0.95c			0.038	0.014	0.001
	7.5	0.77b	0.60a					
Hue angle of the rind		59.4	59.6	64.5b	53.4a	0.261	0.001	0.422
Rind colour rating[3]		2.0	2.0	2.8a	1.1a	1.000	0.001	0.255

1 Two-way ANOVA with Factor A being canopy position and Factor B being storage temperature.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 4.5. Quality of 'Nules Clementine' mandarin fruit sampled from inside (shaded) and outside (non-shaded) canopy positions in the Robertson area in 2005, and stored for 10 weeks at -0.5°C or 7.5°C plus one week at 20°C.

Response variable	Canopy position	Canopy position (Factor A)		Storage temperature (°C) (Factor B)		P-value[1]		
		Inside	Outside	7.5	10	A	B	AxB
Rind breakdown (%)		10.5	12.6	9.8	13.4	0.479	0.219	0.926
Dry rind (%)		8.1	12.8	0.7a[2]	20.2b	0.171	0.001	0.215
Decay (%)		3.8	3.7	1.6a	5.9b	0.984	0.048	0.837
Puffiness (%)		0.5	0.5	0.7	0.3	0.987	0.494	0.362
SSC (°Brix)		10.1a	11.0b	10.5	10.5	0.001	0.949	0.412
Titratable acid (%)		0.46b	0.38a	0.43	0.40	0.014	0.265	0.438
Core drying (%)		37.5b	12.0a	26.0	23.5	0.003	0.737	0.737
Rind colour rating[3]		1.0	1.0	1.0	1.0	nd[4]	nd	nd

1 Two-way ANOVA with Factor A being canopy position and Factor B being storage temperature.
2 Values in the same row followed by different letters indicate significant differences ($P<0.05$) according to the LSD test.
3 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).
4 No statistical analyses done because all fruit were deep orange and fully coloured.

CHAPTER 5

THE EFFECT OF HARVEST DATE ON FRUIT CHARACTERISTICS OF 'NULES CLEMENTINE' MANDARIN (*CITRUS RETICULATA* BLANCO) AND POST STORAGE QUALITY, WITH SPECIAL REFERENCE TO RIND BREAKDOWN

Abstract

'Nules Clementine' mandarin (*Citrus reticulata* Blanco) develops various postharvest disorders during cold storage, including rind breakdown. Rind breakdown is thought to be influenced by, among other factors, harvest date. In this experiment 'Nules Clementine' mandarin fruit were harvested on three different dates during the commercial harvest window of the cultivar and stored at 7.5 °C for 10 weeks then subjected to a subsequent shelf-life period of 1 week at 20 °C before examinations were conducted. The experiment was conducted over three growing seasons, with a different production area selected per season. The fruit maturity at harvest, measured in terms of soluble solids content (°Brix) and titratable acid content, was similar in fruit harvested on different dates. However, rind colour slightly advanced to a yellow or more orange colour over the different harvest dates. Data recorded after cold storage plus shelf-life indicated no consistent trend of increasing or decreasing rind breakdown over the harvest period. A large variation in rind breakdown occurred among areas and seasons, making it difficult to conclude which population was more susceptible to rind breakdown. Attempts to correlate fruit characteristics measured at harvest to the incidence of rind breakdown after cold storage were unsuccessful. Therefore, at this stage the antioxidant capacity and rind pigments measured at harvest cannot be used as indicators for a fruit's susceptibility to post-storage rind breakdown. It was concluded that to reduce rind breakdown in 'Nules Clementine' mandarin, it was important to use optimum postharvest handling and storage techniques.

Introduction

'Nules Clementine' mandarin (*Citrus reticulata* Blanco*)*, hereafter referred to as 'Nules', develops various physiological disorders in cold storage. These include rind breakdown, chilling injury, puffiness and core drying (Murata, 1997). Rind breakdown is a physiological disorder of importance in 'Nules' fruit exported from South Africa because its occurrence over the past 12 years has caused severe losses to the citrus industry (Van Rensburg and Bruwer, 2000). Rind breakdown appears following leakage of oil from oil glands in the

flavedo. The oil leaks into and oxidizes the albedo. Oxidized tissue appears as brown spots on the flavedo (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Occurrence of this disorder has no effect on the internal quality of the fruit, but greatly reduces its market value due to deterioration of the rind.

Several factors have been reported to be associated with rind breakdown on 'Nules', among which is harvest date (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Harvest date is an important factor in 'Nules' because this cultivar has a protracted flowering period, which results in up to three fruit set periods (Saunt, 2000). This flowering pattern and subsequent fruit set results in an extended harvest period. Barry and Rabe (2004) mentioned that in the Western Cape province of South Africa, 'Nules' is harvested over a lengthy period, with the harvest window starting in May and extending into June.

As a result of the long harvest window of 'Nules', fruit quality from the early harvest may be different to those from the later harvest. Saunt (2000) reported that 'Nules' fruit from the first set are smaller and have a smooth rind, becoming larger and coarser with each subsequent fruit set. Other researchers working on different citrus cultivars reported that fruit harvested periodically showed physiological changes, over the harvest period, associated with maturity (Holland et al., 1999; Kato et al., 2004).

Harvest maturity of fruit influences storage life and post-storage quality (Reid, 1992). Van Rensburg et al. (2004) reported that 'Nules' fruit harvested late in the harvest window were more susceptible to rind breakdown than fruit harvested earlier, but there are no published data collaborating this finding.

Other rind disorders that may be morphologically similar to rind breakdown of 'Nules' and which affect other citrus types have also been reported. These occur at non-chilling temperatures, and they include rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Petracek et al., 1995), postharvest pitting of 'Temple' tangor (*C. reticulata* Blanco x *C. sinensis* L.) (Petracek et al., 1997) and 'Fallglo' mandarin (*C. reticulata* Blanco x *C. reticulata* Blanco x *C. paradisi* Macf.) (Petracek et al., 1998a), superficial flavedo necrosis (noxan) of 'Shamouti' orange (*C. sinensis* Osbeck) (Ben Yehoshua et al., 2001), and rind staining of 'Navelina' orange (Agusti et al., 2001; Alférez et al., 2003).

Several factors affecting the mentioned rind disorders have been investigated. However, little or no attention has been given to the effect of harvest date on their occurrence. Sala and Lafuente (2004) mentioned that more mature fruit had a lower activity of antioxidant enzymes and consequently more susceptible to develop rind damage than less mature fruit. Nevertheless, substantial literature exists suggesting that occurrence of these disorders may be due to changes in relative humidity (Lafuente and Sala, 2002; Alférez et al., 2003), modification of internal gas composition as influenced by waxing (Petracek et al. 1997; Petracek et al. 1998b), storage temperature and water stress (Ben Yehoshua et al., 2001). The influence of harvest date on the occurrence of rind breakdown is a factor that needs to be better explored.

Rind breakdown results in damage to plant tissue, and it is known that damage to plant tissue is associated with active oxygen species (Sala and Lafuente, 2004, and references therein). Plant cells are protected against the effect of active oxygen species by a complex antioxidant system (Sala, 1998). Researchers have investigated the role of antioxidants in the occurrence of rind disorders. Sala (1998) concluded that oxidative stress may be involved in cold-induced peel damage and that chilling tolerant mandarin cultivars had a more efficient antioxidant enzyme system. Sala and Lafuente (2004) concluded that antioxidants may be involved in the occurrence of rind staining in 'Navelina' orange. In apples (*Malus domestica* Borkh) late harvested fruit were found to be resistant to scald and also contained higher levels of antioxidants compared to early harvested fruit (Vasilakasis and Manseka, 1995).

The main objective of this study was to elucidate the effect of harvest date on rind breakdown of 'Nules'. As a secondary objective, differences in rind pigments and antioxidant capacities at harvest will be measured to determine whether these biochemical properties were associated with the development of rind breakdown after storage.

Materials and methods

Plant material and Sampling detail

This experiment was conducted on 'Nules Clementine' mandarin budded on Troyer citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] rootstock over three seasons at three different sites. Site details and treatments are summarised in Table 5.1. In all seasons, fruit were harvested on three dates representing early, mid and late harvests within the commercial harvest window.

Fruit degreening

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 g·L^{-1}), Deccomone® (2.4-D sodium salt) (5 mL·L^{-1}), Citricure® (guazatine) (2.5 mL·L^{-1}) and Sporekill® (dimethyldidecyl ammonium chloride) (1.5 mL·L^{-1}). The fungicides were mixed in water with a wetting agent, Bladbuff®, added at a concentration of 0.3 mL·L^{-1}. After drenching, the fruit were held at ambient temperature (~20 °C) for 24 hours, before being moved to a degreening chamber. Degreening was conducted using ~3 ppm ethylene at 20 °C and at 90% RH. In the 2002 season, fruit harvested in weeks 17 and 18 were degreened for 72 hours, and fruit harvested in week 20 were degreened for 24 hours. In the 2003 season, fruit harvested in weeks 18 and 23 were degreened for 72 hours and fruit harvested in week 22 were degreened for 60 hours. Fruit from Robertson in 2004 did not require degreening. After degreening fruit were held at ambient temperature (~20 °C) for 12 to 24 hours before packing.

Fruit packing

Fruit from Saron (2002) and Franschhoek (2003) were packed at a pack house in Franschhoek, and fruit from Robertson (2004) were packed in a packhouse on the farm where the fruit was sampled. During packing fruit were moved through a warm water (~40 °C) bath containing Sanazil® (chloramizol) at 0.67 g·L^{-1}. The fruit were then dried in a hot (~42 °C) tunnel, after which a light polyethylene wax (Decowax®) was applied. After waxing, the fruit were again dried in a hot (~52 °C) air tunnel and then sorted and packed into fruit cartons. Fruit from Saron were packed in open display 600 x 400 mm 15 kg cartons containing ~150 fruit per carton, whereas fruit sampled from Franschhoek (2003) and Robertson (2004) were packed into MO5I open display 5 kg plum cartons containing ~50 fruit per carton.

Fruit storage

Fruit from each harvest date were stored at 7.5 °C for 10 weeks. After this initial cold storage period, a shelf-life period of 1 week at 20 °C was used before evaluations were conducted.

Data collection

Fruit and rind variables measured at harvest

Rind colour of 30 fruit was rated using the CRI rind colour chart set number 36; where 8 = green immature fruit with no colour break, 1 = deep orange and fully coloured fruit (CRI, 2004). Hue angle of the rind was measured on 30 fruit from each harvest date using a colourimeter (Minolta CR10, Japan); where 0° = red, 90° = yellow, 180° = green (McGuire, 1992). Equatorial fruit diameter (mm) of 30 fruit from each harvest date was measured using a digital caliper (Mitutoyo CD-15c, Japan). Juice content (% w/w) was determined from a sample of 30 fruit. Soluble solids content (SSC) (°Brix) was determined from a pooled juice sample from 30 fruit at each harvest date using a digital refractometer (ATAGO DBX-55, Japan). Titratable acid content (%) was determined using a pooled juice sample from 30 fruit at each harvest date by titrating 25 mL of juice with 0.1 N sodium hydroxide to an end-point of pH 8.2. The result was converted to citric acid by the equation: titratable acid content = (mL NaOH/25 mL) x (0.1 N NaOH/0.1562). This sample was considered sufficient to indicate maturity of the fruit at harvest. Rind strength, of fruit from the 2003 season, was measured on five fruit per replicate using a texture analyzer (TA-XT2, Stable Micro Systems Ltd., England). The rind of the fruit was pierced with a 2 mm-thick tip and the force required to penetrate the rind was recorded. Rind moisture of fruit from the 2003 season was determined by removing ten fresh rind discs from the five fruit per replicate (two discs per fruit) using a size 12 cork borer. These discs were weighed fresh then oven-dried at 70 °C for 72 hours and re-weighed after drying. The difference between the fresh weight and dry weight expressed as a percentage indicated the rind moisture content. Mineral nutrient status of fruit from Franschhock in the 2003 season was determined by obtaining rind samples from the equatorial region of five fruit per replicate. Mineral analysis was conducted by BemLab in Somerset West, South Africa.

Selected biochemical rind properties determined at harvest

Antioxidant capacity of the flavedo was determined from five fruit per replicate at each harvest date using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Preparation of standard curve: A 2 mM solution of Trolox (6- 2,5,7,8- tetramethychroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical Co.) was prepared in methanol and

used as an antioxidant standard. From the Trolox stock standard, a series of dilutions (in methanol) were prepared viz 0, 0.2, 0.6, 1.0, 1.4, 1.8 and 2 mM.

ABTS (2,2'-azino-bis3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich Chemical Co. was dissolved in distilled deionized water to a concentration of 8 mM. The ABTS radical cation, $ABTS^{\bullet+}$, was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (~20 °C) for 12 hours. After this period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2.9 mL of the diluted ABTS radical cation to 0.1 mL of each of the serial Trolox dilutions. The mixture was shaken, allowed to react for 6 minutes and an absorbance reading measured at 734 nm using a spectrophotometer (Cary 50 conc UV-visible, Varian, Musgrave, Australia). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Rind sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The flavedo strips were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Before analysis, the frozen strips were ground to a fine powder in a coffee grinder (Molinix 648, France). Liquid nitrogen was added periodically to prevent the sample from thawing during grinding. A sub-sample of 0.2 g was extracted using 1% HCl in 95% methanol. The extraction of antioxidants, in this experiment, was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged (Sorvall RC-58 refrigerated centrifuge, Wilmington, USA) for 5 minutes at 121 *g* and 4.5 °C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 0.1 mL of the antioxidant extract from the citrus rind was sampled and 2.9 mL of the ABTS radical cation was added to this sample. The mixture was allowed to react for 6 minutes, after which absorbance was measured at 734 nm.

Antioxidant capacity (mM Trolox equivalents·g^{-1}sample) was calculated as

(slope x $abs_{734\ nm}$ + C)/(g sample used in analysis)

Where: slope = slope of the standard curve

$abs_{734\ nm}$ = absorbance at 734 nm

C = y intercept on standard curve.

Rind pigments: Total chlorophyll and carotenoid contents were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Lichtenthaler (1987).

Rind sample preparation and pigment extraction: Rind samples were prepared into a fine powder as for antioxidant extraction. The fine powder was then freeze-dried and again stored at -80 °C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL of cold 95% ethanol + butylated hydroxytoluene (BHT) (100 mg·L^{-1}) + diethyldithiocarbamate (DDC) (200 mg·L^{-1}). The sample was then vigorously stirred twice, for 1 minute in each case, on a vortex (G-560E, Bohemia, N.Y). Thereafter, the sample was placed in the dark at 4 °C and allowed to extract for 90 minutes. The sample was then filtered through an ashless filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649 and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and total carotenoids (C_{x+c}) were calculated using the following equations:

$$C_a = 13.36A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = 5.24A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000A_{470} - 2.13\,C_a - 97.64\,C_b}{209}$$

Fruit and rind variables measured after cold storage plus a shelf-life

Rind colour, soluble solids content, and titratable acid content were again determined after cold storage, as previously described. Rind pigments and the antioxidant capacity of fruit that developed rind breakdown after storage and fruit that did not develop the disorder were determined in 2004 using fruit from the Robertson area. These biochemical rind properties were determined as previously described from a rind sample of five fruit with rind breakdown

and five fruit without rind breakdown per replicate. Fruit was classified as having rind breakdown when discoloured spots were seen on the rind (Fig. 1). The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the spots regardless of size. Fruit was classified as puffy when there was disintegration of the albedo resulting in separation of the flavedo from the pulp (Kuraoka et al., 1977). The disorder was measured using 10 fruit per replicate. Decay incidence, green and blue mould caused by *Penicillium digitatum* and *P. italicum* respectively, was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated juice vesicles resulting in decreased extractable juice (Murata, 1997).

Statistical design and analysis

The experiment was laid out as a completely randomised design. Data collected at harvest as well as after cold storage and after shelf-life were analysed using a one-way ANOVA on STATISTICA® (Tulsa, OK). Treatment means were compared using the LSD method. Rind pigments and antioxidant capacity data collected from fruit with and without rind breakdown were analysed as a split-plot design, with harvest date being the main-plot factor and the presence of rind breakdown on the fruit being the sub-plot factor, using ANOVA on STATISTICA®, and treatment means compared using the LSD method.

Results

Fruit characteristics at harvest

Maturity variables and fruit characteristics

Rind colour generally improved with later sampling as colour rating and hue angle decreased over time (Table 5.2, 5.3, and 5.4). Although fruit were sampled on different dates over three seasons, soluble solids, titratable acid and juice content did not consistently change over time. However, the juice content was relatively higher (61.3%, 54.3%, and 52.6%) for early harvested fruit than late harvested fruit (54.9%, 53.2% and 48.7%) [Table 5.2, 5.3, and 5.4]. Fruit of similar commercial size class [caliber 1 (65-68 mm) and 2 (59-64 mm)] were sampled from each area in each season. Fruit fresh mass generally varied according to fruit diameter.

Mineral nutrient status, selected biochemical variables and rind strength

The concentration of most mineral nutrients (N, P, Ca and B) was significantly higher in early harvested fruit than fruit from the later harvest times (Table 5.5). However, K was lower in the middle harvest but showed no significant difference between the first and the last dates. The antioxidant capacity and the total carotenoids did not show a significant change (P>0.05) over the harvest dates, although antioxidant capacity was lower for the last harvest date (P = 0.065). However, the total chlorophylls decreased significantly with harvest date as expected. Rind moisture content showed a slight increase over the harvest dates, with significantly higher rind moisture content recorded in fruit harvested in June than in fruit from the earlier harvests. Rind strength showed no significant change over the harvest dates.

Fruit quality after cold storage and shelf-life

Rind colour

The rind of early harvested fruit from Saron (2002) and Franschhoek (2003) had significantly higher colour ratings and hue angles compared with fruit harvested later from the same areas (Tables 5.6 and 5.7). However, in fruit from Robertson which were harvested late in the season and were at full colour development, the rind colour rating was lower in early harvested fruit compared to fruit harvested later (Table 5.8), but the hue angle of the rind was not significantly affected by harvest date.

Internal quality

The soluble solids content measured after cold storage plus shelf-life tended to be lower in early harvested fruit than in fruit harvested later (Tables 5.6, 5.7 and 5.8). This observation was significant only in fruit harvested in Franschhoek. The titratable acid content showed little change over harvest time in fruit from Saron (2002) (Table 5.6). However, titratable acidity was lower (P=0.051 and P=0.001) in late harvested fruit than for early harvested fruit in 2003 (Table 5.7) and 2004 (Table 5.8), respectively. Core drying, a disorder that was only observed in the 2003 season (Table 5.7), was higher (p=0.055) in late harvested fruit compared to fruit harvested earlier.

Rind breakdown

Harvest date did not significantly affect the occurrence of rind breakdown in fruit harvested from Saron in 2002 (Table 5.6). However, there were indications that early harvested fruit could develop higher levels of the disorder than fruit harvested later (P=0.188). In

Franschhoek in 2003, early harvested fruit developed higher levels of rind breakdown than fruit harvested later (Table 5.7). A different and opposite trend was observed in fruit harvested from Robertson in 2004 (Table 5.8), where the incidence of rind breakdown was significantly higher for late harvested fruit.

Decay and puffiness

Harvest date did not have a significant influence on decay development on fruit from all the areas in which the trial was conducted (Table 5.6, 5.7 and 5.8). Puffiness was only observed in fruit harvested from Franschhoek in 2003 (Table 5.7). This disorder was significantly lower in early harvested fruit than in later harvested.

Rind moisture

Rind moisture content measured in fruit after cold storage plus shelf-life was not significantly influenced by harvest date in fruit from Franschhoek (2003) and Robertson (2004) (Tables 5.7 and 5.8).

Rind pigments and antioxidant capacity of fruit from Robertson with and without rind breakdown

In 2004, total chlorophylls were not significantly influenced by harvest date nor were they influenced significantly by the occurrence of rind breakdown on fruit (Table 5.9). Harvest date did not significantly influence the carotenoid content of fruit. However, fruit with rind breakdown had lower total carotenoid content than fruit without the disorder. Late harvested fruit had a higher antioxidant capacity than fruit harvested earlier, and fruit with rind breakdown had a lower antioxidant capacity than fruit without rind breakdown.

Discussion

Fruit harvested at different times during the harvest window of 'Nules' generally exhibited similar physiological characteristics at harvest, with only rind colour tending to advance over harvest time. This trend was consistent for all the areas, suggesting that fruit harvested on the different dates during the harvest window of 'Nules' could have been of similar physiological maturities measured in terms of soluble solids and titratable acid content. This result is not necessarily unexpected with 'Nules' because the cultivar has a protracted flowering period resulting in three fruit set periods (Saunt, 2000). In this study commercial harvests were conducted based on rind colour, and only fruit that had reached colour break or beyond were

harvested at each harvest date. Greener fruit were allowed to remain on the tree and were harvested when the rind colour was suitable. It cannot be confirmed in this study, but remains possible, that fruit harvested on the different dates could have been from different set periods. As a result the fruit matured at different times, which could explain the small change in physiological maturity over time. Other researchers harvesting citrus at different times reported differences in fruit physiological maturity which were expressed as rind colour changes from green to yellow (Holland et al., 1999; Kato et al., 2004), increases in total soluble solids and a reduction in acidity (Holland et al., 1999).

Although fruit showed little difference in harvest maturity, detailed investigation into mineral nutrient status and selected biochemical properties of the rinds of fruit from the three harvest dates conducted in Franschhoek showed greater differences between the fruit (Table 5.5). The observed decrease in the concentration of mineral nutrients in the rind of early harvested 'Nules' compared to fruit harvested later concurs with findings earlier reported for 'Navel' orange (Storey and Treeby, 2000). The total carotenoid content did not show a definite trend of increasing or decreasing over the harvest time, which could be related to the fruit showing no particular trend in physiological maturity, as indicated by soluble solids and titratable acid content. Researchers who have shown differences in physiological fruit maturity over harvest time also reported increases in the carotenoid content of citrus fruit (Kato et al., 2004) and antioxidant capacity of apple peel (Barden and Bramlage, 1994; Vasilakakis and Manseka, 1995).

Our work showed that the antioxidant capacity of the citrus rind decreased (P=0.055) in late harvested fruit from Franschhoek (Table 5.5). Results reported by Nagy (1980) showed that vitamin C, which also has antioxidant properties, decreased in the juice of citrus fruit during ripening. However, rind antioxidant capacity measured after cold storage, in fruit sampled from Robertson (Table 5.9) showed a trend opposite to that reported in fruit from Franschhoek at harvest. The rind antioxidant capacity of fruit sampled from Robertson was higher in late harvested fruit compared to fruit harvested earlier. The reason for this contradiction cannot be fully established in this study, since the antioxidant capacity of Robertson fruit from each harvest was not measured at harvest or during storage. It was therefore, a limitation of this study to do single point in time measurements of antioxidant capacity, because oxidative homeostasis requires a balance between the production of free radicals and the ability of the cell to remove them (Mittler, 2000). Antioxidant capacity

measurements taken only before or after a particular treatment do not reflect this balance and therefore may be misleading. Future experiments should, therefore, include multiple sampling to reflect the change in antioxidant capacity. The total chlorophyll content decreased significantly over the harvest times, which may have been the reason for the advancement in rind colour over time. This result was not necessarily unexpected since colour change, from green to orange during fruit maturation is associated with a decrease in chlorophyll content and an increase in carotenoids (Spiegel-Roy and Goldschmidt, 1996). In our work the chlorophylls decreased but the carotenoids did not necessarily increase, and this pigment response was sufficient for colour change to be detected with the colorimeter.

From the data reported after cold storage and shelf-life, development of rind breakdown on 'Nules' did not show a consistent trend of increasing or decreasing over harvest date. Two of the areas (Saron and Franschhoek) investigated in our study showed higher rind breakdown levels in early harvested fruit than in fruit harvested later. However, fruit harvested very late in the season (9 July 2004) from Robertson had a high rind breakdown incidence compared to fruit harvested earlier. Furthermore, the incidence of rind breakdown varied greatly among the different areas, with fruit from Saron being more prone to the disorder than fruit from Franschhoek and Robertson. This difference, between areas, in fruit susceptibility to rind breakdown may indicate the role of climatic or cultural practices in the development of rind breakdown. It has been reported that fruit maturing during a warm autumn is more susceptible to rind breakdown (Van Rensburg and Burwer, 2000; Van Rensburg et at., 2004). Due to the lack of consistency in rind breakdown incidence over time and the sporadic occurrence of the disorder, overall findings of this study do not support earlier reports by Van Rensburg et al. (2004) where it was concluded that late harvested 'Nules' developed more rind breakdown than fruit harvested earlier.

In the present study investigation was extended to look at rind moisture and its possible role on the occurrence of rind breakdown, since other rind disorders have been linked to moisture stress across the rind (Ben Yehoshua et al., 2001; Lafuente and Sala, 2002). It appeared as if rind moisture was not the cause for rind breakdown, since fruit harvested on different dates had similar rind moisture contents after storage but exhibited different levels of rind breakdown.

This research also attempted to correlate some of the variables measured at harvest with rind breakdown incidence recorded at the end of shelf-life. All the variables tested were non-significant and had low correlation coefficients (data not shown). This suggests that none of the variables measured at harvest could be used as an indicator of a fruit's susceptibility to rind breakdown after cold storage plus shelf-life.

The involvement of rind pigments in the occurrence of rind breakdown should not, however, be underestimated as 'Nules' fruit that developed rind breakdown after storage had 29% lower total carotenoid content compared to 'Nules' fruit that did not develop rind breakdown (Table 4.9). The difference in total carotenoid content between fruit with and without rind breakdown, reported in this study, does not show whether the lower carotenoid content observed in fruit with the disorder was the cause of rind breakdown or a consequence thereof. However, due to the fact that plastids, containing pigments, in the citrus rind are confined to the single epidermal layer whereas the oil glands are imbedded in the compactly arranged colourless parenchyma cells (Spiegel Roy and Goldschmidt, 1996), it would seem a direct relation between rind pigments and the occurrence of rind breakdown is unlikely. Therefore, low carotenoid content measured in fruit with rind breakdown may not be the cause of the disorder. The antioxidant capacity of fruit with rind breakdown was 12.2% lower than in fruit without the disorder. This difference may seem small but was statistically significant. Again, these data do not show whether the lower antioxidant capacity reported in fruit with rind breakdown caused the disorder or if the occurrence of rind breakdown lowered the antioxidant capacity. Furthermore, since fruit harvested late from Robertson had a higher antioxidant capacity after storage yet developed higher levels of rind breakdown than fruit harvested earlier, it would seem the antioxidants measured may not play a direct role in the development of rind breakdown. No previous research illustrating the role of antioxidants and rind pigments on the occurrence of rind breakdown in 'Nules' could be found. However, work conducted on rind staining of 'Navelina' oranges, a rind disorder associated with collapsed oil glands and therefore may be morphologically similar to rind breakdown of 'Nules', led to the conclusion that antioxidants may play a role in the occurrence of the disorder (Sala and Lafuente, 2004).

Puffiness and core drying are disorders that are largely attributed to advanced maturity among other contributing factors (Murata, 1997). In our research, puffiness was lower in early harvested fruit, but this observation may not be because later harvested fruit were more

mature since it was shown earlier that physiological fruit maturity did not show a change over harvest time. Core drying was not significantly affected by harvest date, but the levels of this disorder were high in early and late harvested fruit. These response variables, puffiness and core drying, appear not to be related to rind breakdown.

Conclusions

Rind breakdown incidence recorded on 'Nules' fruit after cold storage plus shelf-life did not show a consistent trend of increasing or decreasing with harvest date for fruit with similar physiological maturities. This rind breakdown occurrence pattern suggested that harvest date, not linked to fruit physiological maturity, cannot be used reliably to indicate susceptibility to rind breakdown. Differences in the rind breakdown incidence between areas may indicate that climatic factors play a role in the development of the disorder. The role of antioxidants and rind pigments in the development of rind breakdown could not be clarified from this study. Differences in the total carotenoid content and the antioxidant capacity between fruit with and without rind breakdown did not indicate whether low carotenoid content and antioxidant capacity caused rind breakdown, or if these differences were the result of rind breakdown manifestation. However, it was shown that the antioxidant capacity and rind pigments measured only at harvest cannot be used as indicators of a fruit's potential to develop rind breakdown in storage. At this stage it would seem that management of rind breakdown can best be achieved by application of optimal postharvest handling and storage techniques for 'Nules' harvested at different times. The research conducted has shown the influence of harvest date on the development of rind breakdown without addressing the effect of fruit physiological maturity on the occurrence of the disorder, as fruit harvested on the different dates had similar physiological characteristics at harvest. Therefore, the need for a study specifically addressing this phenomenon is essential.

Fig. 1. Rind breakdown in ‘Nules Clementine’ mandarin.

Table 5.1. Summary of trial sites, plant material and harvest dates.

	2002	**2003**	**2004**
Site	**Saron**	**Franschhoek**	**Robertson**
Tree age	14 yrs	10 yrs	19 yrs
Ridging	Yes	Yes	No
Spacing (m)	5 x 3	5 x 3	5 x 2
Harvest date[1]	24 April	1 May	15 June
	3 May	30 May	22 June
	20 May	6 June	9 July
Degreening[2]	Yes	Yes	No[3]

1 At each harvest date fruit were selectively harvested based on colour. Only fruit that had reached colour break or better were sampled.
2 The degreening process followed is explained in the text.
3 Fruit from Robertson were harvested very late in the season and had attained good colour on the tree so did not require degreening.

Table 5.2. Characteristics of 'Nules Clementine' mandarin fruit harvested in Saron on different dates, in the 2002 season.

Variable	Harvest date		
	24 April	**3 May**	**20 May**
Rind colour rating[1]	6.1	5.9	5.1
Hue angle of rind	101.6	95.2	90.8
SSC (°Brix)	9.3	9.3	9.8
Titratable acid (%)	0.98	0.94	0.92
Juice content (%)	61.3	54.4	54.9
Fresh mass (g)	155.4	146.0	117.6
Equatorial diameter (mm)	68.5	68.6	63.4

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.

Table 5.3. Characteristics of 'Nules Clementine' mandarin fruit harvested in Franschhoek on different dates, in the 2003 season.

Variable	Harvest date		
	1 May	**30 May**	**6 June**
Rind colour rating[1]	5.1	4.9	5.1
Hue angle of rind	77.0	78.0	68.3
SSC (°Brix)	10.1	10.7	9.8
Titratable acid (%)	0.83	0.84	0.74
Juice content (%)	54.3	56.0	53.2
Fresh mass (g)	105.1	108.4	119.7
Equatorial diameter (mm)	63.3	64.3	67.9

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.

Table 5.4. Characteristics of 'Nules Clementine' mandarin fruit harvested in Robertson on different dates, in the 2004 season.

Variable	Harvest date		
	15 June	**22 June**	**9 July**
Rind colour rating[1]	2.9	1.7	1.9
SSC (°Brix)	12.0	13.2	12.0
Titratable acid (%)	0.84	0.81	0.81
Juice content (%)	52.6	53.5	48.7
Fresh mass (g)	98.8	107.5	100.3
Equatorial diameter (mm)	61.4	62.7	60.5

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.

Table 5.5. Mineral nutrient status and selected biochemical and physiological variables in the rind of 'Nules Clementine' mandarin harvested in Franschhoek on different dates, in the 2003 season.

Variable	Harvest date			P-value
	1 May	30 May	6 June	
N (mg·100 g^{-1} fresh mass)	284.0b[1]	233.6a	238.0a	0.001
P (mg·100 g^{-1} fresh mass)	24.0b	18.5a	19.5a	0.004
K (mg·100 g^{-1} fresh mass)	299.0b	218.8a	271.4b	0.001
Ca (mg·100 g^{-1} fresh mass)	176.6c	134.4b	101.2a	0.001
B (mg·kg^{-1} fresh mass)	7.8b	7.2b	5.5a	0.002
Antioxidant capacity (mM Trolox equivalents·g^{-1} sample)	4.8	4.9	4.2	0.065
Total chlorophylls (µg·g^{-1} dry weight)	15.1b	10.8b	5.0a	0.002
Total carotenoids (µg·g^{-1} dry weight)	14.2	17.1	13.5	0.127
Rind moisture content (%)	73.0a	74.2a	76.9b	0.001
Rind strength (N)	10.7	10.9	9.7	0.141

1 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 5.6. Quality variables of 'Nules Clementine' mandarin fruit harvested from Saron area, on different dates and cold stored at 7.5 °C for 10 weeks followed by 1 week at 20 °C in the 2002 season.

Response variable	Harvest date			P-value
	24 April	3 May	20 May	
Rind colour rating[1]	1.5b[2]	1.0a	1.0a	0.001
Hue angle of rind	63.2b	58.3a	56.0a	0.001
SSC (°Brix)	9.2	11.8	10.7	nd[3]
Titratable acid (%)	0.60	0.58	0.59	nd
Rind breakdown (%)	64.7	43.8	47.2	0.188
Decay (%)	0.7	1.0	2.5	0.340

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Statistics not done because an unreplicated pooled juice sample from 20 fruit was used per treatment.

Table 5.7. Quality variables of 'Nules Clementine' mandarin fruit harvested from Franschhoek area on different dates and cold stored at 7.5 °C for 10 weeks followed by 1 week at 20 °C in the 2003 season.

Response variable	Harvest date			P-value
	1 May	30 May	6 June	
Rind colour rating[1]	2.2b[2]	1.0a	1.1a	0.002
Hue angle of rind	61.6b	54.1a	52.6a	0.001
SSC (°Brix)	9.3a	10.2b	10.6b	0.001
Titratable acid (%)	0.52	0.44	0.44	0.051
Core drying (%)	8.0	0.0	24.0	0.055
Rind breakdown (%)	11.1b	4.0a	0.3a	0.001
Decay (%)	5.1	4.2	4.2	0.837
Puffiness (%)	2.9a	12.8b	19.4b	0.001
Rind moisture (%)	72.4	72.4	71.9	0.862

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 5.8. Quality variables of 'Nules Clementine' mandarin fruit harvested from Robertson area on different dates and cold stored at 7.5 °C for 10 weeks followed by 1 week at 20 °C in the 2004 season.

Response variable	Harvest date			P-value
	15 June	22 June	9 July	
Rind colour rating [1]	1.0a[2]	1.4b	1.4b	0.001
Hue angle of rind	54.6	53.9	53.6	0.361
SSC (°Brix)	11.8	12.5	12.0	0.223
Titratable acid (%)	0.65b	0.59b	0.39a	0.001
Rind breakdown (%)	7.2a	8.2a	38.2b	0.001
Decay (%)	4.4	4.1	8.5	0.180
Rind moisture (%)	69.1	68.9	69.5	0.878

1 Rind colour rating determined using the Outspan rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

Table 5.9. Rind pigments and antioxidant capacity of fruit with and without rind breakdown harvested on different dates from Robertson in 2004 and cold stored for 10 weeks at 7.5 °C plus addition 1 week at 20 °C.

	Total chlorophylls chlorophyll (μg·g^{-1} dry weight)	**Total carotenoids chlorophyll (μg·g^{-1} dry weight)**	**Antioxidant capacity (mM Trolox equivalents·g^{-1} sample)**
Harvest date (Factor A)			
15 June	15.0	56.0	4.8a[1]
22 June	14.9	55.3	5.0a
9 July	14.7	49.6	6.2b
Rind breakdown (Factor B)			
With RB	15.1	44.5a	5.0a
Without RB	14.7	62.7b	5.7b
P-values			
A	0.843	0.350	0.002
B	0.214	0.001	0.003
AxB	0.092	0.838	0.981

1 Values in the same column followed by different letters indicate significant differences (P<0.05) according to the LSD test.

CHAPTER 6

EFFECT OF ETHYLENE GAS DEGREENING ON THE POST STORAGE QUALITY OF 'NULES CLEMENTINE' MANDARIN (*CITRUS RETICULATA* BLANCO), WITH SPECIAL REFERENCE TO RIND BREAKDOWN AND CHILLING INJURY.

Abstract

'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit were harvested at optimal maturity, with a citric acid level of <1.0%, and then subjected to various degreening durations of, 0 hours, 48 hours and 72 hours, before 10 weeks storage at -0.5 °C or 7.5 °C. Ten days after harvest, when all the degreening treatments were completed and fruit stored at the respective temperatures for a short duration, an evaluation was conducted. It was established that degreening had a marginal effect on internal quality but greatly reduced chlorophylls and increased the total carotenoid content in the citrus rind. Degreening did not have a significant effect on the antioxidant capacity of 'Nules Clementine' mandarin. Results obtained after storage plus shelf-life showed that degreening did not have a significant effect on the occurrence of rind breakdown. Temperature was identified as the largest contributor to the occurrence of rind breakdown. This disorder was prevalent at 7.5 °C and not at -0.5 °C. The other rind disorder of interest was chilling injury, and this disorder only developed on fruit stored at -0.5 °C. Furthermore, fruit degreened for 72 hours tended to develop higher levels of chilling injury than non-degreened fruit or fruit degreened for a shorter duration. This observation was not statistically significant in the 2002 season, whereas, in the 2003 season, the differences between degreening treatments were significant but the trend was not very distinct. Attempts to correlate rind pigments or the antioxidant capacity to rind breakdown after storage were unsuccessful, suggesting that these variables measured only at harvest or soon thereafter cannot be used as indicators for fruit's susceptibility to rind breakdown. It was concluded that ethylene degreening is not the main cause for rind breakdown, and that from a post harvest point-of-view, storage temperature is the largest contributor to the development of rind breakdown. It is therefore recommended that more stringent temperature management practises be adopted, and that a storage temperature of 7.5 °C should be avoided.

Introduction

Rind breakdown on 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) is a postharvest physiological disorder that has caused severe financial loss to the South African citrus industry (Van Rensburg and Bruwer, 2000). Rind breakdown appears following leakage of oil from oil glands in the flavedo. Oil leaks into and oxidises the albedo resulting in discoloured, brown spots on the flavedo (Van Rensburg et al., 2004). The occurrence of this disorder may be influenced by various factors among which is ethylene gas degreening (Van Rensburg et al., 2004).

Citrus is non-climacteric so produces relatively low levels of ethylene during maturation and senescence (Kader, 1992). Although endogenous levels of ethylene are low, citrus fruit are known to respond to exogenously applied ethylene (Cohen, 1978; Porat et al., 1999). The exogenous application of ethylene is a common practise in early-maturing citrus cultivars, which usually meet commercial internal maturity before their rinds attain the desired colour for harvest. Ethylene is then used to enhance rind degreening, to make the fruit more appealing to the consumer (Cohen, 1978; Yamauchi at al., 1997; Porat et al., 1999). The degreening process of citrus involves postharvest application of ethylene, to fruit, at a specific concentration, air temperature and RH (Saltveit, 1999). It has been reported that the observed improvement in rind colour of citrus from green to yellow following exposure to ethylene is largely due to accelerated breakdown of chlorophyll (Mizuntani et al., 1992; Yamauchi et al., 1997). Goldschmidt et al. (1993) showed that during the degreening process there is promotion of carotenoid biosynthesis, which also contributes to the improvement of citrus rind colour from green to yellow.

When not done correctly degreening can have adverse effects on fruit quality. Variable fruit colour, green spotting, brown calyx, green rings and wilting are some of the damaging effects of degreening (Krajewski and Pittaway, 2002). Other unwanted responses of citrus fruit to ethylene degreening include development of physiological disorders and postharvest diseases (Kader, 1985). The influence of ethylene gas degreening on post-storage decay development varies according to the host-pathogen association (Palou et al. 2003). Ethylene degreening can enhance decay development on citrus fruit, particularly stem-end rot caused by *Diplodia natalensis* (Brown and Burns, 1998; Porat et al., 1999). However, Porat et al. (1999) also reported that degreening reduced the appearance of mould rots on citrus caused by *Penicillium digitatum* and *P. italicum*. Other researchers demonstrated that decay

development on stone fruit, peaches [*Prunus persica* (L.)], plums (*P. salicina* Lindel), nectarines [*P. persica* (L.) Batsch. var. *nucipersica* (Suckow) Schneid], apricots (*P. armeniaca* L.) and sweet cheries (*P. avium* L.), as well as on table grapes (*Vitis vinifera* L.) was not affected by continuous exposure to ethylene (Palou et al., 2003).

The effect of ethylene degreening on physiological disorders of citrus is variable. Van Rensburg et al. (2004) reported that long ethylene exposure increased the risk of rind breakdown in 'Nules Clementine' mandarin fruit. However, ethylene pre-treatment was found to reduce the incidence of rind staining, a physiological rind disorder associated with collapsed oil glands, which may be morphologically similar to rind breakdown of 'Nules', in 'Navelina Navel' orange [*C. sinensis* (L.) Osbeck] (Lafuente and Sala, 2002; Sala and Lafuente, 2004). Ethylene exposure was also found to enhance chilling injury symptoms to citrus fruit (Yuen et al., 1995). However, Bower et al. (1999) reported results to the contrary, their work showed that degreening reduced chilling injury of lemon (*C. limon* Burm. f.), 'Marsh' grapefruit (*C. paradisi* Macf.) and 'Navel' orange.

Chilling injury of citrus cultivars and rind staining of 'Navelina Navel' orange have been associated with oxidative stress (Sala, 1998; Sala and Lafuente, 2004). Furthermore, it was shown that ethylene gas degreening of 'Navelina Navel' orange reduced the incidence of rind staining (Lafuente and Sala, 2002; Sala and Lafuente, 2004). This result was attributed to higher glutathione reductase activity in the rinds of fruit pre-treated with ethylene (Sala and Lafuente, 2004). The effect of ethylene on quality of 'Nules Clementine ' mandarin has been reported, however, detailed investigations into the effect of ethylene on rind antioxidants has not been conducted.

Therefore, the aim of this study was to test the hypothesis that degreening accentuates rind breakdown of 'Nules Clementine' mandarin fruit. The study further investigates whether ethylene gas degreening has an effect on the rind antioxidant capacity of 'Nules Clementine' mandarin fruit, in order to establish if an association exists between the antioxidant capacity and the occurrence of rind breakdown in 'Nules Clementine' mandarin.

Materials and methods

Plant material and sampling

'Nules Clementine' mandarin fruit were harvested from 14-year-old trees in Saron on 24

April 2002, and from 10-year-old trees in Franschhoek on 1 May 2003. At both sites the trees were budded on Troyer citrange (*C. sinensis* x *Poncirus trifoliata*) rootstock, planted on ridges and the spacing distance was 5 x 3 m. A total of ~1000 kg of fruit was harvested from Saron and ~400 kg of fruit was harvested from Franschhoek. Fruit was harvested into 20 kg plastic crates. After harvest fruit were immediately transported to Franschhoek for degreening and packing.

Fruit degreening (treatments)

All harvested fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (Benomyl) (1 $g \cdot L^{-1}$), Deccomone® (2.4-D sodium salt) (5 $mL \cdot L^{-1}$), Citricure® (Guazatine) (2.5 $mL \cdot L^{-1}$) and Sporekill® (dimethyldidecyl ammonium chloride) (1.5 $mL \cdot L^{-1}$). The fungicides were mixed in water with a wetting agent, Bladbuff®, added at a concentration of 0.3 $mL \cdot L^{-1}$. After drenching the fruit was held at ambient temperature (~20°C) for 24 hours. Thereafter, fruit was divided into two groups of equal mass (~500 kg), in 2002. In the 2003 season fruit were divided into three groups of equal mass (~130 kg). Fruit were then moved to a degreening chamber. Degreening was conducted, for 48 hours and 72 hour, using ~3 ppm ethylene at 20 °C and at an RH of ~90% (the 48 hours degreening treatment was only included in the 2003 season). The control fruit was not degreened. After degreening fruit was held at ambient (~20 °C) for 12 to 24 hours before packing.

Fruit packing

Fruit from both areas in both seasons were packed at a commercial pack house in Franschhoek. During packing fruit from each degreening treatment and the control was moved through to a warm water (~40 °C) bath containing Sanazil® (chloromizol) at 670 $mg \cdot L^{-1}$. The fruit was then dried in a hot (~42 °C) tunnel, after which a light polyethylene wax (Decowax) was applied. After waxing, the fruit were again dried in a hot (~52 °C) air tunnel and then sorted and packed into open display 600 x 400 mm 15 kg cartons containing ~150 fruit per carton in 2002. Fruit sampled from Franschhoek in the 2003 season were packed into MO5I open display 5 kg plum cartons containing ~50 fruit per carton. A total of seven replicate cartons were packed for each treatment.

Fruit storage

The fruit from the different degreening treatments and the control were stored for a maximum

period of 10 weeks at -0.5 and 7.5 °C, and then subjected to a shelf-life period of 1 week at 20 °C before evaluations were conducted. In the 2003 season a maturity evaluation was conducted 10 days after harvest, when all degreening treatments were complete and fruit had been stored at the respective temperatures for a short duration.

Data collection

Internal fruit quality and rind properties at harvest

Rind colour was rated using 30 fruit. The CRI rind colour chart set number 36 was used for the rating, where 8 = poorly coloured, dark green with no colour break and 1 = deep orange and fully coloured fruit (CRI, 2004; Appendix 1). Hue angle of the rind was measured on the same 30 fruit used in colour rating. Hue angle was measured using a clourimeter (Minolta CR 10, Japan); where 0° = red, 90° = yellow, and 180° = green (McGuire, 1992). Soluble solids content (SSC) (°Brix) was determined on a pooled juice sample from 30 fruit using a digital refractometer (ATAGO DBX-55, Japan). Titratable acid content (%) was determined using a pooled juice sample from 30 fruit. 25 mL of juice was titrated with 0.1 N NaOH to an end-point of pH 8.2. The result was converted to citric acid equivalents by the equation: tiratable acid content = (mL NaOH/ 25 mL) x (0.1 N NaOH/0.1562). Juice content (% w/w) was determined from a sample of 30 fruit. Fresh mass (g) was determined by taking the average mass of 10 randomly selected fruit. Equatorial fruit diameter (mm) of 30 fruit was measured using a digital calliper (Mitutoyo, CD-15c, Japan).

Internal fruit quality and rind properties determined 10 days after Harvest

Rind colour rating, hue angle of the rind, SSC (°Brix), titratable acid (%) were determined as previously discussed. Rind strength was measured on five fruit per replicate using a texture analyser (TA-XT2 manufactured by Stable Micro Systems Ltd, England). The rind of the fruit was pierced with a 2-mm thick tip and the force required to penetrate the rind was recorded.

Selected biochemical rind properties determined 10 days after harvest

The antioxidant capacity of the flavedo was determined using five fruit from each of the seven replicates per treatment using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Preparation of standard curve: A 2 mM solution of Trolox (6- 2,5,7,8- tetramethylchroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical Co.) was prepared in methanol and used as an antioxidant standard. From the Trolox stock standard, a series of dilutions (in methanol) were prepared viz 0, 0.2, 0.6, 1.0, 1.4, 1.8 and 2 mM.

ABTS (2,2'-Azino-bis3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich chemical Co. was dissolved in distilled deionised water to a concentration of 8 mM. The ABTS radical cation $ABTS^{\bullet+}$ was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (~20 °C) for 12 hours. After this period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2.9 mL of the diluted ABTS radical cation to 0.1 mL of each of the serial Trolox dilutions. The mixture was shaken, allowed to react for 6 minutes and an absorbance reading measured at 734 nm using a spectrophotometer (Cary 50 conc UV-visible, Varian, Musgrave, Australia). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The strips were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Before analysis, the frozen strips were ground to a fine powder in a coffee grinder (Molinix 648, France). Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. A sub-sample of 0.2 g was extracted using 1% HCl in 95% methanol. The extraction of antioxidants, in this experiment, was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged (Sorvall RC-58 refrigerated centrifuge, Wilmington, USA) for 5 minutes at 121 *g* and 4.5 °C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 0.1 mL of the antioxidant extract from the citrus rind was drawn and 2.9 mL of the ABTS radical cation was added to this sample. The mixture

was allowed to react for 6 minutes, after which the absorbance was measured at 734 nm.

Antioxidant capacity (mM Trolox equivalents·g^{-1} sample) was calculated as

(slope x $abs_{734\ nm}$ + C)/(g sample used in analysis)

Where: Slope = slope of the standard curve

$abs_{734\ nm}$ = sample absorbance at 734 nm

C = y intercept on standard curve.

Rind pigments: The total chlorophylls and carotenoid content were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Litchtenthaler (1987).

Sample preparation and pigment extraction: Rind samples were prepared into a fine powder as for antioxidant extraction. The fine powder was then freeze dried and again stored at -80 °C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL of cold 95% ethanol + butylated hydroxytoluene (BHT) (100 mg·L^{-1}) + diethyldithiocarbamate (DDC) (200 mg·L^{-1}). The sample was then vigorously stirred twice, for one minute in each case, on a vortex (G-560E, Bohemia, N.Y, USA), after which the sample was placed in a dark at 4 °C and allowed to extract for 90 minutes. The sample was then filtered through an ashless filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649, and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and the total carotenoids (C_{x+c}) were calculated using the following equations:

$$C_a = 13.36A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = 5.24A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000A_{470} - 2.13\,C_a - 97.64\,C_b}{209}$$

Fruit and rind variables measured after cold storage plus shelf-life

Rind colour, soluble solids content, and titratable acid were again determined as after cold storage, as previously described. Fruit was classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the spots, regardless of size.

Fruit was classified as having chilling injury when there was superficial brown discolouration on the rind (Murata, 1997). The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the blemishes, regardless of size. Decay incidence, green and blue mould caused by *Penicillium digitatum* and *P. italicum* respectively, was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated of juice vesicles resulting in decreased extractable juice (Murata, 1997).

Statistical design and analysis

The experiment was laid out as a completely randomised design. Data collected 10 days after harvest, in the 2003 season, were analysed using a two-way ANOVA on STATISTICA® (Tulsa, OK); factor A being degreening treatment and factor B being storage temperature. Treatment means were compared using the LSD method. Each treatment was replicated seven times with one carton of fruit comprising a single replicate. The same analysis was used after cold storage plus shelf-life on data collected in both seasons.

Results

Rind and fruit characteristics at harvest

Fruit from Saron (2002) were sampled at a rind colour rating of 6.1, a SSC of 9.3 °Brix and a citric acid content of 0.98% (Table 6.1). Fruit sampled from Franschhoek in 2003 had a rind colour rating of 5.1, a SSC of 10.1 °Brix and a citric acid content of 0.83%. These data indicate that fruit sampled were at optimum maturity.

Rind and fruit characteristics measured 10 days after harvest

To allow the influence of the degreening treatment to come into effect, fruit characteristics were again measured 10 days after harvest, in the case of Franschhoek fruit in 2003, only.

Rind colour

A significant interaction occurred between degreening duration and storage temperature on colour development measured as hue angle with the colorimeter (Table 6.2). The rinds of non-degreened fruit kept at either -0.5°C or 7.5°C had a significantly higher hue angle than degreened fruit kept at the same temperatures. In non-degreened fruit, storage at 7.5 °C resulted in a lower hue angle than in fruit stored at -0.5 °C. Degreened fruit stored at either -

0.5 °C or 7.5 °C had similar hue angles. Only degreening, and not storage temperature significantly affected the rind colour rating. Colour rating decreased significantly with increasing degreening duration.

Internal quality

There was a significant interaction between degreening and storage temperature for SSC (Table 6.2). Degreened fruit stored at 7.5 °C had lower SSC than fruit stored at -0.5 °C. However, the reverse occurred in non-degreened fruit where SSC was higher in fruit stored at 7.5 °C than in fruit stored at -0.5 °C. In fruit stored at -0.5 °C, degreening for 72 hours resulted in higher SSC compared to no degreening. The titratable acid content was not affected by either degreening duration or storage temperature.

Rind strength

Fruit degreened for 72 hours tended to have a stronger rind than non-degreened fruit and fruit degreened for 48 hours (Table 6.2). Fruit stored at 7.5°C had a lower rind strength compared to fruit stored at -0.5°C.

Rind pigments and the antioxidant capacity

A significant interaction between degreening duration and storage temperature occurred for total chlorophyll content of the fruit rind (Table 6.3). Non-degreened fruit had a significantly higher chlorophyll content than degreened fruit, irrespective of the storage temperature. The effect of storage temperature was inconsistent; non-degreened fruit stored at -0.5°C had a significantly higher chlorophyll content than fruit stored at 7.5°C, whereas, the chlorophyll content of degreened fruit at both storage temperatures was similar. In fruit stored at 7.5°C, degreening for 48 or 72 hours resulted in a higher total carotenoid content than non-degreened fruit at the same temperature. However, degreening did not have the same effect in fruit stored at -0.5°C. The total carotenoid content of fruit kept at -0.5°C was similar among the control and degreening duration treatments. There was a clear trend of higher carotenoid content in fruit stored at 7.5 °C compared to fruit stored at -0.5 °C, irrespective of degreening duration. Neither degreening duration nor storage temperature had a significant effect on the antioxidant capacity of the fruit rind (Table 6.3).

Rind and fruit characteristics after cold storage plus shelf-life

Rind breakdown

In both seasons, only storage temperature had a significant effect on the occurrence of rind breakdown (Table 6.4 and 6.5). Rind breakdown was prevalent on fruit stored at 7.5 °C for 10 weeks with zero or low levels observed on fruit stored at -0.5 °C for the same duration. Degreening did not significantly influence rind breakdown, even though there was a tendency for degreened fruit to develop slightly higher levels of this disorder.

Chilling injury

In the 2002 season, only storage temperature had a significant effect on the occurrence of chilling injury (Table 6.4), whereas in the 2003 season, an interaction between degreening duration and storage temperature was observed on the occurrence of this disorder (Table 6.5). Chilling injury only occurred in fruit stored at -0.5 °C, whereas no chilling injury developed in fruit stored at 7.5 °C in both seasons. In the 2003 season fruit degreened for 72 hours developed more chilling injury than non-degreened fruit or fruit degreened for a shorter duration.

Decay

In the 2002 season, fruit stored at 7.5 °C developed significantly higher levels of decay than fruit stored at -0.5 °C (Table 6.4), although the levels were numerically low. In the 2003 season, decay was not significantly affected by any of the treatments (Table 6.5).

Rind colour

In both seasons, rind colour development of fruit after storage showed a similar response (Table 6.4 and 6.5). Generally, fruit stored at -0.5 °C had a significantly higher rind colour rating and higher hue angle, than fruit stored at 7.5 °C, irrespective of degreening duration, i.e fruit was less orange. In both seasons, degreening resulted in lower rind colour rating and hue angle than no degreening, for fruit stored at the same temperature. However, non-degreened fruit stored at 7.5 °C was more orange than degreened fruit stored at -0.5 °C.

Internal quality

In the 2002 season, SSC and TA data were not analysed statistically as an unreplicated pooled juice sample per treatment was used. In the 2003 season, there was a significant interaction between storage temperature and degreening duration (Table 6.5). Although the differences

between treatments were significant they were numerically small and did not show an evident trend. Fruit stored at -0.5 °C maintained a higher titratable acid level than fruit stored at 7.5 °C, irrespective of the degreening duration (Table 6.5). A similar trend was observed in the 2002 season. Degreened fruit stored at 7.5 °C had significantly higher acid level than non-degreened fruit stored at the same temperature. Core drying was only observed in the 2003 season. This disorder was prevalent in fruit stored at 7.5 °C, irrespective of degreening duration treatment, but did not occur in fruit stored at -0.5 °C. Degreening for 48 or 72 hours greatly reduced the incidence of core drying compared to no degreening.

Rind strength and rind moisture

Only storage temperature affected rind strength and rind moisture content. Fruit stored at -0.5 °C had a stronger rind with lower moisture content than fruit stored at 7.5 °C (Table 6.5).

Puffiness

Puffiness decreased with increasing degreening duration (Table 6.5); fruit degreened for 72 hour had significantly less puffiness than degreening for 48 hour of no degreening. Fruit stored at 7.5 °C had significantly higher levels of puffiness compared to fruit stored at -0.5 °C (Table 6.5).

Discussion

Ethylene gas degreening did not accentuate rind breakdown of 'Nules Clementine' mandarin fruit harvested at optimum maturity and stored at 7.5°C or -0.5°C for a period of 10 weeks. However, during weekly observations of fruit stored at 7.5 °C it was noted that rind breakdown appeared about 1 week earlier on degreened fruit, with non-degreened fruit eventually developing the disorder to the same level over time in storage (data not shown). Van Rensburg et al. (2004) reported that ethylene degreening aggravated rind breakdown but did not mention the storage period over which this observation was made. Evidently, the results of the present study do not support findings by Van Rensburg et al. (2004). The reason for this contradiction in results cannot be fully established. However, it remains possible that storage period over which data were collected could be the cause for the difference. Results reported over a shorter storage duration could be different from those reported over a 10 weeks storage period as was the case in our study. Work conducted on rind staining of 'Navelina Navel' orange, which is also a rind disorder associated with collapsed oil glands as is the case with rind breakdown, showed that treating fruit with

ethylene considerably reduces the disorder (Lafuente and Sala, 2002; Sala and Lafuente, 2004). It is clear that the occurrence of rind staining in 'Navelina Navel' orange and rind breakdown in 'Nules Clementine' mandarin in response to ethylene is different. This suggests that the disorders, rind staining and rind breakdown, are different. To date it has not been established how the different rind disorders, occurring on different citrus types, described in literature are associated with one another.

In response to temperature, 'Nules Clementine' mandarin fruit stored at -0.5 °C developed none or very low levels of rind breakdown compared to fruit stored at 7.5 °C. Results obtained in chapter 5 of this thesis showed that rind breakdown is most readily expressed in fruit stored at 7.5°C. A similar development of rind disorders in response to temperature has also been reported in 'Shamouti' orange (Ben Yehoshua, et al., 2001), where fruit stored at 5 or 6 °C developed less noxan than fruit stored at 20 °C, and 'Navelina Navel' orange (Lafuente and Sala, 2002), where fruit stored at 2 °C developed less rind staining than fruit stored at 22 °C.

Fruit stored at -0.5 °C did not develop rind breakdown, but at this temperature chilling injury was prevalent. The study further showed that prolonged degreening for up to 72 hours increased the risk of chilling injury in fruit stored at -0.5 °C compared to fruit degreened for a shorter duration or not degreened at all. It is not uncommon for ethylene gas degreening to increase the incidence of chilling injury on citrus, as similar results have been reported on 'Shamouti' orange (Porat et al., 1999) and on other citrus cultivars (Yuen et al., 1995). However, Bower et al. (1999) reported results to the contrary where ethylene reduced chilling injury due to a decrease in the chlorophyll:carotenoid ratio. In our work the chlorophyll:carotenoid ratio was markedly reduced by degreening but the chilling injury incidence increased.

It was a secondary objective of this study to establish whether trends observed in the development of rind breakdown and chilling injury after storage could be associated with the rind antioxidant capacity. No association could be established because similar antioxidant capacities were recorded in fruit from the different degreening treatments, 10 days after harvest and similar levels of rind breakdown were recorded after storage. Differences in chilling injury levels after storage could not be associated with the antioxidant capacity of fruit measured 10 days after harvest. The limitation of this study was that the long term effect

of degreening on the antioxidant capacity was not determined because this variable was not measured during the storage period. Yet it is known that oxidative homeostasis requires a balance between the production of free radicals and the ability of the cell to remove them (Mittler, 2000). Antioxidant capacity measurements taken only before or after a particular treatment do not reflect this balance and may, therefore, be misleading. The results reported in the present study are similar to those reported by Sala and Lafuente (2004), where ethylene gas degreening for four days did not significantly affect the activities of active oxygen species scavenging enzymes in 'Navelina Navel' orange fruit compared to fruit treated with air for the same duration.

In fruit stored at 7.5 °C and evaluated ten days after harvest, degreening improved rind colour through chlorophyll degradation and stimulation of carotenoid synthesis. This result was expected, as similar results have been previously reported where ethylene gas degreening improved rind colour of citrus fruit (Yamauchi et al., 1997; Saltveit, 1999). However, in fruit stored at -0.5 °C and evaluated ten days after harvest, degreening improved rind colour through chlorophyll degradation, which removed the green colour, without carotenoid synthesis since the levels of this pigment were similar between the degreening durations (Table 6.3). This response highlighted the role of temperature in carotenoid syntheses and degradation, and can possibly be attributed to enzyme kinetics. Enzymes responsible for carotenoid synthesis may be non-functional at -0.5°C therefore no additional carotenoids were synthesized, resulting in similar levels among the degreening durations. After 10 weeks storage, the poor colour in fruit stored at -0.5°C may have been due to carotenoid degradation, where there is breakdown of this pigment at sub-zero temperatures. The deep orange colour developed in fruit stored at 7.5°C was possibly due to carotenoid synthesis at this temperature. Chapter 5 of this thesis shows this trend, where there was synthesis of carotenoids at higher temperatures and breakdown of carotenoids at -0.5°C. Other results showed poor colour development in 'Palmer Navel' sweet orange stored at sub-zero temperatures and this was attributed to carotenoid degradation (Van Wyk 2004). Petracek and Montalvo (1997) reported that degreening of 'Fallglo' mandarin was hindered by storage at 4.5 °C in fruit not exposed to ethylene.

The internal quality measured, in terms of SSC and titratable acid, showed little change with degreening and storage temperature. This response may be due the non-climacteric nature of citrus fruit, which undergo very slow changes in internal quality during storage (Davies and

Albrigo, 1994). Plaza et al. (2004) reported similar results, where late harvested ‘Clementine’ mandarin fruit degreened with ethylene had similar soluble solids and titratable acidity as non-degreened fruit after the marketing period.

The titratable acid content of ‘Nules Clementine’ mandarin was lower in fruit stored at 7.5 °C compared to fruit stored at -0.5 °C irrespective of the degreening treatment. Probably, the higher storage temperature increased respiration (Murata, 1997), and with extended storage the increased respiration rate resulted in citric acid consumption by using it as a metabolic substrate for respiration (Plaza et al., 2004).

Conclusions

In conclusion, ethylene gas degreening did not aggravate rind breakdown in 'Nules Clementine' mandarin fruit harvested at optimum maturity and stored at -0.5 °C or 7.5 °C for a period of 10 weeks. Suggesting that ethylene gas degreening is not the cause for this disorder. Rind breakdown was most readily expressed in fruit stored at 7.5 °C than in fruit stored at -0.5 °C. It would seem ‘Nules Clementine’ mandarin fruit, when exposed to certain environmental conditions, can potentially develop rind breakdown when stored at 7.5°C, regardless of degreening. Furthermore, the present study could not establish an association between rind pigments, the rind antioxidant capacity, measured 10 days after harvest, and the occurrence of rind breakdown. Therefore, it cannot be concluded in this study whether rind breakdown was due to an oxidative stress. At this stage, changes in the degreening process will not be a solution to rind breakdown, but adopting more stringent temperature control measures during fruit storage could reduce the incidence of this disorder.

Table 6.1. Characteristics at harvest of 'Nules Clementine' mandarin fruit harvested from Saron and Franschhoek in the 2002 and 2003 seasons, respectively.

Variable	Saron (2002)	Franschhoek (2003
Rind colour rating[1]	6.1	5.1
Hue of the rind(°)	101.6	77.0
SSC[2] (°Brix)	9.3	10.1
Titratable acid (%)	0.98	0.83
Juice content (%)	61.3	54.3
Fresh mass (g)	155.4	105.1
Equatorial diameter (mm)	68.5	63.3

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = porly colourd dark green fruit with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Apendix 1).
2 Soluble solids content

Table 6.2. Effect of degreening duration and storage temperature on fruit characteristics measured 10 days after harvest from fruit sampled in Franschhoek in the 2003 season.

Response variable	Storage temperature (°C)[1]	Degreening duration (h) (Factor A)			Storage temperature (°C) (Factor B)		Prob>F[3]		
		0	48	72	-0.5	7.5	A	B	AxB
Hue angle of the rind	-0.5	87.3c[2]	73.7a	70.7a			0.001	0.056	0.025
	7.5	81.1b	73.3a	71.2a					
Rind colour rating		3.4c	2.6b	2.3a	2.9	2.7	0.001	0.105	0.081
SSC[4] (°Brix)	-0.5	9.8ab	10.0bc	10.2c			0.315	0.471	0.001
	7.5	10.2c	9.7a	9.7a					
Titratable acid (%)		0.79	0.81	0.78	0.79	0.80	0.471	0.511	0.895
Rind stength (N)		11.5a	11.4a	13.0b	12.5b	11.3a	0.001	0.002	0.486

1 If itemised, interaction occurred between factor A and B.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Two-way ANOVA with factor A being degreening duration and factor B being storage temperature.
4 Soluble solids content

Table 6.3. Effect of degreening duration and storage temperature on selected rind biochemical properties measured 10 days after harvest from fruit sampled in Franschhoek in the 2003 season.

Response variable	Storage temperature (°C)[1]	Degreening duration (h) (Factor A)			Storage temperature (°C) (Factor B)		Prob>F[3]		
		0	48	72	-0.5	7.5	A	B	AxB
Total chlorophylls ($\mu g \cdot g^{-1}$ dry weight)	-0.5	16.4d[2]	2.6ab	1.4a			0.001	0.288	0.001
	7.5	13.0c	3.5b	2.5ab					
Total carotenoids ($\mu g \cdot g^{-1}$ DW)	-0.5	10.7a	10.6a	11.4ab			0.001	0.001	0.001
	7.5	13.2b	21.0c	19.8c					
Antioxidant capacity (mM Trolox equivalents$\cdot g^{-1}$ sample)		5.2	5.1	5.3	5.1	5.3	0.789	0.128	0.309

1 If itemised interaction occurred between factor A and B.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Two-way ANOVA with factor A being degreening duration and factor B being storage temperature

Table 6.4. Effect of degreening duration and storage temperature on the quality of 'Nules' Clementine mandarin fruit sampled from Saron in the 2002 season and stored for 10 weeks at -0.5 °C or 7.5 °C plus an additional 1 week at 20 °C.

Response variable	Storage temperature (°C)[1]	Degreening duration (h) (Factor A)		Storage temperature (°C) (Factor B)		Prob>F[3]		
		0	72	-0.5	7.5	A	B	AxB
Rind breakdown (%)		30.4	32.9	0.5a[2]	62.7b	0.698	0.001	0.819
Chilling injury (%)		21.2	25.5	46.7b	0.0a	0.319	0.001	0.319
Decay (%)		1.2	0.3	0.0a	1.5b	0.164	0.021	0.163
Rind colour rating[4]	-0.5	4.8d[5]	3.0c			0.001	0.001	0.001
	7.5	1.9b	1.5a					
Hue angle of the rind	-0.5	93.1d	72.7c			0.001	0.001	0.001
	7.5	68.4b	63.2a					
SSC (°Brix)		10.4	9.2	10.1	9.5	nd[6]	nd	Nd
Titratable acid (%)		0.71	0.67	0.75	0.64	nd	nd	Nd

1 If itemised interaction occurred between factor A and B.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Two-way ANOVA with factor A being degreening duration and factor B being storage temperature.
4 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004).
5 Values in the same row and column followed by different letters indicate significant differences (P<0.005) according to the LSD test.
6 Data not analysed statistically as an unreplicated, pooled juice sample from 20 fruit was used per treatment.

Table 6.5. Effect of degreening duration and storage temperature on the quality of 'Nules' Clementine mandarin fruit sampled from Franschhoek in the 2003 season and stored for 10 weeks at -0.5 °C or 7.5 °C plus an additional 1 week at 20 °C.

Response variable	Storage temperature (°C)[1]	Degreening duration (h) (Factor A)			Storage temperature (°C) (Factor B)		Prob>F[3]		
		0	48	72	-0.5	7.5	A	B	AxB
Rind breakdown (%)		5.0	6.9	6.7	0.0a[2]	12.4b	0.337	0.001	0.337
Chilling injury (%)	-0.5	13.1b[4]	10.9b	16.8c			0.031	0.001	0.031
	7.5	0.0a	0.0a	0.0a					
Decay (%)		3.1	4.3	3.4	3.0	4.2	0.584	0.191	0.700
Rind colour rating[5]		4.6d	3.0c	3.0c			0.001	0.001	0.001
		2.0b	1.5a	1.4a					
Hue angle of the rind	-0.5	86.1d	73.9c	72.4c			0.001	0.001	0.001
	7.5	62.5b	60.4ab	59.7a					
SSC[6] (°Brix)	-0.5	9.6bc	9.7c	9.8c			0.008	0.005	0.006
	7.5	9.2ab	9.9c	9.1a					
Titratable acid (%)	-0.5	0.60c	0.60c	0.60c			0.001	0.001	0.001
	7.5	0.36a	0.50b	0.50b					
Core drying (%)	-0.5	0.0a	0.0a	0.0a			0.018	0.001	0.018
	7.5	28.0b	4.0a	8.0a					
Rind moisture (%)		69.9	70.0	70.4	68.8a	71.3b	0.741	0.001	0.549
Rind strength (N)		9.4	9.6	9.9	10.6b	8.6a	0.700	0.001	0.364
Puffiness (%)		6.5b	4.6ab	2.8a	3.7a	5.6b	0.005	0.040	0.232

1 If itemised interaction occurred between factor A and B.
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Two way ANOVA with factor A being degreening duration and factor B being storage temperature.
4 Values in the same row and column followed by different letters indicate significant differences (P<0.005) according to the LSD test.
5 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = green immature fruit with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004).
6 Soluble solids content

CHAPTER 7

EFFECT OF STORAGE TEMPERATURE AND STORAGE DURATION ON THE POST-STORAGE QUALITY OF 'NULES CLEMENTINE' MANDARIN (*CITRUS RETICULATA* BLANCO), WITH SPECIAL REFERENCE TO RIND BREAKDOWN AND CHILLING INJURY

Abstract

'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit are susceptible to the physiological disorder rind breakdown. The occurrence of this disorder is known to be influenced by storage temperature and storage duration. However the cause and physiology of rind breakdown is not well understood. The objective of this study was to establish the influence of storage temperature and storage duration on rind antioxidant capacity and the occurrence of rind breakdown on 'Nules Clementine' mandarin fruit. Fruit were harvested within the optimum maturity window, from Saron in 2002 and from Franschhoek in 2003. Fruit were degreened, packed and stored at -0.5 °C, 4.5 °C, 7.5 °C or 11 °C for 20, 40, 60 or 80 days plus a shelf-life period of 1 week at 20 °C before evaluations. Rind breakdown levels were significantly higher in fruit stored at 7.5 °C and increased over time compared to fruit stored at the other temperatures. Chilling injury occurred in fruit stored at -0.5 °C and levels increased over time, with zero or very low levels recorded at higher storage temperatures. This experiment also monitored changes over time in rind pigments and rind antioxidant capacity of fruit stored at the different temperatures to establish whether these biochemical properties were associated with the occurrence of rind breakdown. The antioxidant capacity of fruit stored at 4.5 °C and 7.5 °C increased over time in storage, whereas in fruit stored at -0.5 °C the antioxidant capacity decreased significantly after 80 days of storage, and showed no definite trend of increasing or decreasing in fruit stored at 11 °C. This suggested that antioxidants may not play a direct role in the occurrence of rind breakdown. By contrast, changes over storage time in rind antioxidant capacity and the occurrence of chilling injury indicated that this disorder can possibly be attributed to an oxidative stress. Total carotenoids of fruit stored at 7.5 °C and 11 °C increased over time, whereas carotenoids of fruit stored at -0.5 °C and 4.5 °C showed a slight decrease and no significant change, respectively. The total chlorophylls degraded over time at the respective temperatures with no chlorophylls present after 80 days storage. From these results a direct association between rind pigments and the occurrence of rind breakdown could not be established. While significant differences occurred in the soluble solids content and titrable citric acid level, the absolute values were

marginal and not of commercial importance. It was concluded that optimum fruit quality of 'Nules Clementine' mandarin fruit can be achieved by cold storage at 4.5 °C for less than 60 days.

Introduction

Rind breakdown of 'Nules Clementine' mandarin (*C. reticulata* Blanco) is characterised by collapsed oil glands in the flavedo resulting in oil leakage from the oil glands, into the adjacent albedo where the oil oxidises and becomes visible as brown spots on the fruit surface (Van Rensburg et al., 2004). The occurrence of rind breakdown can be influenced by, among other factors, storage temperature and storage duration (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

Citrus fruit are non-climacteric with low respiration rates during maturation and senescence, suggesting that they can be stored for relatively long periods (Davies and Albrigo, 1994). However, differences exist among species and cultivars (Chalutz et al., 1985) and as a result, no single storage protocol is applicable to all citrus cultivars. Davies and Albrigo (1994) suggested that citrus types not sensitive to chilling can be stored at temperatures below 4 °C, whereas cultivars sensitive to chilling injury should be stored at temperatures above 10 °C.

Harvested fruit are living organs, as they continue to respire and lose moisture in storage (Burdon, 1997). These and other ongoing metabolic processes in a fruit during storage can result in changes, detrimental to fruit quality, which may be pathological or physiological (Burdon, 1997). These detrimental changes can be exacerbated by fruit storage at sub-optimal condition, in terms of storage environment (temperature and relative humidity) and storage duration (Burdon, 1997). Van Rensburg et al. (2004) mentioned that rind breakdown of 'Nules Clementine' mandarin fruit is aggravated by high storage temperature and long storage duration. However, in this communication specific temperatures and durations were not provided.

Other rind disorders, associated with collapsed oil glands, that may be morphologically similar to rind breakdown of 'Nules Clementine' mandarin and which affect other citrus types have also been reported. The incidence of these disorders is higher at non-chilling temperatures (>15 °C) compared to lower temperatures, and they include rind pitting of 'Marsh' grapefruit (*C. paradisi* Macf.) (Petracek et al., 1995) and 'Fallglo' mandarin [*C.*

reticulata Blanco x (*C. reticulata* Blanco x *C. paradisi* Macf.)] (Petracek et al., 1998), superficial flavedo necrosis (noxan) of 'Shamouti' orange [*C. sinensis* (L.) Osbeck] (Ben Yehoshua et al., 2001), and rind staining of 'Navelina' orange (Agusti et al., 2001; Alférez et al., 2003). Low storage temperature generally suppresses fungal decay development (Eckert and Brown, 1986; Shellie and Skiria, 1998). However, in citrus fruit, due to the presence of chilling injury or other rind disorders at low temperatures opportunistic infection can occur, resulting in higher decay levels in fruit stored at low than at high temperatures (Cohen and Schiffmann-Nadel, 1978; Chalutz et al., 1985).

Apart from physiological and pathological disorders, storage temperature and storage duration can also affect biochemical properties of citrus fruit. Rind colour development may be influenced by storage temperature, with better rind colour (more orange) development reported in fruit stored at high temperature than at lower or subzero temperatures (Cohen and Schiffmann-Nadel, 1978; Van Wyk, 2004). Prolonged storage at chilling temperatures can induce oxidative stress in fruit (Wise and Naylor, 1987; Du and Bramlage, 1994). Oxidative stress occurs when the production of active oxygen species exceeds the capacity of the cell to remove them and maintain a cellular redox homeostasis (Hodges et al., 2004; Toivonen, 2004). This stress can be measured directly, by detecting the accumulation of active oxygen species, increases in lipid peroxidation products, enhanced membrane leakage or the accumulaion of brown pigments, or indirectly, by detecting changes in the antioxidant components (Toivonen, 2004). Symptoms of oxidative stress can include the development of postharvest disorders (Hodges at al., 2004). In citrus fruit, the postharvest disorders chilling injury and rind staining of 'Navelina' orange have been associated with oxidative stress (Sala, 1998; Sala and Lafuente, 2004).

The effect of storage temperature and storage duration on the development of rind breakdown of 'Nules Clementine' mandarin fruit has been investigated. However, the influence of these factors on the rind antioxidant capacity has not been investigated and is therefore unknown. The aim of this study was, therefore, to investigate the effect of storage temperature and storage duration on the rind antioxidant capacity and the occurrence of rind breakdown of 'Nules Clementine' mandarin fruit.

Materials and methods

Plant material and sampling

About 1600 kg of 'Nules Clementine' mandarin fruit were harvested from ~50 trees in Saron, Western Cape province, South Africa on 24 April 2002. In the following season about 500 kg of fruit were sampled from ~50 trees in Franschhoek Western Cape province, South Africa on 1 May 2003. The orchard used in Saron had 14-year-old trees and the one used in Franschhoek had 10-year-old trees. At both sites the trees were budded on Troyer citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] rootstock, planted on ridges and with a planting distance of 5 x 3 m. Fruit were harvested into 20 kg lug boxes and immediately transported to a commercial packhouse in Franschhoek for degreening and packing.

Fruit degreening and packing

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 $g \cdot L^{-1}$), Deccomone® (2.4-D sodium salt) (5 $mL \cdot L^{-1}$), Citricure® (guazatine) (2.5 $mL \cdot L^{-1}$) and Sporekill® (dimethyldidecyl ammonium chloride) (1.5 $mL \cdot L^{-1}$). The fungicides were mixed in water with a wetting agent, Bladbuff®, added at a concentration of 0.3 $mL \cdot L^{-1}$. After drenching the fruit was held at ambient temperature (~20 °C) for 24 hours before being moved to a degreening chamber. Degreening was conducted for 72 hours using ~3 ppm ethylene at 20 °C and at 90% RH. After degreening, fruit were again held at ambient temperature (~20 °C) for 12 to 24 hours before packing. During packing fruit were moved through a warm water (~40 °C) bath containing Sanazil® (chloramizol) at 0.67 $g \cdot L^{-1}$. The fruit were then dried in a hot (~42 °C) tunnel, after which a light commercial polyethylene wax (Decowax®) was applied. After waxing the fruit were again dried in a hot (~52 °C) air tunnel and then sorted and packed into fruit cartons. A total of 80 cartons were packed from the fruit sampled at each area. Fruit from Saron were packed in open display 600 x 400 mm 15 kg cartons containing ~150 fruit per carton, whereas fruit sampled from Franschhoek in the 2003 seasons were packed into MO5I open display 5 kg plum cartons, containing ~50 fruit, per carton.

Treatments

After packing fruit were stored at different temperatures for different durations. The 80 cartons of fruit packed from each area were then divided into four groups of 20 cartons each and stored at -0.5, 4.5, 7.5 or 11 °C. The 20 cartons of fruit at each storage temperature were

further divided into four groups of 5 cartons each (replications) corresponding to storage periods of 20, 40, 60 or 80 days. A shelf-life period of 7 days at 20 °C was implemented after the initial cold storage period before evaluations were conducted.

Data collection

Internal fruit quality and rind variables at harvest

Rind colour of 30 fruit was rated using the CRI rind colour chart set number 36, where 8 = poorly coloured, dark green with no colour break, and 1 = deep orange and fully coloured fruit (CRI, 2004; Appendix 1). Hue angle of the rind was measured on the same 30 fruit used for colour rating using a colorimeter (Minolta CR10, Japan). where 0° = red, 90° = yellow, and 180° = green (McGuire, 1992). Equatorial fruit diameter (mm) was measured on 30 fruit using a digital calliper (Mitutoyo CD-15c, Japan). Soluble solids content (SSC) (°Brix) was determined on a pooled juice sample from 30 fruit using a digital refractometer (ATAGO DBX-55, Japan). Titratable acid content (%) was determined on a pooled juice sample from 30 fruit. 25 mL of juice was titrated with 0.1 N NaOH to an end-point of pH 8.2. The result was converted to citric acid equivalents by the equation: tiratable acid content = (mL NaOH/ 25 mL) x (0.1 N NaOH/0.1562). Fresh mass (g) was determined by taking the average mass of 10 randomly selected fruit.

Internal fruit quality and rind variables after storage plus a shelf-life

Rind colour, SSC, and titratable acid content were again determined after each storage period on 10 fruit per replicate as previously described. Fruit were classified as having rind breakdown when discoloured spots were seen on the rind. The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the spots, regardless of size. Fruit was classified as having chilling injury when there was superficial brown discolouration on the rind (Murata, 1997). The incidence of this disorder was measured on ~40 fruit per replicate by determining the percentage of fruit with the blemishes, regardless of size. Fungal decay, green mould caused by *Penicillium digitatum*, incidence was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated juice vesicles resulting in decreased extractable juice (Murata, 1997).

Selected biochemical rind properties determined after storage plus shelf life

The antioxidant capacity of the flavedo was determined from the rinds of five fruit per replicate per storage temperature and duration using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Preparation of standard curve: A 2 mM solution of Trolox (6-2,5,7,8-tetramethylchroman-2-carboxylic acid, obtained from Sigma Aldrich Chemical Co.) was prepared in methanol and used as an antioxidant standard. From the Trolox stock standard, a series of dilutions (in methanol) were prepared vis 0, 0.2, 0.6, 1.0, 1.4, 1.8 and 2 mM.

ABTS (2,2'–Azino-bis3-ethylbenz-thiazoline-6-sulfonic acid) obtained from Sigma Aldrich Chemical Co. was dissolved in distilled deionised water to a concentration of 8 mM. The ABTS radical cation $ABTS^{\bullet+}$ was produced by reacting equal volumes of the ABTS stock solution and 3 mM potassium persulfate. The mixture was placed in the dark at room temperature (~20 °C) for 12 hours. After this period, the ABTS radical cation was diluted in a phosphate buffer at pH 7.4 to a final absorbance of 1.8.

A standard curve was prepared by adding 2.9 mL of the diluted ABTS radical cation to 0.1 mL of each of the serial Trolox dilutions. The mixture was shaken, allowed to react for 6 minutes and an absorbance reading measured at 734 nm using a spectrophotometer (Cary 50 conc UV-visible, Varian, Musgrave, Australia). Fresh chemicals and a new standard curve were prepared for each day of analysis.

Rind sample preparation and antioxidant extraction: A zester was used to remove thin layers of citrus flavedo with part of the albedo attached, from the equatorial position of the fruit. The strips were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Before analysis, the frozen strips were ground to a fine powder in a coffee grinder (Molinix 648, France). Liquid nitrogen was added periodically to prevent the sample from thawing during the grinding. A sub-sample of 0.2 g was extracted using 1% HCl in 95% methanol. The extraction of antioxidants was done in three phases. The sample was first extracted with 10 mL of solvent for 2 hours on a shaker (Janke and Kunkel IKA-WERK KS 500), and then centrifuged (Sorvall RC-58 refrigerated centrifuge, Wilmington, USA) for 5 minutes at 121 *g* and 4.5 °C after which the supernatant was retained. The residue was again extracted with 10 mL of solvent, placed on a shaker for 5 minutes and then centrifuged, as before. The

supernatant was again retained as in the previous step. The second step was repeated, all three supernatants were combined and the residue discarded.

To determine the antioxidant capacity, 0.1 mL of the antioxidant extract from the citrus rind was added to 2.9 mL of the ABTS radical cation. The mixture was allowed to react for 6 minutes, after which the absorbance was measured at 734 nm.

Antioxidant capacity (mM Trolox equivalents·g^{-1} sample) was calculated as:

(slope x $abs_{734\ nm}$ + C)/(g sample used in analysis)

Where: Slope = slope of the standard curve

$abs_{734\ nm}$ = sample absorbance at 734 nm

C = y intercept on standard curve.

Rind sample preparation and pigment extraction: The total chlorophylls and carotenoid content were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Litchtenthaler (1987). Rind samples were prepared into a fine powder as for antioxidant extraction. The fine powder was then freeze dried and again stored at -80 °C until analysis. During analysis, 0.2 g of freeze-dried sample was extracted in 10 mL of cold 95% ethanol + butylated hydroxytoluene (BHT) (100 mg·L^{-1}) + diethyldithiocarbamate (DDC) (200 mg·L^{-1}). The sample was then vigorously stirred twice, for one minute in each case, on a vortex (G-560E, Bohemia, N.Y, USA). Thereafter, the sample was placed in a dark at 4 °C and allowed to extract for 90 minutes. The sample was then filtered through an ashless filter paper and the residue discarded. The supernatant was poured into a cuvette and the absorbance measured at 470, 649, and 664 nm. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and the total carotenoids (C_{x+c}) were calculated using the following equations:

$$C_a = 13.36A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = 5.24A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{1000A_{470} - 2.13\,C_a - 97.64\,C_b}{209}$$

Statistical design and analysis

The experiment was analysed as a completely randomised design. Data collected after each storage period plus shelf-life was analysed using a two-way ANOVA on STATISTICA® (Tulsa, OK). A 4 x 4 factorial set of treatments was used with factor A being the storage temperature and factor B being the storage duration. Treatment means were compared using the LSD method. The were five single-carton replicates per treatment.

Results

Rind and fruit characteristics at harvest

Fruit were sampled from Saron 2002 at a rind colour rating of 6.1, a SSC of 9.3 °Brix and a citric acid content of 0.98% (Table 7.1). Fruit sampled from Franschhoek in 2003 had a rind colour rating of 5.1 SSC of 10.1 °Brix and a citric acid content of 0.83%.

Rind and fruit characteristics after each storage period plus shelf-life

Rind breakdown

Rind breakdown development on fruit from Saron (Table 7.2) and on fruit from Franschhoek (Table 7.3) showed a similar trend. In both areas, the levels of this disorder were highest in fruit stored at 7.5 °C and increased over time, with zero or low levels (<4%) occurring in fruit stored at -0.5 and 4.5 °C. Fruit harvested from Saron and stored at 11 °C developed rind breakdown and the levels increased with extended storage (Table 7.2). However, rind breakdown at 11 °C was significantly lower than in fruit stored at 7.5 °C, even after 80 days of storage. Fruit harvested from Franschhoek and stored at 11 °C developed low levels (0.3%) of rind breakdown even after 80 days of storage (Table 7.3).

Chilling injury

In both seasons and production areas, chilling injury occurred predominantly in fruit stored at -0.5 °C with zero or low levels occurring in fruit stored at the higher temperatures (Tables 7.2 and 7.3). In fruit sampled from Saron, this disorder increased significantly with extended storage at -0.5 °C (Table 7.2). In the case of fruit from Franschhoek, chilling injury levels remained low during storage and only increased significantly after 80 days storage (Table 7.3).

Decay

Generally low levels (<1%) of decay, identified as blue mould caused by *P. digitatum*, were recorded in fruit sampled from Saron (Table 7.2). Storage temperature did not have a significant effect on decay, whereas extended storage significantly increased the incidence of decay compared to shorter storage durations. In fruit sampled from Franschhoek, a significant interaction between storage temperature and storage duration was observed on the occurrence of decay (Table 7.3). Generally, the decay levels were low (<3%) and showed no particular trend across storage temperatures for up to 60 days of storage, and then levels increased to ~6% in fruit stored at -0.5, 4.5, and 7.5 °C. Decay levels remained low (<2%) in fruit stored at 11 °C even with extended storage.

Rind colour

A significant interaction was observed between storage temperature and storage duration on the rind colour rating of fruit from both Saron and Franschhoek (Tables 7.2 and 7.3). The rind colour rating decreased with increasing storage temperature irrespective of storage duration, in fruit from both areas (Tables 7.2 and 7.3). In fruit sampled from Saron, the distinction in rind colour rating of fruit stored at the different temperatures was evident early (20 days) in storage, with minimal changes occurring over time in storage at the respective temperatures (Table 7.2). The rind colour rating of fruit harvested from Franschhoek and stored at 7.5 and 11 °C decreased over storage time, from 1.8 to 1.3 and from 1.8 to 1.2 respectively after 80 days storage. By contrast, in fruit stored at -0.5 °C and 4.5 °C the rind colour rating increased slightly over time, from 2.2 to 3.0 and from 2.0 to 2.3 respectively after 80 days storage (Table 7.4). In fruit sampled from Saron, only temperature had a significant effect on the hue angle of the rind (Table 7.2). There was a decrease in hue angle with increasing storage temperature. In fruit sampled from Franschhoek, there was a significant interaction between storage temperature and storage duration on colour development measured with the colorimeter (Table 7.3). Generally, the hue angle of the rind decreased with increasing temperature, irrespective of the storage duration. The hue angle of the rind in fruit stored at 7.5 and 11 °C decreased over time, whereas, in fruit stored at -0.5 and 4.5 °C, there was a marginal change, which did not show a definite trend of increasing or decreasing over time.

Puffiness

Puffiness was only observed in fruit harvested from Franschhoek (Table 7.4). During storage no puffiness was recorded for up to 60 days. Thereafter, the disorder developed with levels increasing significantly with increasing storage temperature.

Internal quality

The SSC and titratable acid content of fruit harvested from Franschhoek were significantly affected by storage temperature as well as by storage duration (Table 7.4). The SSC and titratable acid level was significantly higher in fruit after a short storage duration (20 days) than in fruit stored for longer periods. Both the SSC and titratable acid levels remained more or less constant with increasing storage temperature. Furthermore, the maximum difference in SSC and acidity of fruit at the different temperatures was 0.4 °Brix and 0.08%, respectively. No core drying was observed in fruit after 20 and 40 days storage, thereafter significant levels appeared (Table 7.4).

Rind moisture

A significant interaction was observed between storage temperature and storage duration on the rind moisture content of 'Nules Clementine' mandarin fruit (Table 7.4). However, the variations in rind moisture did not show a definite trend between the storage temperatures over time.

Selected biochemical rind properties

The total chlorophylls in the rind declined significantly over time in storage, with no chlorophylls detected in the rinds of fruit after 80 days of storage (Table 7.5). Storage temperature did not significantly affect the total chlorophyll content of fruit. A significant interaction between storage temperature and storage duration occurred for total carotenoid content of the rind (Table 7.5). Generally, the carotenoid content of the fruit rind increased with increasing storage temperature irrespective of storage duration. However, this trend was not very distinct early in storage (20 days), but became more apparent over time in storage. Furthermore, the carotenoid content significantly increased over time in fruit stored at 7.5 and 11 °C, with marginal changes which did not show a definite trend of increasing or decreasing over time in fruit stored at 4.5 and -0.5 °C. In fruit stored at 11 °C the carotenoid content was significantly higher after 60 days storage than after 80 days. A significant interaction between

storage temperature and storage duration was observed for the antioxidant capacity of the rind (Table 7.5). In fruit stored at 4.5 and 7.5 °C the antioxidant capacity increased significantly over time in storage, whereas in fruit stored at -0.5 °C the antioxidant capacity tended to decrease over time and showed no definite trend of increasing or decreasing over time in fruit stored at 11 °C.

Discussion

Rind breakdown incidence was highest in fruit stored at 7.5 °C compared to fruit stored at higher or lower temperatures. This disorder developed progressively during storage. The development of rind breakdown over time confirms earlier reports where rind breakdown was aggravated by extended storage (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). However, the occurrence of rind breakdown in response to storage temperature reported in this study is not consistent with reports by Van Rensburg and Bruwer (2000), where high shipping temperatures were found to increase the incidence of rind breakdown. The reason for this contradiction cannot be fully established as the Van Rensburg and Bruwer (2000) did not provide the temperatures at which fruit was stored. It is not uncommon for rind disorders to develop more rapidly in a particular temperature range, above or below which levels are greatly reduced. Chalutz et al. (1985) showed that chilling injury, expressed as rind pitting, in grape fruit, 'Shamouti' and 'Valencia' orange was highest in fruit stored at 6 °C compared to fruit stored above or below this temperature. In the present study it cannot be fully explained why rind breakdown was higher at 7.5 °C than at the other temperatures. Enzyme activity is known to have an optimum temperature above or below which activity is reduced (Salisbury and Ross, 1991). Therefore, it should be investigated whether the development of rind breakdown is enzymatic. Other rind disorders, which include rind pitting of 'Marsh' grapefruit (Petracek et al., 1995), and 'Fallglo' mandarin (Petracek et al., 1998), superficial flavedo necrosis (noxan) of 'Shamouti' orange (Ben Yehoshua et al., 2001), and rind staining of 'Navelina' orange (Agusti et al., 2001; Alférez et al., 2003) occur at high levels in fruit stored at non-chilling temperatures (>15 °C) compared to fruit stored at lower temperatures (<4 °C). However, in the present study the influence of non-chilling temperatures on the occurrence of rind breakdown was not tested.

Chilling injury was prevalent in fruit stored at -0.5 °C and increased over time in storage. A low incidence or no chilling injury occurred in fruit stored at higher temperatures. These

results support earlier reports where chilling injury in citrus fruit was reduced by increasing storage temperature (Chalutz et al., 1985; Schirra, 1992).

The role of oxidative stress on the occurrence of rind disorders has been investigated in other citrus cultivars and it was reported that antioxidants may play a role in protecting citrus fruit from chilling injury and rind staining (Sala, 1998; Sala and Lafuente, 2000; Sala and Lafuente, 2004). In the present study attention was focused on the effect of storage temperature and storage duration on the rind antioxidant capacity. The results showed that the ability of 'Nules Clementine' mandarin fruit stored at 7.5 °C to metabolise active oxygen species increased over time in storage, compared to fruit stored at -0.5 and 11 °C. However, since 7.5 °C was the temperature at which rind breakdown was most readily expressed, this result showed that the ability of the fruit to remove active oxygen species did not protect it from rind breakdown. Therefore, antioxidants may not play a direct role in the occurrence of rind breakdown, and consequently the occurrence of this disorder may not be due to an oxidative stress. However, the reduction in antioxidant capacity after 80 days of storage in fruit stored at -0.5 °C, which was concomitant with a significant increase in chilling injury, suggests that antioxidants play a role in the occurrence of this disorder. This result confirms earlier reports by Sala (1998) as well as by Sala and Lafuente (2000).

Decay, caused by *P. digitatum*, recorded in this study remained low, and only increased after 80 days storage in fruit stored at -0.5, 4.5 and 7.5 °C. No significant increase occurred in fruit stored at 11 °C even after 80 days. The possible reason for an increase in decay with extended storage was that the natural disease resistance of the fruit rind may have been reduced over time in storage possibly due advanced of senescence (Eckert and Brown, 1986; Schirra, 1992). It has been reported that decay development is suppressed in fruit stored at low temperatures (Eckert and Brown, 1986; Shellie and Skiria, 1998). However, conflicting results were reported in the present study, since fruit stored at 11 °C showed lower decay levels than fruit stored at lower temperatures.

Fruit stored at 7.5 and 11 °C were better coloured than fruit stored at -0.5 and 4.5 °C. The reason for improved colouration in fruit stored at higher temperature was carotenoid synthesis in storage. Between 20 and 80 days storage at 7.5 and 11 °C, there was a 79 and 58 % increase in total rind carotenoids of fruit stored at these respective temperatures. In fruit stored at -0.5 °C poor colour development was due to carotenoid degradation, as there was a 16%

decrease in total rind carotenoids in fruit stored at this temperature over the same time period of between 20 and 80 days. This result concurs with earlier findings on 'Navel' orange (*C. sinensis* [L.] Osbeck), where poor colour development was reported in fruit stored at subzero temperatures, and this trend was attributed to carotenoid degradation (Van Wyk, 2004).

Puffiness is a disorder that has been largely attributed to advanced maturity, tree vigour, and also to high humidity in the store room (Murata, 1997). However, our work showed that extended storage and increasing storage temperature increased the incidence of puffiness.

Storage temperature had a marginal effect, with no definite trend, on SSC and titratable acid content. This response may be due to citrus being non-climacteric, with low starch reserves, and hence, undergoing slow changes in internal quality during storage (Davies and Albrigo, 1994). However, extended storage was associated with reduced SSC and acid content. It has been reported that extended storage can result in citric acid consumption by using it as a metabolic substrate for respiration (Davies and Albrigo, 1994; Plaza et al., 2004), and this is a likely explanation for the results measured on 'Nules Clementine' mandarin fruit.

Rind moisture content showed no definite trends over time in fruit stored at the different temperatures. This suggests that, unlike other rind disorders that have been linked to moisture stress across the rind (Ben Yehoshua et al., 2001; Lafuente and Sala, 2002), rind moisture does not appear to be the cause for rind breakdown on 'Nules Clementine' mandarin fruit.

Conclusions

The main findings of this study were that rind breakdown and chilling injury are most readily expressed in fruit stored at 7.5 °C and -0.5 °C, respectively, compared to fruit stored at the other temperatures. Furthermore, the occurrence of these disorders was progressive, as they increased over time in storage. Differences in the levels of rind breakdown and chilling injury, between fruit from Saron and Franschhoek in 2002 and 2003, respectively, indicated that temperature was not the main cause for the occurrence of these disorders. Certain environmental or cultural practises may make the fruit more susceptible to rind breakdown and chilling injury, which are only expressed during storage at particular temperatures. In this study there was no evidence to suggest that rind breakdown of 'Nules Clementine' mandarin may be due to oxidative stress. However, the occurrence of chilling injury was associated with a decrease in the rind antioxidant capacity. Therefore, it cannot be ruled out that the

occurrence of this disorder was due to an oxidative stress. The cause for rind breakdown is unknown, but temperature management can greatly reduce the levels of these disorders in storage. Optimum post-storage quality on ‘Nules Clementine’ mandarin was achieved in fruit stored at 4.5 °C for less than 60 days.

Table 7.1. Rind and fruit characteristics at harvest of 'Nules Clementine' mandarin harvested in Saron and Franschhoek in the 2002 and 2003 seasons, respectively.

Variable	Saron (2002)	Franschhoek (2003)
Rind colour rating[1]	6.1	5.1
Hue angle of the rind	101.6	77.0
SSC (°Brix)	9.3	10.1
Titratable acid (%)	0.98	0.83
Juice content (%)	61.3	54.3
Fresh mass (g)	155.4	105.1
Equatorial diameter (mm)	68.5	63.3

1 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, and 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 7.2. Effect of storage temperature and storage duration on the quality of 'Nules Clementine' mandarin fruit after cold storage plus shelf-life, in fruit sampled from Saron in the 2003 season.

Response variable	Storage temperature (°C)[1]	Storage duration (Days) (Factor A)				Storage temperature (°C) (Factor B)				Prob>F[3]		
		20	40	60	80	-0.5	4.5	7.5	11.0	A	B	AxB
Rind breakdown (%)	-0.5	0.0a[2]	0.0a	0.0a	0.5a					0.001	0.001	0.001
	4.5	0.0a	2.1a	0.6a	3.7a							
	7.5	1.2a	24.0b	27.5bc	62.7d							
	11.0	0.4a	6.1a	8.0a	34.4c							
Chilling injury (%)	-0.5	2.6a	13.0b	32.4c	46.7d					0.001	0.001	0.001
	4.5	0.0a	0.0a	0.9a	0.0a							
	7.5	0.0a	0.0a	0.0a	0.0a							
	11.0	0.0a	0.0a	0.0a	0.0a							
Decay (%)		0.0a	0.1a	0.4ab	0.9b	0.1	0.2	0.4	0.9	0.002	0.051	0.986
Rind colour rating[4]	-0.5	2.7fg	2.8fg	3.4h	3.0g					0.322	0.001	0.001
	4.5	2.2d	2.9fg	2.6efg	2.3de							
	7.5	1.6c	1.2ab	1.3abc	1.5bc							
	11.0	1.1a	1.0a	1.0a	1.0a							
Hue angle of the rind		66.6	68.0	66.6	66.2	74.4c	72.8c	63.7b	56.5a	0.299	0.001	0.064

1 If itemised interaction occurred between factor A and B

2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

3 Two-way ANOVA with factor A being storage duration and factor B being storage temperature

4 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, and 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 7.3. Effect of storage temperature and storage duration on the quality of 'Nules' after cold storage plus shelf life, in fruit sampled from Franschhoek.

Response variable	Storage temperature (°C)[1]	Storage duration (Days) (Factor A)				Storage temperature (°C) (Factor B)				Prob>F[3]		
		20	40	60	80	-0.5	4.5	7.5	11.0	A	B	AxB
Rind breakdown (%)	-0.5	0.0a[2]	0.0a	0.0a	0.a					0.001	0.001	0.001
	4.5	0.0a	0.0a	0.0a	0.0a							
	7.5	0.0a	0.0a	1.7b	13.7c							
	11.0	0.0a	0.0a	0.0a	0.3a							
Chilling injury (%)	-0.5	0.0a	0.7ab	0.7ab	9.6c					0.001	0.001	0.001
	4.5	0.0a	0.0a	0.0a	1.0b							
	7.5	0.0a	0.0a	0.0a	0.0a							
	11.0	0.0a	0.0a	0.0a	0.0a							
Decay (%)	-0.5	0.0a	1.0ab	0.3ab	5.2c					0.001	0.040	0.011
	4.5	1.3a	0.0a	2.1ab	6.7c							
	7.4	1.2ab	2.7b	2.0ab	6.4c							
	11.0	0.7ab	1.7ab	2.3ab	1.8ab							
Rind colour rating[4]	-0.5	2.2ef	2.6gh	2.8hi	3.0i					0.840	0.001	0.001
	4.5	2.0def	2.1def	2.4fg	2.3fg							
	7.5	1.8d	1.7cd	1.4bc	1.3ab							
	11.0	1.8d	1.2ab	1.0a	1.2ab							
Hue angle of the rind	-0.5	71.7hi	69.9hi	70.2hi	72.6i					0.001	0.001	0.001
	4.5	68.4gh	65.9fg	64.4ef	65.0ef							
	7.5	64.0ef	63.1ef	59.5cd	56.8bc							
	11.0	62.0de	54.3ab	51.8a	51.7a							

1 If itemised interaction occurred between factor A and B

2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

3 Two way ANOVA with factor A being storage duration and factor B being storage temperature

4 Rind colour rating determined using the CRI rind colour chart set number 36, where 8 = poor coloured, dark green with no colour break, 1= deep orange and fully coloured fruit (CRI, 2004; Appendix 1).

Table 7.4. Effect of storage temperature and storage duration on the internal quality and rind moisture content of 'Nules' after cold storage plus shelf life, in fruit sampled from Franschhoek.

Response variable	Storage temperature (°C)[1]	Storage duration (Days) (Factor A)				Storage temperature (°C) (Factor B)				Prob>F[3]		
		20	40	60	80	-0.5	4.5	7.5	11.0	A	B	AxB
Puffiness (%)	-0.5	0.0a[2]	0.0a	0.0a	0.6ab					0.001	0.001	0.001
	4.5	0.0a	0.0a	0.0a	2.1b							
	7.5	0.0a	0.0a	0.0a	4.7c							
	11.0	0.0a	0.0a	0.0a	12.0d							
SSC[4] (%)		10.1b	9.8a	9.9a	9.8a	10.0b	9.8a	9.7a	10.1b	0.002	0.001	0.134
Titratable acid (%)		0.78d	0.69c	0.64b	0.58a	0.71c	0.67bc	0.63a	0.68bc	0.001	0.001	0.064
Core drying (%)		0.0a	0.0a	8.0b	10.0b	1.0	7.0	5.0	5.0	0.001	0.142	0.229
Rind moisture (%)	-0.5	67.8ab	69.1abcde	69.5bcde	67.6a					0.001	0.001	0.001
	4.5	70.0def	71.5f	70.7ef	69.5bcde							
	7.5	71.4f	68.7abcd	71.4f	68.4abcd							
	11.0	70.6ef	68.6abcd	69.6cde	68.2abc							

1 If itemised interaction occurred between factor A and B

2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

3 Two way ANOVA with factor A being storage duration and factor B being storage temperature.

4 Soluble solids content

Table 7.5. Effect of storage temperature and storage duration selected biochemical rind properties of 'Nules' after cold storage plus shelf life, in fruit sampled from Franschhoek.

Response variable	Storage temperature (°C)[1]	Storage duration (Days) (Factor A)			Storage temperature (°C) Factor B				Prob>F[3]		
		20	60	80	-0.5	4.5	7.5	11.0	A	B	AxB
Chlorophyll content (μg·g^{-1} DW)		1.4b[2]	1.5b	0.0a	1.2	1.0	0.9	0.8	0.001	0.546	0.398
Carotenoid content (μg·g^{-1} DW)	-0.5	23.2ab	20.5a	19.5a					0.001	0.001	0.001
	4.5	31.2c	29.6bc	36.2c							
	7.5	29.9bc	44.6de	51.4ef							
	11.0	36.9cd	69.1g	58.3f							
Antioxidant capacity (mM Trolox equivalents·g^{-1} sample)	-0.5	4.7b	4.7b	3.4a					0.001	0.001	0.001
	4.5	4.8b	5.0b	7.3d							
	7.5	4.8b	5.3c	7.6d							
	11.0	3.4a	5.4c	3.3a							

1 If itemised interaction occurred between factor A and B
2 Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.
3 Two-way ANOVA with factor A being storage duration and factor B being storage temperature

CHAPTER 8

OVERALL DISCUSSION AND CONCLUSION

The objective of this study was to quantify the effects of various factors on the development of rind breakdown of 'Nules Clementine' mandarin fruit, as well as to investigate an association between rind pigments and rind antioxidant capacity on the development of this disorder. This study showed that 'Nules Clementine' mandarin was more susceptible to rind breakdown than 'Oroval Clementine' mandarin. Furthermore, harvest date, storage temperature and storage duration significantly affected the development of rind breakdown of 'Nules Clementine' mandarin fruit. The other factors investigated, ethylene gas degreening and canopy position significantly affected rind pigments and in some cases also significantly affected the antioxidant capacity, but did not affect the development of rind breakdown.

The study showed that the two 'Clementine' mandarin selections studied, 'Nules' and 'Oroval', had similar characteristics at harvest but different sensitivities to rind breakdown. These findings confirm earlier reports by Van Rensburg and Bruwer (2000) and Van Rensburg et al. (2004) where 'Nules Clementine' mandarin fruit was also found to be more susceptible to rind breakdown. It was suggested that the reason for 'Oroval Clementine' mandarin fruit being more resistant to rind breakdown was the dark orange rind colour, due to higher carotenoids, developed by mature fruit (Wahl and La Grange, personal communication). However, this relationship could not be established in the present study. It is suggested that future research on rind breakdown of 'Nules Clementine' mandarin should also investigate certain markers in fruit that may be associated with rind breakdown. Molecular markers are now used in laboratories worldwide to identify genes involved in the development of postharvest disorders (Lafuente and Zacarias, 2006). Rind volatiles and changes thereof during storage should also be investigated.

Evident in the present study was seasonal variation in the occurrence of rind breakdown and differences in the sensitivity of fruit to the disorder from different production regions. For this reason it would seem, even though rind breakdown develops in storage, the susceptibility of fruit to rind breakdown is determined by fruit exposure to certain environmental conditions, in the absence of which rind breakdown may not be severe. It is also possible that cultural practices in certain orchards or in some seasons may make the fruit more or less

susceptible to rind breakdown. It is unclear in the present study whether the inherent quality of the fruit was influenced largely by environmental or cultural practices. However, it has been reported in previous research that the primary cause of rind breakdown is climatic. Fruit maturing during a warm autumn are more susceptible to rind breakdown (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). Rind staining of 'Navelina Navel' orange, which is a rind disorder also associated with collapsed oil glands and may therefore be morphologically similar to rind breakdown of 'Nules Clementine' mandarin, was also influenced by climatic factors (Lafuente and Sala, 2002). It was reported that fruit harvested during or after exposure to weather conditions favouring rind dehydration were more susceptible to rind breakdown (Lafuente and Sala, 2002). Agusti et al. (2001) concluded that sudden changes in relative humidity during colour break seem to be responsible for the natural development of rind breakdown of 'Navelate Navel' orange.

The effect of storage temperature and storage duration on the development of rind breakdown was confirmed in this study, with additional data generated. An increase in storage temperature increases the risk of rind breakdown (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004). However, in the present study it was shown that rind breakdown is most readily expressed in fruit stored at 7.5 °C compared to fruit stored above or below this temperature. The reason for this trend could not be established from the biochemical variables measured, and further research is thus required to establish why rind breakdown was highest at this temperature. Optimum quality of 'Nules Clementine' mandarin fruit was achieved by storage at 4.5 °C for <60 days. Previous studies showed that other rind disorders, which include rind pitting of 'Marsh' grapefruit (Petracek et al., 1995) and 'Fallglo' mandarin (Petracek et al., 1998), superficial flavedo necrosis (noxan) of 'Shamouti' orange (Ben Yehoshua et al., 2001), and rind staining of 'Navelina Navel' orange (Agusti et al., 2001; Alférez et al., 2003) also occur at high levels in fruit stored at non-chilling temperatures (>15 °C) compared to fruit stored at lower temperatures (<4 °C).

Ethylene gas degreening and fruit canopy position did not significantly affect the development of rind breakdown. It was shown that these two factors significantly affected rind pigments and in some cases antioxidant capacity, but these factors could not be associated with the development of rind breakdown after storage. Sala and Lafuente (2004) reported that ethylene by itself did not induce changes in the activities of antioxidant enzymes, but significantly reduced the incidence of rind staining in 'Navelina Navel' orange.

A direct association between antioxidant capacity and rind pigments in the development of rind breakdown of 'Nules Clementine' mandarin could not be fully established. It was found that fruit with rind breakdown had a lower antioxidant capacity and lower carotenoid content than fruit without rind breakdown. However, it could not be proven whether a lower antioxidant capacity or lower carotenoid content was the cause of rind breakdown and not a consequence thereof. Furthermore, differences in rind pigments and antioxidant capacity during storage could not be associated with the development of rind breakdown. From these results it is tempting to conclude that no relationship exists between rind antioxidant capacity and the development of rind breakdown. However, due to certain limitations of this study it would be inappropriate to arrive at such a conclusion. Firstly, a one dimensional (TEAC) assay was used to determine the antioxidant capacity, whereas it is appreciated that antioxidants cannot be measured in a one-dimensional assay due to their different characteristics and functions (Frankel and Meyer, 2000; Prior et al., 2005). Furthermore, antioxidant capacity measured only at harvest, after a particular treatment or after storage, does not reflect the changes in antioxidant capacity over time, since oxidative homeostasis requires a balance between the production of reactive oxygen species and the capacity to scavenge them (Mittler, 2000). Therefore, single point in time measurements may be misleading as they do not give the full reflection of oxidative balance. Future experiments should be designed with multiple sampling times to reflect the dynamic nature of oxidative balance (Toivonen, 2004).

Undoubtedly progress has been made in understanding rind breakdown of 'Nules Clementine' mandarin and some of the factors associated with the disorder. This progress should, however, not be allowed to hide the need for further fundamental research on rind breakdown as the cause of rind breakdown is still unknown and no commercial treatment is available for the control of the disorder. Future research on antioxidants, incorporating multiple sampling times and using more than one assay, is suggested. It is also suggested that research focusing on other biological/physiological aspects of the rind be conducted. These include rind water relations during handling and storage. Investigating rind volatiles may also help shed some light on the underlying causes of the disorder. Hopefully rind breakdown will then be better understood and the development of commercial treatments will become possible. At this stage, optimum fruit quality will be achieved in fruit degreened and stored at 4.5 °C for <60 days.

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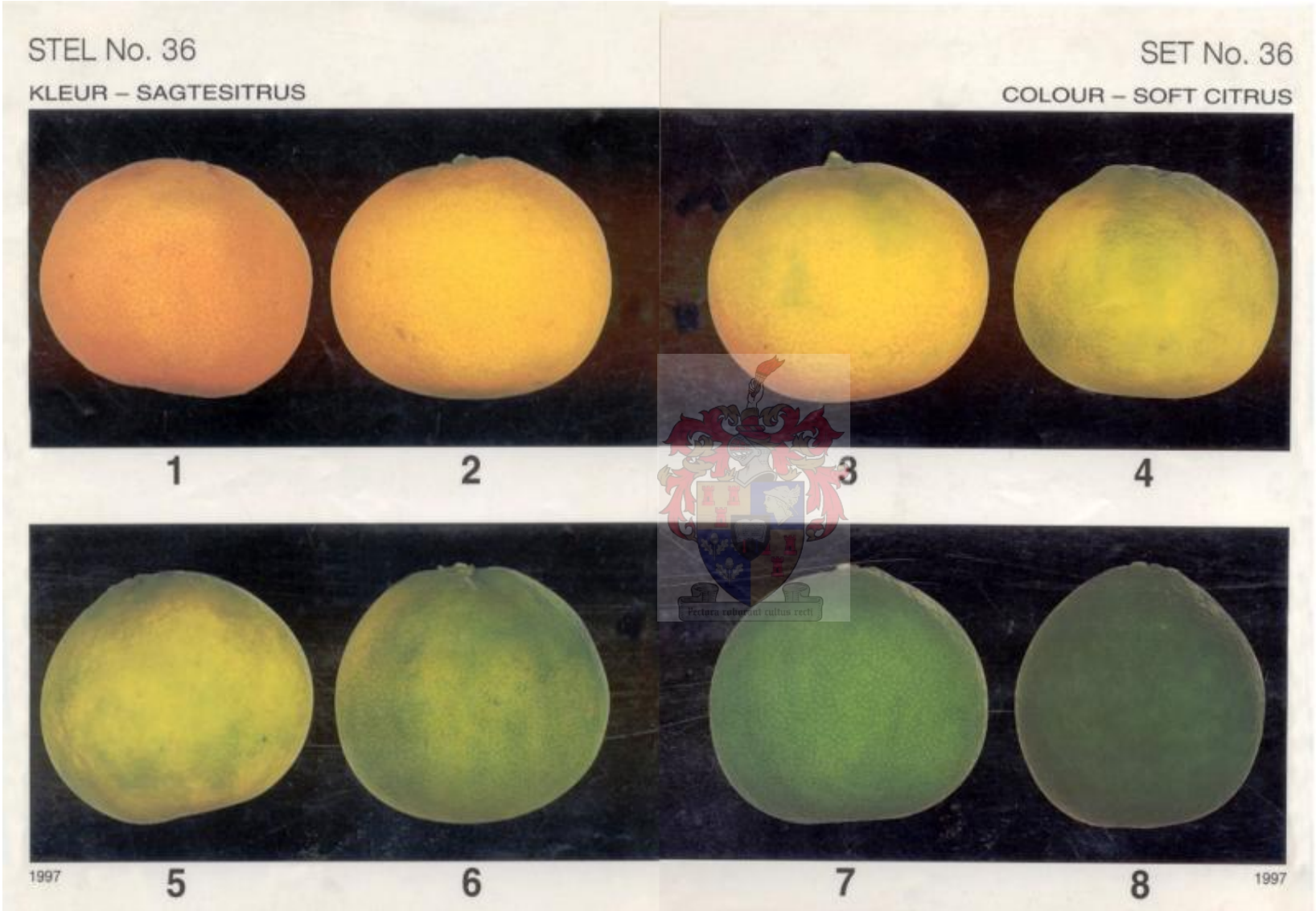

Appendix 1. Rind colour rating chart for soft citrus (CRI, 2004).