

**Internal flesh browning of ‘Cripps’ Pink’ apple
(*Malus domestica* Borkh.)
as influenced by pre-harvest factors
and the evaluation of near infrared reflectance spectroscopy
as a non-destructive method for detecting browning.**

by

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Declaration

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SUMMARY

The successful marketing of 'Pink Lady'TM apples produced in South Africa is under pressure due to internal flesh browning (IFB) development. IFB incidence is increased by long term controlled atmosphere (CA) storage at low temperatures and overmaturity of fruit. Australian research has identified pre-harvest factors which influence the susceptibility of fruit towards internal browning. The first aim of this study was to investigate temperature, mineral concentration, soil type and tree age as factors which influence incidence of browning under South African growing conditions. The second aim of this study was to evaluate NIR spectroscopy (NIRs) as a non-destructive tool for sorting out internally brown fruit before shipment.

The first trial investigated the effect of temperature during different growth stages on the incidence of browning types for Elgin and the Koue Bokkeveld. Diffuse browning was found in both seasons while radial browning was only found for the 2011/2012 season. Incidence of diffuse browning related to the difference between the maximum and minimum temperature during the cell division phase (0-50 DAFB) and high temperatures during the maturation phase (last 60 days before harvest). Radial browning related to maximum temperatures during the early cell expansion phase (100-150 DAFB). A third type of browning which exhibited browning patterns of both browning types was identified and named "combination" browning and was related to fruit size but not pre-harvest temperature. The second trial investigated the influence of tree age, soil type and mineral concentration of fruit on the incidence of browning. Soil type and tree age affected incidence of diffuse browning but not that of radial browning. Fruit harvested from orchards with sandy soil and young trees were more mature and were prone to develop diffuse browning. Higher potassium (K) concentration and K:Magnesium (Mg) ratios were related to non-brown fruit and the K:Mg ratio had a strong negative correlation with browning incidence.

NIR spectra were collected from fruit after 7 months CA storage (7M) at -0.5 °C and after 7 months CA at -0.5 °C + 4 weeks regular atmosphere (RA) 0.5 °C + one week shelf-life at 20 °C (7M4W7D) in pursuit of the second aim of this study. Incidence of diffuse- and "combination" browning increased from 7M to 7M4W7D while incidence of radial browning did not. Brown and non-brown fruit were successfully identified by means of PLS-DA at both storage stages. NIRs calibration and validation models showed that NIRs can predict total soluble solids concentration (TSS) concentration of 'Cripps' Pink' apples at 7M.

Anatomical investigation of tissue affected by "combination" browning with scanning electron microscopy (SEM) showed collapsed cells and large intercellular spaces below the fruit peel, as found for diffuse browning, and cell fracture and collapse of cortical tissue near the vascular bundles, as found for tissue affected by radial browning. Tissue of samples affected

by “combination” browning showed symptoms of both diffuse and radial browning thus, confirming that fruit affected by “combination” browning were susceptible to both browning types during long term storage.

OPSOMMING

Die suksesvolle bemarking van 'Pink Lady'TM appels in Suid-Afrika word benadeel deur interne verbruining. Verlente opbergingsperiodes by lae temperature (<0 °C) onder beheerde atmosfeer (BA) toestande, asook oorrypheid tydens oes verhoog die insidensie van verbruining. Australiese navorsing het vooroes faktore geïdentifiseer wat verbruining beïnvloed. Die doel van hierdie studie was om die invloed van temperatuur, grondtipe, boomouderdom en die minerale samestelling van die vrug op die insidensie van verbruining te ondersoek onder Suid-Afrikaanse produksietoestande. Die tweede doel was om die gebruik van naby-infrarooirefleksie spektroskopie (NIRs) te ondersoek as 'n moontlike tegniek vir die nie-destruktiwe identifisering van vrugte geaffekteer deur interne verbruining. Die eerste proef het die invloed van temperatuur gedurende verskillende ontwikkelingsstadia van die vrug op die insidensie van verbruiningstipes ondersoek in Elgin en die Koue Bokkeveld. Diffuse-verbruining het in albei seisoene voorgekom terwyl radiale-verbruining net in 2011/2012 voorgekom het. Diffuse-verbruining het 'n verband getoon met die verskil tussen maksimum en minimum temperature gedurende die selverdelingsfase (0 – 50 DNVB) en hoë temperature gedurende rypwording (60 dae voor rypwording). Hoë temperature gedurende die selvergrotingsfase (50 – 100 DNVB) het die insidensie van radiale-verbruining beïnvloed. 'n Derde tipe verbruining wat patrone van beide verbruiningstipes besit is geïdentifiseer en "kombinasie-verbruining" genoem. "Kombinasie-verbruining" was positief gekorreleer met vruggrootte maar nie met voor-oes temperatuur nie.

Die tweede proef het die invloed van grondtipe, boomouderdom en die minerale samestelling van die vrug op insidensie van verbruining ondersoek. Vrugte vanuit boorde met jong bome of sanderige grond was ryper en meer vatbaar vir diffuse-verbruining. Die insidensie van radiale-verbruining het nie verskil tussen boorde met verskillende grondtipes of boomouderdomme nie. 'n Hoër kalium (K) inhoud en 'n K:Magnesium (Mg) verhouding was waargeneem in nie-bruin vrugte en die K:Mg verhouding het 'n sterk negatiewe korrelasie met die insidensie van verbruining getoon.

NIR spektra is versamel vanaf vrugte na 7 maande BA opberging (7M) by -0.5 °C en na 7 maande BA + 4 weke in gewone atmosfeer (GA) by -0.5 °C + 1 week raklewe by 20 °C (7M4D7D) om die tweede doel van hierdie studie aan te spreek. Die insidensie van diffuse- en "kombinasie-verbruining" het vermeerder vanaf die 7M na die 7M4W7D tydperk, maar nie die insidensie van radiale-verbruining nie. Spektra van bruin en nie-bruin vrugte is suksesvol geïdentifiseer na die 7M en die 7M4W7D tydperk met behulp van PLS-DA. NIR kalibrasie en validasie modelle het aangedui dat totale oplosbare vastestowwe (TOVS) suksesvol voorspel kan word deur NIRs.

Weefsel van verbruiningstipes is ondersoek met behulp van skanderings-elektron mikroskopie om te bevestig of “kombinase-verbruining” deur beide verbruiningstipes beïnvloed word op anatomiese vlak. Ineengestorte korteks selle en groot intersellulêre ruimtes onder die skil is gevind in weefsel geaffekteer deur diffuse-verbruining. Selkrake en ineengestorte korteksselle langs die vaskulêre bondels is gevind in weefsel geaffekteer deur radiale-verbruining. Kenmerke van beide verbruiningstipes is gevind in weefsel geaffekteer deur “kombinasie-verbruining”. Dit bevestig dat vrugte wat geaffekteer is deur “kombinasie-verbruining” wel vatbaar was vir radiale- en diffuse-verbruining.

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GENERAL INTRODUCTION

Internal flesh browning (IFB) of 'Cripps' Pink' apples is a postharvest disorder associated with overmaturity of fruit and long term controlled atmosphere (CA) storage which is problematic in most production regions (Jobling and James, 2008; Majoni, 2012). Browning occurs when cell membranes are disrupted or damaged and the enzyme polyphenol oxidase (PPO) comes into contact with its substrate, phenolic compounds, to form brown coloured polymers (Mathew and Parpia, 1971; Mayer, 1987; Nicolas et al., 1994). The cause of membrane disruption or disintegration differs between browning types which leads to different patterns of browning based on the affected area of the fruit tissue. Radial browning affects cortical tissue closest to the vascular bundles and diffuse browning affects the cortical cells below the peel, moving towards the centre of the fruit (James, 2007).

Radial- and diffuse browning do not only differ according to browning patterns inside the fruit but also according to causal factors and induction of browning development in fruit. Radial browning is the result of senescent breakdown (Wilkinson and Fidler, 1973) and could be the result of harvesting fruit at post-optimal maturity. Increased concentration of CO₂ (>1 %) in CA storage seems to increase the likelihood of 'Cripps' Pink' apples developing radial browning during long term storage (Jobling and James, 2008). Diffuse browning is caused by chilling injury (Bramlage et al., 1980; James et al., 2005) and/or overmaturity of fruit in combination with a long storage period (4-6 months) at CA conditions (Majoni, 2012). During long term storage a build-up of CO₂ in fruit causes stress which cause reactive oxygen species to accumulate and in turn cause the breakdown of cell membranes (de Castro et al., 2007). Low storage temperature may also induce a stress response in fruit and cause a build-up of reactive oxygen species (ROS) and an impeded antioxidant turn-over (Bartosz, 1997; Wismer, 2003). Environmental or management practises which lead to overmaturity of fruit (Jobling and James, 2008; Steyn et al., 2004) or sensitivity towards chilling injury during storage (DeEll et al., 2005; Lau, 1998) may predispose fruit to browning development during long term storage.

Susceptibility towards chilling injury or senescent breakdown is influenced by pre-harvest factors such as temperature (DeEll et al., 2005) and mineral content of fruit (Marcel, 1995). Australian research has shown that accumulated growing day degrees above 10 °C (GDD_{>10 °C}) can be used to discern between Australian production regions which are susceptible to development of radial and diffuse browning (James, 2007). Fruit grown in warm production regions which accumulate more than 1200 GDD_{>10 °C} during the growing season (September to April) are prone to radial browning development while fruit grown in cold production regions, less than 1200 GDD_{>10 °C} during the growing season, are prone to

diffuse browning development during long term CA storage (James et al., 2010). In a previous study of internal browning in South Africa it was found that diffuse browning is the main type of browning to develop (Crouch, 2014; Majoni, 2012) even though South African production regions are considered 'warm' ($GDD_{>10^{\circ}C} >1200$) (Engelbrecht et al., 2011). Temperature influences many aspects of fruit development (Bergh et al., 1990). Various temperature parameters influence physiological fruit growth and development during different stages, which in turn could influence the browning development (Lau, 1998).

Internal flesh browning is well known for its unpredictable nature and variation in incidence from one season to the next. While many possible factors influence browning and the different types of browning, the stress induced disintegration of membranes caused by high levels of CO_2 in CA storage is the key to induction of this disorder (de Castro et al., 2007; Hodges, 2003; Jobling and James, 2008). Membrane integrity is influenced by mineral composition of the fruit in the form of availability and concentration of minerals such as calcium (Ca) or boron (B) (Poovaiah, 1993; Wojcik and Wojcik, 2003). Ca and B stabilize the cell wall structure (Neilson and Neilson, 2003) and Ca also stabilizes phospholipids in the cell membrane structure (Marinos, 1962). Mineral content of fruit is subject to soil type and tree age (Sposito, 1984; Tromp, 1980). In orchards with sandy soils minerals are easily leached and vary throughout the season and from one season to the next (Rengasamy and Churchman, 1999). In orchards with clay soils the reservoir of minerals in the soil is bigger and not easily leached. Clay soils may hold minerals too tightly in some cases that roots cannot extract the minerals from the soil (Sposito, 1984). Young trees show vigorous growth which leaves fruit with a deficit of minerals as shoots of young trees compete better for photosynthates compared to fruit (Hanger, 1979). Fertilization of nitrogen (N) during late summer supports vigorous shoot growth, which competes with fruit for other minerals and leaves fruit with mineral deficits (Perring and Jackson, 1975). Minerals interact with each other by increasing or decreasing the flow of another mineral to the fruit (Ferguson and Watkins, 1981). As Ca and Mg are cations with the same charge they compete for the same positions within the fruit cells. Fruit disorders have been linked to mineral content of the fruit (de Freitas et al., 2010). The effect of tree age and soil type on the mineral composition of fruit may influence the susceptibility of fruit towards development of internal browning as minerals such as phosphorus (P) and Ca have shown relationships with low temperature breakdown and senescence breakdown (Perring, 1968).

As internal flesh browning is not visible from the outside of the fruit it can only be found when fruit are destructively investigated for browning patterns on the inside of the fruit. Browning is mostly found after shipment to overseas markets or when a consumer finds it at home during shelf-life (Majoni, 2012). This leads to great financial losses and a decrease in popularity of the fruit with consumers. Non-destructive detection of disorders such as watercore and

browning of table grapes has been made possible with the use of near infrared reflectance spectroscopy (NIRs) (Birth and Olsen, 1964; Daniels, 2013). NIRs is a process where the reflectance, transmittance and absorption of light in the near-infrared range is affected by internal factors of the fruit and displays some of the chemical and physical parameters (Nicolai et al., 2007). Analytical tools, such as partial mean square (PLS) models can be built to correlate light scattering, caused by internal compounds of fruit, with physiological characteristics of the fruit (Wold, 1982). The success of these models is influenced by the penetration depth of the light (Hother et al., 1995) and the detection of physical components which are related to the physiological disorder (Xing and Guyer, 2008). It is important to identify fruit with internal browning symptoms as well as fruit which have the potential to develop browning so that these fruit could be removed before shipment to avoid financial losses.

This study had three main aims: (1) to determine the influence of temperature parameters during different growth periods on the development of IFB types and incidence, (2) to determine the effect of soil type, tree age and mineral composition of fruit on the incidence of IFB type and incidence and 3) to investigate NIRs as a non-destructive tool for identification of internal browning. The studies were carried out on fruit grown in the Koue Bokkeveld and Elgin in the Western Cape, South Africa in the 2011/2012, 2012/2013 seasons and also in the 2013/2014 season for the last objective.

This study aims to identify causal factors of internal browning of 'Cripps' Pink' apples under South African growing conditions as well as ascertain if it is possible to detect internal flesh browning non-destructively with NIR.

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LITERATURE REVIEW

INTERNAL FLESH BROWNING OF ‘CRIPPS’ PINK’ APPLES AS INFLUENCED BY MINERAL NUTRITION AND CLIMATE AND THE EVALUATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY AS A NON-DESTRUCTIVE METHOD FOR DETECTING INTERNAL BROWNING.

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1. INTRODUCTION

1.1 'Cripps' Pink' cultivar and the Pink Lady™ trademark

The Cripps' Pink apple cultivar was developed as a cross between Golden Delicious and Lady Williams in 1973 (Cripps et al., 1993). The great taste of 'Golden Delicious' and the good storage quality of 'Lady Williams' has been combined into a product which has been so successful commercially that a new registered trademark has been developed to market high quality 'Cripps' Pink' apples as 'Pink Lady'™ apples. This trademark belongs to Apple and Pear Australia Ltd. Marketing of the 'Pink Lady'™ brand has led to higher prices for farmers producing this quality standard of 'Cripps' Pink' apples (Studdert, 2002; Wilkinson, 2000). Characteristics specific to 'Pink Lady'™ apples are a blush percentage of 40 % or more, flesh firmness of 7.0 kg measured with a 7.9 mm penetrometer tip and a desired total soluble solid requirement of 15.0 % (Hurndall and Fourie, 2003). With its medium chilling requirement, these apples can be successfully grown in most growing regions. Consistent superior quality regardless of where the fruit are grown was expected from growers. However, each growing region has its own challenges regarding the production of high quality 'Cripps' Pink' apples (de Castro et al., 2007; James, 2007; James et al., 2005). The biggest problem regarding 'Cripps' Pink' apples is internal browning which occurs after a prolonged period of cold storage in a controlled atmosphere (CA) environment under low temperatures (Jobling and James, 2008).

1.2 Internal flesh browning

Internal flesh browning (IFB) of 'Cripps' Pink' apples occurs because of cold injury (James et al., 2005) and overmaturity of fruit (Majoni, 2012; Wilkinson and Fidler, 1973), a deficit in Ca content of fruit at harvest (de Castro et al., 2008) and prolonged storage under CA conditions (Jobling et al., 2004; Crouch et al., 2014; Zanella et al., 2004). This disorder leads to the development of three different types of browning. Discoloration because of CO₂ damage has been broadly studied and described in other apple cultivars (Clark and Burmeister, 1999; Grant et al., 1996; Streif and Saquet, 2003; Voltz et al., 1998). CO₂ damage of 'Cripps' Pink' does not differ significantly from CO₂ damage in other apple cultivars and will, therefore not be studied with the same depth as the other two types of internal browning, namely radial flesh browning (RFB) and diffuse flesh browning (DFB). RFB can be distinguished from DFB by the different pattern of affected tissue (James, 2007). Fruit predisposed to RFB have a brown discoloration of cortical tissue directly next to the vascular bundles (Fig. 1). In DFB vascular tissue remains unaffected and the cortical tissue starts to discolour to brown under the fruit peel (Fig 1). DFB can affect all remaining cortical tissue except for tissue next to the vascular bundles in severe cases. Research has been

conducted to correlate probable causes with IFB incidence of 'Cripps' Pink' apple (de Castro et al., 2007; Crouch et al., 2014; James, 2007; Jobling et al., 2004; Majoni, 2012). However it is important to ascertain research gaps and limitations and determine its relevance under South African conditions.

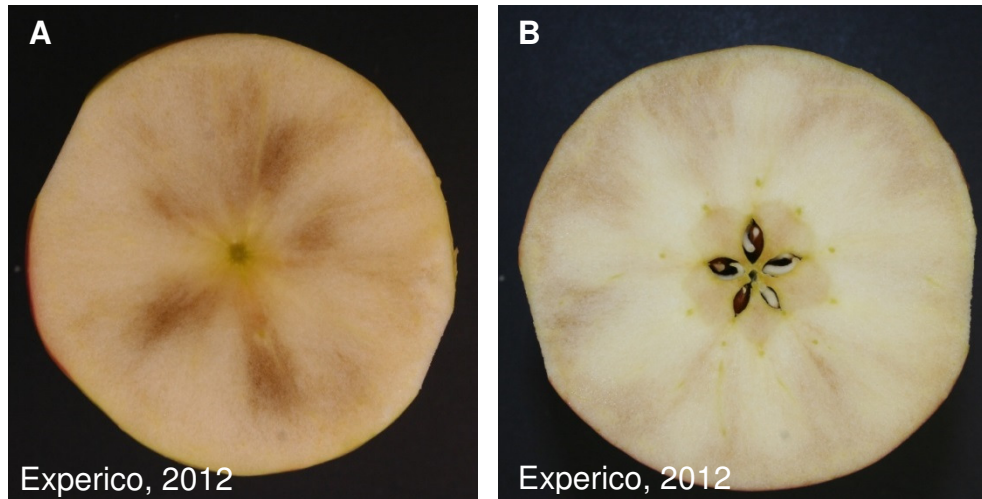


Figure 1: A) Radial browning and B) diffuse browning found in a 'Cripps' Pink' apple harvested in 2011/2012 in the Western Cape, South Africa.

2. Minerals

2.1 Effect of mineral content of fruit on internal flesh browning incidence

Physiological postharvest disorders are only found in a small percentage of susceptible fruit instead of being a general occurrence under all stored fruit (Bramlage, 1993). This leads to question of the effect of pre-harvest attributes, such as mineral content of fruit, on the postharvest quality of fruit. Correlations between fruit mineral content and fruit quality cannot assure a causal relationship between a specific mineral and a disorder except for those found annually on a consistent basis (Marcelle, 1995). Minerals such as calcium (Ca), magnesium (Mg), potassium (K), nitrogen (N), phosphorous (P) and micro minerals such as boron (B), zinc (Zn) and manganese (Mn) are known to correlate with postharvest quality of fruit. Lau and Looney (1978) found a higher concentration of N, Mn, and Zn and lower Mg and K concentrations in 'Golden Delicious' fruit with CO₂ injury. In some cases correlations between mineral content of fruit and disorder incidence may be as direct as associations of minerals with cell membranes and cell function and in other cases indirect such as association through their effect on shoot growth, blush development and interactions with other minerals (Cakmak, 1994; de Castro et al., 2008; Mason et al., 1975; Perring, 1968). Minerals such as Mg (de Freitas et al., 2010), Ca (Bangert, 1979) and B (Wojcik and Wojcik, 2003) have a direct role in membrane stability and could influence susceptibility of fruit towards IFB development by altering membrane strength. It is necessary to investigate the

role of different minerals in fruit to understand how minerals may have an effect on postharvest storage quality of fruit. Therefore, the following summary of important minerals and their role in fruit is discussed.

2.1.1 Nitrogen

Nitrogen influences maturity indices such as fruit firmness and eating quality parameters such as dry matter content and refractometric index. In 'Cox Orange Pippin' and 'Golden Delicious' soluble sugar concentration and acidity decreases with an increase in N content of fruit (Marcelle, 1995). A higher N concentration causes excelled growth resulting in larger fruit cells which contributes to a decrease in firmness at harvest (Perring, 1968). An increase in postharvest disorders as well as susceptibility towards pathogen infection can be related to excessively high fruit N content (Bramlage, 1993; Shear and Faust, 1980). It is known that advanced maturity at harvest influences internal flesh browning (Crouch et al., 2014). Blush development is delayed by high N content and yellowing of fruit during storage may be impeded by this high N content (Fallahi et al., 1997; Raese and Drake, 1997). Blush development is important for 'Cripps' Pink' growers as high coloured fruit generate a higher income (Hurndall and Fourie, 2003)

High N in trees lead to vigorous growth which can lower Ca content of fruit through increased competition between shoots and fruit for Ca (Perring and Jackson, 1975). The increase in fruit size caused by high N of trees, lowers fruit Ca by dilution (Perring and Jackson, 1975).

2.1.2 Calcium

The Ca content of fruit is strongly related to postharvest quality. With an increase in Ca content of fruit a decrease in susceptibility of fruit towards postharvest disorders as well as a decrease in susceptibility to pathogen infection is found (Conway and Sams, 1983; Poovaiah, 1993). Although it is known that high Ca concentrations may reduce the incidence of IFB it is also true that a low Ca concentration in fruit does not result in higher IFB occurrence (de Castro et al., 2007). This highlights the importance of other minerals in the development of this particular disorder.

The role of Ca in fruit comprises mainly of the sustaining and strengthening of cell walls and cell membranes as well as mediating the transfer of extra cellular signals into intracellular biochemical reactions (Marinos, 1962; Poovaiah, 1985). With a decrease in fruit Ca concentration to a point where a deficit occurs, cell membranes are weakened making cells more susceptible to cell breakdown (Bangert, 1979; Shear, 1975). At this point the pool of free Ca external to the plasma membrane lessens to such an extent that signaling is

disrupted and cell activity is dysfunctional (Ferguson and Drobak, 1988). Extracellular Ca concentrations decrease as fruit ripens (Ferguson et al., 1995). This decline may be due to changes in cell wall pectin molecules when more binding sites for Ca become exposed because of pectin breakdown during early stages of ripening and thus resulting in a shift in equilibrium between bound and free Ca (Ferguson et al., 1995). A positive correlation between Ca content of fruit and fruit firmness exists because of the maintaining effect which Ca has on cell walls (Marinos, 1962). Ca may influence harvest maturity due to its retarding effect on the climacteric rise as it decreases the climacteric maximum by decreasing fruit respiration and ethylene emission (Marcelle et al., 1989). High Ca concentrations in fruit lead to a decrease in *lipoxygenase* activity, 1-aminocyclopropane-1-carboxylic-acid (ACC) content and therefore ethylene emission which delays senescence (Marcelle, 1991). In fruit maturity indices, Ca is negatively correlated to content in soluble sugars, the refractometric index and the percentage of dry matter.

Fruit with IFB after storage had lower Ca concentrations (de Castro et al., 2008). In studies by de Castro et al. (2008) the influence of antioxidants on browning incidence were investigated. A negative relation between the concentrations of the antioxidant ascorbic acid and brown tissue was found. Majoni et al. (2013) investigated how maturity of 'Cripps' Pink' relates to IFB and found that overmature fruit were characterized by membrane disruption and fruit exhibiting DFB showed an increase in lipid peroxidation and decrease in ascorbic acid levels and linolenic acid. As antioxidants protect the cell membrane from oxidative stress and ascorbic acid in combination with Ca preserves the stability of membranes. The possibility of a deficit in Ca and ascorbic acid at storage should therefore be considered as a cause of IFB. In 'Cripps' Pink' Ca levels of 1.5 % should be maintained in leaves and Ca should be included in a spray program (Hurdall and Fourie, 2003).

2.1.3 Potassium

Fruit low in potassium (K) content is more susceptible to low temperature breakdown (Bramlage, 1993) and cold injury is one of the leading causes of DFB (Bramlage et al., 1980; James et al., 2005). Resistance towards chilling injury in fruit high in K is associated with an increase in phospholipids, membrane permeability and improvement of the biochemical and biophysical properties of cells (Hakerlerker et al., 1997). K increases resistance towards chilling temperatures by regulating osmotic and water potential of cell sap and reducing electrolyte leakage (Singer et al., 1996). K is important for the eating quality of the fruit as it influences the acidity and sugar concentration of fruit of which the balance between acids and sugars are most important (Marcelle, 1995). Titratable acidity (TA) plays an important role in respiration as these organic acids are intermediates in the citric acid cycle. Changes in titratable acidity are related to the rate of metabolism of the fruit (Clarke et al., 2003) and

TA decreases with an increase in duration of storage time (Ghafir et al. 2009). Although high K of fruit would be associated with high TA, which is needed for long term storage as a pool to sustain respiration, high concentrations of K in fruit greatly increases the risk for storage disorders by inducing a Ca deficiency (Bramlage, 1993). An optimum K concentration in fruit would be such that low temperature breakdown is not induced by too low K levels and Ca deficiency is not induced by too high concentrations of K. An optimal concentration of fruit should promote TA accumulation in fruit and thus maintain a good storage potential of fruit.

2.1.4 Phosphorus

Low P levels of fruit are related to increased susceptibility of fruit towards low temperature breakdown and senescent breakdown (Bramlage, 1993). P correlates positively with firmness of fruit and influences the crispness, eating quality and storage potential of the fruit (Faust, 1989). As DFB is related to chilling injury it is especially important to note that P has a negative correlation with susceptibility of fruit towards low temperature and senescence breakdown (Marcelle, 1995), potentially due to its involvement as energy currency ATP, much needed for recovery during chilling injury (Jackson, 2003; Neilson and Neilson, 2003; Yogaratnam and Sharples, 1982).

2.1.5 Magnesium

'Cripps' Pink' is susceptible towards Mg deficiency which may be exacerbated by high K levels (Hurndall and Fourie, 2003). Mg has a detrimental effect on storage quality of fruit (Bramlage, 1993), although pre-harvest it is important in photosynthesis (Ferguson and Watkins, 1989). It is an antagonist of K and as it competes with Ca for binding sites during its transport it suppresses Ca uptake into fruit tissue leaving the fruit susceptible to postharvest disorders and diseases (Marcelle, 1995). Transport of Mg takes place towards damaged tissue (Perring, 1985; Perring and Pearson 1986). Bitterpit of apples is associated with high Mg and low Ca concentrations (Faust and Shear, 1968) as high Mg will reduce uptake of Ca into fruit tissue (Ferguson and Watkins, 1989) and competes with Ca for binding sites in the cell membranes (de Freitas et al., 2010). Weakening of cell membranes associated with this competition between magnesium and calcium may cause susceptibility towards IFB.

2.1.6 Boron

Boron is an important mineral for the growth of pollen tubes as the mineral stimulates the growth rate of the pollen tubes (Obermeyer et al., 1996). It forms complexes with constituents of cell walls and is involved in cell elongation and nucleic acid metabolism. Deficiencies may lead to the development of a reduction in cell wall elasticity (Findekle and

Goldbach, 1996) so that leaves become brittle. B sprays during full bloom or after harvest have decreased membrane permeability of fruit at harvest and internal browning incidence in 'Conference' pears (Wojcik and Wojcik, 2003).

2.2 Mineral interactions

From the information gathered from various sources, it cannot be said that a specific mineral is the cause of IFB. It is important to look at interactions between minerals and how combinations of minerals lead to the development of the disorder.

In trials done by de Castro et al. (2008) fruit with CO₂ injury, which is similar to IFB, had higher N, Mn and Zn concentrations and lower concentrations of K and Mg. It is known that high K concentrations may induce Ca deficiency (Voogt, 1998). While Ca has a sure role in the retention of postharvest quality its functionality is obscured by varying concentrations of other elements (Bramlage, 1993). Low N:Ca ratios are associated with good storage quality of fruit (Bramlage, 1993). A high K content in relation with a low Ca content of fruit increases the risk of postharvest disorders as well as diseases (Marcelle, 1995), thus creating a desired low K:Ca ratio at harvest should be established to optimize storage quality. The K content of trees change the distribution of Mg, and low K content of leaves diverts a large part of Mg to fruit or other storage organs (Lüdders et. al, 1973). High levels of Mg in fruit reduce the uptake of Ca into fruit (Ferguson and Watkins, 1989). Excessive Mg in relation to Ca or K can lead to the development of deficiency symptoms of Ca or K (Tisdale et al., 1985). K is known to translocate sugars to sink tissue, thus supplying fruit with photosynthates needed for normal cell function (Itoh and Kumura, 1987). Even though adequate K content of fruit is important to support foliage and normal cell function it is rather the effect of high K on increased fruit growth and dilution of Ca content of fruit or the effect of low K and its association with increased Mg content of fruit which competes with Ca for binding sites which leave fruit susceptible to storage disorders (de Freitas et al., 2010).

Diffuse flesh browning incidence is correlated with decreasing ratios of Ca:P and Ca:Mg (James, 2007). The role of Ca in delaying senescence by reducing micro-viscosity of membranes (Poovaiah, 1993) may impede the development of DFB during long term storage.

Table 1: Influence of different minerals at different levels on aspects of fruit development which may influence internal browning of 'Cripps' Pink' apples.

Mineral	Content	Influence on fruit quality	Reference
Nitrogen	High	Decrease in TSS and TA Excelled growth = larger fruit = lower firmness Delayed blush development Increased vigour = lower Ca by dilution	Marcelle, 1995 Perring, 1968 Fallah et al., 1997; Rease and Drake, 1997 Perring and Jackson, 1975
Calcium	High	Decreased susceptibility towards postharvest disorders Decrease in LOX activity, ACC concentration and thus ethylene emission	Conway and Sams, 1983
	Low	Weakening of cell membranes, disrupted signalling, dysfunctional cell activity	Ferguson and Drobak, 1988
Potassium	High	Induces Ca deficiency	Bramlage, 1993
	Medium	Increasing K = increasing TA	Marcelle, 1995
	Low	Increase susceptibility of fruit towards low temperature breakdown	Bramlage, 1993
Phosphorus	Low	Increased susceptibility of fruit towards low temperature breakdown and senescence breakdown	Bramlage, 1993
Magnesium	Low	Breakdown of feeding-root system which leads to decrease in mineral uptake from soil	Shear, 1980
	High	Competes with Ca for binding sites in cell membranes and reduces the uptake of Ca	de Freitas et al., 2010 Ferguson and Watkins (1989)
Boron	Low	Reduced cell wall elasticity	Obermeyer et al., 1996

2.3 Growth Factors

Mineral content of fruit can be influenced by various growth factors. Crop load, light distribution, harvest maturity, blush development and rootstock are some of the factors determined by growers or growing conditions which influence mineral uptake and distribution to fruit and will be further discussed.

2.3.1 Crop load

In work done by de Castro et al. (2007) it was found that during years when trees had a light crop load IFB incidence was lower than in years with high crop loads. In contrast to this lower cropping trees could show more IFB because of their vigorous growth and higher shoot:fruit ratios which influences the mineral movement into fruit negatively (Hanger, 1979; Perring and Jackson, 1975). In more vigorous growing trees a strong competition exists between fruit and shoots. Shoots are a stronger sink than fruit competing for minerals, thus leaving fruit with a mineral deficit, particularly Ca (Hanger, 1979). A light crop load leads to the development of larger more dense fruit which give more resistance to gas diffusion, thus leading to the development of anoxic conditions in the fruit (Little and Holmes, 2000; Voltz et al., 1999). Larger fruit also have lower Ca concentrations because of the effect of dilution (Perring and Jackson, 1975). Radial flesh browning incidence has been correlated with low crop load and an associated decrease in fruit Ca concentration (James, 2007).

The reasons behind the light crop load should be brought into consideration as the fruit mineral content from a light crop due to thinning will differ from the fruit mineral content of a light crop due to a natural occurrence. In fruit from light cropping trees due to thinning, the Ca content of fruit are the same as fruit from heavy cropping trees but the K, P and Mg content of fruit are higher (Sharples, 1964 and 1968).

With heavy cropping trees more fruit develop on the tree which forces fruit to compete for minerals, thus causing a decrease in the fruit mineral content especially Ca. Over cropping of trees decreases the ascorbic acid concentration of fruit (Kondo, 1992). In work done by Marcelle (1995) on 'Cox Orange Pippin' and 'Golden Delicious' it was found that years with a high yield tend to have fruit with a low fresh fruit weight or fruit diameter. It was found that larger fruit had lower Ca concentrations. Fruit size and yield was not always correlated but the occurrence of a negative correlation between Ca concentration and yield appeared to be more consistent (Marcelle, 1995).

2.3.2 Fruiting position, leaf area, seed number and pollination

Location within the canopy has been shown to affect the fruit mineral content and generally fruit higher in the tree canopy will have a lower Ca content than fruit lower in the tree (Barritt et al., 1987; Ferguson and Triggs, 1990; Jackson et al., 1971; Voltz et al., 1993). Fruit on bearing positions on two-year-old wood has a lower Ca concentration than fruit on bearing positions on three-year-old spurs (Schumacher et al., 1979). Terminally positioned fruit has a higher Ca content than fruit on lower bearing positions and fruit on one-year-old laterals tend to have lower Ca concentrations (Voltz et al., 1994). The major effect of fruiting position lies in the effect which surrounding leaves have on fruit in the fruiting position. With an increase

of leaves surrounding a fruiting position the influx of Ca to the developing fruit will be increased and fruit surrounded by a smaller leaf area will have lower Ca concentrations (Ferguson et al., 1995).

Successful pollination of flowers is necessary for fruit set and the formation of seeds. With better pollination comes better seed formation which leads to fruit with more seeds (Ferguson et al., 1995). Lower Ca concentrations have been found in fruit with less seeds or fruit which developed by parthenocarpic fruit set (Bangert, 1976; Bramlage et al., 1990; Tomala and Dilley, 1990). Fruit with more seeds produce more auxins which increases the flow of calcium to fruit as the fruit become stronger sinks (Bangert, 1976).

As poor bearing positions, leaf area and seed number may lead to the development of fruit with mineral deficiencies these factors may influence the storage quality of fruit (Bangert, 1976).

2.3.3 Rootstock

The rootstock-scion interaction is crucial for production of high quality fruit. As rootstock influences a variety of growth factors such as leaf gas exchange, plant size, timing of fruit set, water relations, mineral uptake, plant size, yield efficiency, blossoming and fruit quality (Nielsen and Kappel 1996; Schmitt et al. 1989); it is important to choose a rootstock-scion combination best suited for the specific cultivar and growing conditions. The mechanisms involved in the manner of rootstock influence on tree vigor is unknown to date but some dwarfing mechanisms have been studied including hydraulic conductivity differences between rootstock and scion (Simons and Chu, 1984; Ussahatanonta and Simons, 1988), lower total sap solute concentration (Jones, 1984), reduced growth and hormone production (Sorice et al. 2002) and low nutrient uptake capacity (Ebel et al. 2000; Simons and Swiader, 1985). Differences between the effect which invigorating and dwarfing rootstocks have on the scion, include a consistently higher stem water potential in scions grafted on invigorating rootstocks (Olien and Lakso 1986).

'Cripps' Pink' is a semi vigorous variety and as the production of good quality fruit becomes more challenging with a more vigorous tree, a semi-vigorous rootstock such as M793 or M25 is used (Hurndall and Fourie, 2003). The early maturation of fruiting wood on a M25 rootstock reduces the vigorous growth of the scion.

The choice of rootstock should be closely correlated with the soil type and planting distance so that optimal vigor according to soil type and light distribution throughout the whole tree is met.

2.3.4 Soil type and soil management

The soil in which the tree is planted is the basis for most mineral uptake from stored minerals as well as applied minerals by fertilization. Soil is a complex of substances which all interact with mineral elements. The most important differences in soil types regarding their effect on growth and fruit quality would be the cationic exchange quotient, pH of the soil and the soil texture. As inorganic soil particles are negatively charged, mineral cations such as ammonium (NH_4^+), calcium (Ca^{2+}) and K^+ will be absorbed to the negative surface charge of the particles (Sposito, 1984). Clay soils will hold on to soil water longer while sandy soil will lose water more quickly because of the finer texture of the clay in comparison to the coarse texture of the sand (Rengasamy and Churchman, 1999). Clay soils have a larger cation capacity, thus a larger mineral pool for a tree to extract from but, also a higher potential for minerals to interact with each other and compete for uptake (Rengasamy and Churchman, 1999). Sandy soils have a smaller capacity for cations and minerals, and are easily leached from the soil leaving them out of reach of tree roots and are then unobtainable (Rengasamy and Churchman, 1999). However, minerals are more readily available and uptake by trees takes place using less energy than in clay soils due to its low or no charge which holds onto these cations in clay soils (Rengasamy and Churchman, 1999). Soils with a high organic composition have good water holding capacity and a high cation exchange capacity (Carter et al., 1986).

Soil pH affects the availability of different minerals. Slightly acidic soils favour root growth as well as the weathering of mother material that releases Mg, Ca, K and/or Mn, thus making these soils richer in the minerals of which the mother material comprised of (Sposito, 1984). The solubility of sulfates, phosphates and carbohydrates are advanced in slightly acid soils which increases the availability of these minerals to the roots (Sposito, 1984)

During fertilization it should be kept in mind that 'Cripps' Pink' is insensitive towards high N in soil which leads to a loss in fruit quality both internally (internal browning) and externally (hammering) (Hurdall and Fourie, 2003).

2.4 Relationship between mineral nutrition with fruit development

The above mentioned influences on fruit mineral content are all external factors and factors such as development of the fruit through the growing season should be considered.

Throughout the development of fruit a series of mineral sprays are given to increase fruit quality at harvest. P sprays during the early growth stages of rapid cell division are effective as it results in an increase in storage quality and reduction in low temperature breakdown of fruit (Johnson and Yogaratnam 1978; Yogaratnam and Sharples, 1982). This is due to the effect which P has on cell division and membrane lipids as well as the rapid development of

the fruit core in relation with the cortex which leads to higher concentrations of P and Mg residing in the core zone of the fruit.

As Ca is an important mineral in the development of internal flesh browning, this literature review briefly focusses on how Ca is taken up and at what growth stages this uptake takes place. Cellular uptake of Ca takes place in two distinct phases, a slow phase which is stretched over a longer period and a short swift phase. During the slow phase a smaller amount of Ca is taken up at a much slower rate into the vacuole and the cytoplasm (Ferguson and Watkins, 1981). In studies to find the extent to which apple fruit tissue take up Ca, Ferguson and Watkins (1981) found that the Ca uptake capacity of 'Cox' fruit tissue declined during initial fruit growth and that fruit tissue is most responsive to Ca uptake during initial fruit development (0-50 DAFB). During the swift phase of Ca uptake, the force of uptake is largely driven by exchange and it is during this time of early cell growth that Mg uptake predominates and inhibits Ca uptake (Ferguson and Watkins, 1981).

Maturation of fruit affects postharvest quality as over maturity leads to susceptibility to a wide array of disorders. South African research has shown that harvest maturity plays a role in fruit susceptibility to IFB (Bergman et al., 2011; Crouch et al., 2014; Majoni et al., 2013). As fruit mature a decrease in the uptake of Ca is found. This is due to the inability of tissue to take up Ca because of a decrease in metabolic activity and a decline in P uptake (Ferguson and Watkins, 1981; Watkins and Ferguson, 1981). As fruit mature ethylene emission increases. The ethylene cycle consists of different precursors such as ACC, which have been related to mineral concentrations such as low P content and the ratio of K to Ca in fruit (Marcelle, 1989). Fruit with higher Ca content produce less ethylene. Other events taking place during maturation such as cell membrane disintegration is also linked to mineral content of fruit. The enzyme *lipoxxygenase* (LOX) catalysis the oxidation of fatty acids found in cell membranes such as linoleic and linolenic acid (Grossman and Leshem, 1978). In the apple cultivar Belle de Boskoop a physiological browning disorder of the fruit core is very closely linked with the activity of *lipoxxygenase* (Feys et al, 1980). The fruit P content and K:Ca ratio has an effect on the LOX activity during storage. A lower LOX activity in fruit is related to a lower K:Ca ratio and a higher P content (Marcelle, 1989; Marcelle, 1991).

2.5 Storage and internal flesh browning

One of the causal factors of IFB is a prolonged storage period (Jobling and James, 2008). Although metabolic activity of fruit is lowered to an almost standstill position, enough activity still takes place to allow for physiological disorders to develop. Stress induced symptoms are a common occurrence as the storage conditions do not allow normal fruit respiration and metabolic activity (Perring and Pearson, 1988).

As IFB is stress induced disorder, antioxidants or the lack thereof, may possibly control the damage caused by the accumulation of active oxygen species (AOS). The two antioxidants mainly responsible for protection of plant cells against oxidative stress are glutathione and ascorbic acid (Noctor and Foyer, 1998). Majoni et al. (2013) found that non-brown fruit harvested at optimum ripeness had the highest glutathione content compared to brown fruit and fruit harvested at post-optimal maturity. Further results from their study showed that overmature fruit were characterized by membrane disruption as fruit exhibiting diffuse flesh browning showed an increase in lipid peroxidation and decreases in ascorbic acid and linolenic acid levels (Majoni et al., 2013). Ca has an interactive relationship with ascorbic acid in the maintaining of membrane stability. Veltman et al., 1999, found that an accumulation of CO₂ in fruit led to a stress response induced by the accumulation of AOS which damages cell membranes. Loss of compartmentalization caused browning of internal tissue in pears (Larrigaudière et al., 2004). Decreasing of ascorbic acid during storage was related to induction of brown core in pears (Larrigaudière et al., 2004).

Low temperature breakdown is a common occurrence in fruit left in storage for prolonged periods at extremely low temperatures and/or controlled atmosphere (Bramlage et al., 1980). Internal breakdown of fruit tissue develops as core flush or browning around the vascular bundles which spreads to the outer part of the fruit (Larrigaudière et al., 2004). It is generally accepted that an increase in K, P and Mg at sufficient Ca levels will lead to a decrease in low temperature breakdown (Perring, 1968). Low Ca content of fruit may lead to acceleration in loss of cell integrity under chilling stress.

High fruit Mg and P content has been related to resistance towards low temperature breakdown in 'Cox Orange Pippin' (Perring, 1968). During cold storage Mg and P is redistributed towards the core of the fruit to protect the fruit from low temperature breakdown. The low oxygen levels associated with controlled atmosphere storage inhibits movement of Mg and P towards the core of the fruit (Perring and Pearson, 1988).

Storage of fruit at CA conditions and low temperature is a useful technique to prolong the storage life of apples while retaining the fruit quality. Postharvest disorders exacerbated by these conditions, such as superficial scald, have been controlled commercially by the use of a synthetic antioxidant, diphenylamine (DPA), as a drench treatment (Hall et al., 1961). Research by de Castro et al. (2007) has shown that DPA could be used for the successful control of CO₂ induced internal browning of 'Cripps' Pink' apples. Due to the DPA's environmental impact (Drzyzga, 2003) the European Union (EU) has lowered the maximum residue limits (MRL) to 0.1 ppm in 2014 and thus, DPA cannot be used as a commercial postharvest treatment any longer. It may therefore necessary to review current storage regimes (van der Merwe, 2005) and to adapt atmospheric and temperature conditions to

better suit prevention of internal browning of 'Cripps' Pink' apples without the use of this synthetic antioxidant

3. Effect of seasonal temperature on internal flesh browning development

Temperature influences every developmental part of the tree (above and below ground) from germination of seeds to bearing of fruit (Palmer et al., 2003). Studies have shown that pre-harvest temperature affect the susceptibility of fruit to postharvest disorders such as low temperature breakdown (DeEll et al., 2005), core flush (Sharples, 1984), soft scald (Bramlage, 1993) and superficial scald (Thomai et al., 1998). A factor with such a wide spread influence should surely have an effect on IFB as a postharvest disorder.

3.1 Aspects of apple production affected by temperature

Temperature affects all aspects of fruit production from development and growth to postharvest storage life. Fruit development is affected by alternations of the rate of all physiological processes such as the rate of pollen tube growth, cell division, expansion and respiration (Palmer et al., 2003).

3.1.1 Breaking of dormancy and bud development

As apples are temperate deciduous specie, a period of winter chill followed by warmer conditions is necessary to break dormancy (Hammond and Seeley, 1978). In warmer apple growing areas such as some of the fruit growing areas of South Africa, higher winter temperatures can influence the accumulation of cold units negatively, thus inadequate chilling may be problematic and lead to delayed foliation symptoms with the concomitant extended flowering period (Erez et al., 1990). As the dormancy requirement has been satisfied, further flower and fruitlet development is dependent on bud temperature (Hamer, 1980). High spring temperatures increase fruit set which in turn leads to smaller fruit size and higher yield (Tukey, 1956). As previously stated, fruit size and yield has an effect on the severity of IFB incidence (de Castro, 2007).

3.1.2 Bloom and tissue development

Many other important events leading to successful bloom such as pollen transfer and the rate of pollen tube growth is temperature dependent (Williams, 1970).

Respiration in plant tissue is temperature dependent as it is associated with the energy needed for the synthesis of cell structures (Lakso, 1994). Studies by Francescone et al. (1997) on the effect of temperature on single leaves showed that the process of cell structure formation is sensitive towards temperature as the cost of maintaining cell function

increases with warmer climates . This single leaf effect may not be of value when examining the effect of temperature on whole canopy gas exchange. While examining the balance between respiration and assimilation, Francesconi et al. (1997) found that CO₂ exchange decreased when air temperature changed from 18 °C to 34 °C in comparison to an increase when temperature lowered from 18 °C to 13 °C. These different findings make pinpointing temperature related events as possible causal agents of IFB difficult.

3.1.3 Fruit growth

Fruit growth includes an period of exponential growth (initial 35-50 days after full bloom (DAFB)), where fruit cell numbers rapidly increase, followed by a linear growth phase where fruit size increases (50-150 DAFB) mainly through cortical cell expansion until harvest maturity is reached (Bain et al., 1951). Kronenberg (1988) identified the month following flowering as well as the period immediately preceding harvest as periods which are sensitive to high temperatures. Controlled-environment studies by Warrington et al. (1999) found that fruit expansion rates were highly temperature responsive during the cell-division phase which lasted up to 40 days after pollination. Fruit growth rates are affected by higher temperatures during early season development in such a way that an increase in cell division in the cortical region takes place with a rise in temperatures (Bergh, 1990). With higher temperatures an increase in fruit expansion was found but the duration of fruit cell division is prolonged with cooler conditions (Bergh, 1990). Although temperature has an effect on fruit growth it is a certainty that other influences such as competition among fruit are integrated with the temperature influence (Kondo et al., 1987).

3.1.4 Fruit ripening and the summer temperature effect

The timing of fruit ripening may be affected by temperatures experienced during early fruit growth (Warrington et al. 1999). Maturity indices such as starch breakdown, firmness, ground colour, red blush and ethylene production were accelerated when temperatures during the first six weeks of fruit growth were high (Tromp, 1997; Warrington et al. 1999). In temperature trials done by Warrington et al. (1999) the conclusion was made that an increase in cell division and thus cell size and number will be accompanied by a higher firmness at harvest. High temperatures used in this trial (22 °C) were comparable to moderate to low summer temperature under South African conditions. Tromp (1997) found that high temperatures during early development was more influential concerning maturity indices than high temperatures applied during the time of onset of maturity.

High summer temperatures have an effect on the flower bud formation of the following year. In studies by Tromp (1976) and Jonkers (1984), flower bud formation was reduced at day

time temperatures of 25-27 °C). This may be due to the effect that high temperatures have on carbohydrate availability. As photosynthesis stops at high temperatures, when the stomata close to reduce water loss, carbohydrate production is also halted (Paul et al., 1990). When carbohydrate assimilation lowers so that a deficit may be foreseen, the reserve carbohydrates would be sent to other plant parts before it is utilized for bud development (Jonkers, 1984).

3.1.5 Fruit quality

Blush development is a commercially important event in 'Cripps' Pink' apples as fruit with a blush percentage of more than 40 % are sold at a higher price under the 'Pink Lady'TM trademark. The red blush found on 'Cripps' Pink' apples develops as a function of pre-harvest temperature and maturity. The formation of anthocyanin such as cyanidin 3-galactoside occurs as a result of an interaction of irradiance, temperature and fruit maturity (Faragher, 1983). Anthocyanin synthesis is stimulated by low night temperatures (<20 °C) and inhibited by high temperatures (Saure, 1990). High temperatures during the day as well as during the night suppress anthocyanin biosynthesis (Creasy, 1968). Studies by Marais et al. (2001) to find the effect of temperature and maturity on the colour development of 'Cripps' Pink' apples revealed that diurnal temperature differences just before harvest play an important role in the synthesis of anthocyanin and thus, blush development on the irradiated side of the fruit. Another aspect studied by these experiments was the difference in response of fruit from two climatically different areas namely the Grabouw (warmer) and Ceres (cold) area. Marais et al. (2001) found that the colour development in fruit from Ceres was more sensitive to high temperatures (20 °C) than those from Grabouw. At 20 °C a small amount of blush developed in fruit from Grabouw and blush colour started to breakdown in fruit from Ceres. With this they concluded that fruit from Grabouw had a greater need for a period of cold before solar radiation to develop the same colour as fruit from the Ceres area. Fruit quality and storability is affected by temperature during the 4-6 weeks before harvest (DeEll et al., 2005). Core flush (Sharples, 1984), water core and superficial scald (Thomai et al., 1998) are some of the disorders which have been found to be correlated with certain temperatures before harvest. Scald on 'Edward VII' apples however, has been found to be related to an increase in soil water deficit from late June to early September in the UK (Sharples, 1984). As Sharples stated, the effect of temperatures may be confounded by fruit maturity at harvest because of the frequent relation of fruit maturity with storage potential. It seems as though the occurrence of radial flesh browning may be associated with senescent breakdown and high levels of CO₂ during storage (Jobling and James, 2008; Wilkinson and Fidler, 1973). The effect of maturity on storability may be temperature dependent as high temperatures before harvest may hasten maturation while cool temperatures may delay ripening (Stanley et al., 2000). Treatments leading to early ripening will cause fruit to mature

during warmer pre-harvest temperatures causing the fruit to be more susceptible to scald and other disorders (Bramlage, 1993). When the growing season is extended and maturity delayed, fruit will ripen during lower pre-harvest temperatures leaving fruit more susceptible to low-temperature breakdown which is a contributor to IFB incidence (Wilkinson and Fidler, 1973). CO₂ injury during storage has been found to be more severe in fruit which experienced a cooler 50 days after full bloom. The cool temperatures during cell division caused an extension of this period which leads to the development of fruit with more and smaller cells, thus having a higher density and reduced gas diffusivity (Little and Holmes, 2000; Lau, 1998).

Both RFB and DFB incidence seem to be exacerbated by over maturity (Bramlage et al., 1980; Wilkinson and Fidler, 1973). Fruit may be left on trees for an extended period before harvest in order to wait for the cold fronts to initiate anthocyanin development (Steyn et al., 2004). For a 'Cripps' Pink' apple to be classed as a 'Pink Lady'[™] apple the red blush should cover 40 % of the fruit surface (Hurndall and Fourie, 2003). This specification has been lowered from the previous 60 % to accommodate growers who previously left fruit on trees to develop blush but also becoming over mature (James et al., 2005). 'Pink Lady'[™] apples have a high density and are sensitive to CO₂ damage (James et al., 2005; Jobling et al., 2004) and IFB incidence is higher in more mature fruit (Crouch et al. 2014, James et al., 2005; Jobling et al., 2004; Majoni, 2012).

3.2 Examining the effect of temperature on fruit growth and development

Different techniques are used for examining the influence of temperature on fruit growth. These include the investigation of correlations between long term phenology, yield and weather data (Jackson and Hamer, 1980) as well as controlled-environment studies where specialized facilities, such as growth chambers, are used to detect direct temperature effects (Miller, 1988). As the use of these specialized facilities are limited, most studies investigate relationships between phenology and yield and weather data on a long term basis (Bergh, 1990).

As temperature has a positive effect on cell growth (Bramlage, 1993) a measurement system of temperature in the form of growing day degrees above 10 °C [$GDD_{10^{\circ C}} = \frac{(\text{minimum temperature} + \text{maximum temperature})}{2} - 10$] was used to correlate a specific number of heat units to the different types of IFB. GDD_{10°C} are measured from full bloom until harvest. This led to a postulation which ascribes areas or seasons where the threshold of 1200 GDD_{10°C} is not exceeded to have a higher risk of developing DFB in contrast to areas which accumulate more than 1200 GDD_{10°C}, which have an increased risk of developing RFB (James, 2007). In trials done to find the correlation between GDD_{10°C} and fruit mass at

harvest extended intervals and the complete fruit growth period proved to be inaccurate for this type of prediction (Palmer et al., 2003). Field studies conducted by Stanley et al. (2000) found a strong correlation between $GDD_{10^{\circ}C}$ measured from pollination until 30 days after pollination (DAP) and fruit weight at harvest. These different findings accentuate the importance of interaction between temperature influence and other physiological or cultural influences and how intertwined these may be.

3.3 Climatically different regions and internal flesh browning

Pre-harvest temperature affects internal flesh browning development as different browning types develop in different climatic areas (James, 2007). Two types of browning related to climatically different regions have been identified (James et al., 2010). It has been reported that radial flesh browning is a senescent disorder as well as a chilling injury (Jobling and James, 2008; Wilkinson and Fidler, 1973). The tissues next to vascular bundles become brown, whereas the rest of the cortical tissue remains unaffected (James and Jobling, 2009). Senescent breakdown, aggravated by toxic levels of CO_2 concentrations within the small vascular cells, is associated with the breakdown of this tissue. Storage conditions as well as orchard conditions (measured in $GDD_{10^{\circ}C}$) determine the development of RFB (James, 2007). Of these two factors the accumulation of $GDD_{10^{\circ}C}$ is said to be the key factor influencing RFB development during storage (James, 2007). RFB develops in regions that accumulate 1200 to 1700 $GDD_{10^{\circ}C}$. In seasons when more than 1700 $GDD_{10^{\circ}C}$ accumulate, radial flesh browning does not develop during storage (Jobling and James, 2008).

DFB on the other hand affects tissues below the peel first while it can be seen throughout the cortical tissue but the vascular tissue remains unaffected. DFB development is mostly caused by chilling injury as it develops at temperatures below 3 °C while fruit prone to RFB can withstand temperatures of down to 1 °C (Bramlage et al., 1980). The larger cells, with thinner cell walls as found in the apple fruit cortex tissue are more prone to collapse due to chilling injury than the small thicker walled cells close to the vascular tissue. These cells collapse due to structural changes during chilling injury resulting in the browning of the cortex tissue. As with RFB it was found that the accumulation of $GDD_{10^{\circ}C}$ was the key factor influencing the development of DFB during storage. Diffuse flesh browning developed in climatic areas which accumulate less than 1100 $GDD_{10^{\circ}C}$ (Jobling and James, 2008).

Although South African apple growing regions would mostly be classified as warm growing regions (Engelbrecht et al. 2011), and so RFB would be the expected type of browning to develop, this is not the case. It was found that fruit used for the experiments from the Vyeboom and Ceres regions developed DFB and only a small percentage of fruit from the Ceres region developed RFB (Bergman et al., 2010 and 2011; Majoni, 2012). As the climatically different regions in the Western Cape do not differ to the same extent as the

regions studied in Australia, trials to define regions and correlate DFB and RFB with the different regions should be done. Apple growing districts in the Western Cape, South Africa, include the Elgin/Vyeboom region, the Koue Bokkeveld and Warm Bokkeveld in the Ceres region and the Langkloof region. The Koue Bokkeveld has been described as a high chilling region and Elgin as a medium chilling region (Midgley and Lötze, 2011). This can be seen from the accumulated Daily Positive Utah Chill units for 2012 where the Koue Bokkeveld accumulated 1276 units and Elgin accumulated 569 units (HORTGRO Science, 2013). Climatically these regions differ in the amount of cold units accumulated, diurnal temperature differences and cumulative $GDD_{10^{\circ}C}$ so that Elgin can be seen as a “warm region” and Koue Bokkeveld as a “cold region” (HORTGRO Science, 2013; Midgley and Lötze, 2011). Climatic differences between regions become applicable on a practical level when Elgin is compared to the Koue Bokkeveld. These two regions differ in their accumulated chilling units to such an extent that the use of chemical rest breakers to enhance bud break is a common practice in Elgin but not in the Koue Bokkeveld (Midgley and Lötze, 2011)

3.4 Pre-harvest temperatures concluding remarks

Seasonal temperature affects fruit development and susceptibility of fruit to postharvest disorders (Sharples, 1968; Thomai et al., 1998). The value of the crop determines harvest time and a highly blushed ‘Cripps’ Pink’ has a higher value. Both low percentage blush colour and flesh browning are influenced by environmental conditions. The influences of climate on fruit maturity and blush development before harvest may be critical when deciding whether to either leave fruit on trees and develop adequate colour (Steyn et al., 2004) and run the risk of browning (Majoni, 2012) or picking at the correct ripeness but losing higher market value due to poor colour development. As harvest maturity, light interception and crop load is under the influence of temperature and or mineral uptake (Shear, 1980), the interaction of temperature and mineral influence should be kept in mind throughout the growing season.

4. Near infrared reflectance spectroscopy (NIRs) as a non-destructive method to assess browning incidence

For ‘Cripps’ Pink’ production to be of economic value to farmers it should reach the high standards of ‘Pink Lady’TM quality (Hurdall and Fourie, 2003) and be able to withstand the long storage time necessary to reach overseas markets at a time when the financial return is the greatest. During this prolonged storage time under CA conditions, IFB develops in susceptible fruit (Jobling and James, 2008; van der Merwe, 2005). To avoid or at least reduce losses in market value due to below standard products, a non-destructive method is

needed to assess single fruit for browning incidence so that fruit subject to internal browning can be removed prior to packaging and shipment. This sorting tool should be accurate and reliable as not to remove sound fruit unnecessarily and to win the confidence of overseas buyers in the quality of South African 'Pink Lady'TM apples.

NIRs uses the near infrared spectrum (800 nm to 2500 nm) to detect abnormalities within tissue through differences in the transmission and absorption of the light through the fruit (Nicolai et al., 2007). Internal quality and disorder incidence such as bruises within fruit are detectable with NIRs as a reduction in near infrared reflectance pattern (Brown et al., 1994). Near infrared reflectance spectroscopy has been reported to be useful in the sorting of intact fruit (Walsh et al., 2004).

4.1 NIRs and internal flesh browning

The type of browning may influence the detectability of internal browning because of the distribution of discoloured tissue and penetration depth of the light path. Internal browning of table grapes has been successfully identified by partial mean squares with discriminant analysis (PLS-DA) as analytical tool to discern between NIRs from brown and non-brown grapes, with a total accuracy of 92 %, after cold storage (Daniels, 2013). With diffuse flesh browning the stem and calyx end of the fruit is mostly affected, with the equatorial part of the fruit being less affected (James and Jobling, 2009). As DFB occurs widely spread throughout the cortical tissue and also affects cortical tissue below the peel surface first, it may be detectable by NIRs (James, 2007). In fruit with RFB the stem end of the fruit is highly affected while the affected area decreases toward the calyx end of the fruit (James and Jobling, 2009). The tissue next to the vascular bundles is affected (James, 2007) and therefore penetration depth may be a problem. The position of the vascular bundles and browning position therefore is unknown from the outside of the fruit and may therefore be missed. NIRs would therefore be less suited to RFB measurement.

4.2 NIRs concluding remarks

The use of NIRs stretches beyond detection of postharvest disorders as it is also able to measure pH, acid levels and soluble solids at the same time with high accuracy (Moons et al., 1997). Other advantages of the NIRs include the repeatability of the measurements, the measurement speed as well as its non-destructive nature (Osborne et al., 1993). NIR as a non-destructive measurement tool of fruit maturity and internal defects, such as IFB, would be a useful tool to incorporate in pack lines to detect browning at an early stage.

5. CONCLUSION

Although ‘Cripps’ Pink’ apples have been characterized as fruit with flesh which resists browning after being cut and exposed to air (Cripps et al., 1993) and of having a high tolerance towards cold storage (Cripps et al., 1993), it develops an internal flesh browning after an extended period in cold storage at CA conditions (Jobling and James, 2008). The ‘Pink Lady’™ trademark is threatened by the incidence of IFB in susceptible fruit.

Internal flesh browning occurrence is sporadic, therefore, it has been suggested that a combination of events and growing conditions contribute to the development of this disorder (James, 2007). Determining the mineral content of fruit affected by IFB and comparing it to that of non-brown fruit will broaden the possible causes and aid in forming an understanding of the events leading to the development of IFB. Other causes of IFB such as overmaturity of fruit (Majoni, 2012; Crouch et al., 2014) and heavy versus light crop loads (Voltz et al., 1999) could also be influenced by the mineral content of fruit (de Castro et al., 2007) and affected by temperature during the growing season (James, 2007).

Non-destructive methods to detect postharvest disorders are becoming a necessity as it detects disorders at an early stage and thus providing the industry with a sorting tool after long term storage before packaging and shipping to avoid or reduce expensive claims.

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PAPER 1

INFLUENCE OF TEMPERATURE ON INTERNAL FLESH BROWNING INCIDENCE OF 'CRIPPS' PINK' APPLES IN TWO CLIMATICALLY DIFFERENT PRODUCTION REGIONS IN SOUTH AFRICA

ABSTRACT

Australian research related the type of internal browning development to growing day degrees above 10 °C (GDD_{10°C}). Two climatically different production regions in the Western Cape of South Africa were used to establish if and how browning incidence differs over two seasons. Fruit were harvested at a starch breakdown of > 50 % from five farms in each region (Elgin and Koue Bokkeveld) and stored under CA conditions (7 months at -0.5 °C) + RA (4 weeks at -0.5 °C) + shelf-life (1 week at ambient) and examined for internal browning incidence and type. Accumulated GDD_{10°C}, average maximum (max) and minimum (min), and max - min temperatures were measured for growth stages and correlated to type of browning incidence. Temperature differences between years rather than temperature differences between regions affected browning incidence. Diffuse browning incidence did not differ between seasons. Radial browning was found for the 2011/2012 season but not for the 2012/2013 season. Small max-min temperature differences during cell division (0 to 50 dafb) correlated with an increased incidence of diffuse browning. Low maximum temperatures during the early cell expansion stage (50-100 dafb) led to an increase in radial browning incidence. Fruit maturity affected diffuse browning susceptibility but did not influence radial browning. "Combination" browning, a new type of browning with affected tissue similar to diffuse- as well as radial browning in one fruit was described, and did not correlate with any of the growth stages described. Radial and diffuse browning development in South Africa is driven by different processes during the growing season and the influence of temperature on cell development during these growth stages. The cause of radial browning appears to be bound by season while diffuse browning seems related to harvest maturity and storage under CA at -0.5° C. Incidence of browning types cannot be predicted by the Australian prediction model.

Keywords: *Malus domestica*, minimum and maximum temperatures, growing day degrees, diffuse browning, radial browning, "combination" browning.

INTRODUCTION

The 'Cripps' Pink' apple cultivar (*Malus x domestica* Borkh.) is characterised mainly by a blush on the peel surface which must not be less than 40 % of the total surface area of the fruit to meet commercial standards to be sold at a premium as 'Pink Lady'TM (Hurndall and Fourie, 2003). This cultivar is grown in different climatic regions around the world. Internal flesh browning (IFB) has been identified in most growing regions and appears more problematic in some regions than others. James et al. (2010) found browning incidence in 'Cripps' Pink' grown in Australia to be correlated with accumulated growing day degrees ($GDD_{>10^{\circ}C}$) during the growing season. This correlation indicated that browning development is associated with climatic conditions during fruit growth and development. Through the results by Jobling and James (2008), a guideline was developed for Australian growers to indicate the type of browning prevalence in various climatic areas. Two different forms of flesh browning were identified on the basis of the affected tissue of the fruit. The two different types of IFB, radial flesh browning and diffuse flesh browning, have been related to different climatic regions in Australia. Fruit grown in the warm Goulburn Valley accumulates more than 1200 $GDD_{>10^{\circ}C}$ during the growing season (September to April) and are prone to the development of radial flesh browning (James et al., 2010). In the colder region of Tasmania, which accumulates less than 1200 $GDD_{>10^{\circ}C}$ during the growing season, fruit is more prone to developing diffuse flesh browning (James et al., 2010). When including California (United States of America) into the model, which has a very high $GDD_{>10^{\circ}C}$ accumulation (> 1700), orchards in this region has been identified as not prone to development of radial or diffuse browning but CO₂ damage may occur (de Castro and Mitcham, 2004).

Temperature throughout the growing season is known to influence biophysical properties of fruit such as cell structure, wax cuticle properties, tissue density and gas diffusivity (Stanley et al., 2000). Therefore, temperature may contribute indirectly towards browning of fruit, rendering it more susceptible towards CO₂ damage or cell membrane breakdown.

The diurnal temperature difference between day and night at the end of season is known to affect blush development (Little and Holmes, 2000). Blush development influences the grower's decision when to harvest since 'Cripps' Pink' is sold at a premium as 'Pink Lady'TM when the blush colour covers more than 40 % of the total surface area (Hurndall and Fourie, 2003). Fruit can therefore either be harvested at optimum maturity, with less blush development, or wait for blush development, with the possibility of harvesting at post-optimum maturity and running the risk of fruit developing IFB (James et al., 2005; Jobling et al., 2004). Blush will only develop in the fruit peel once fruit ripening has been initiated (Chalmers et al., 1973; Faragher, 1983). At physiological maturity before harvest, the

amount of blush or anthocyanin content in the fruit peel is determined by temperature and light (Chalmers et al., 1973; Marais et al., 2001).

Cold fronts just before harvest have been shown to induce the desired blush development specifically in 'Pink Lady™' apples under South African conditions (Steyn et al., 2004). A cold front reaching South African production regions during the harvesting window of 'Cripps' Pink' cannot be guaranteed and varies between seasons. This often leads to a high incidence of over mature fruit in late hanging orchards while producers await the arrival of a cold front. These conditions increase the possibility for fruit browning during long term cold storage under CA conditions.

South African apple production regions such as Elgin, the Koue Bokkeveld, the Warm Bokkeveld and the Langkloof are climatically different according to their chill unit accumulation, average temperatures and rainfall (HORTGRO Science, 2013). The Koue Bokkeveld have been described as high chilling regions and Elgin as a medium chilling region (Midgley and Lötze, 2011). This can be seen from the accumulated Daily Positive Utah Chill units for 2012 where the Koue Bokkeveld accumulated 1276 units and Elgin accumulated 569 units (HORTGRO Science, 2013).

A recent study by Majoni et al. (2013) showed that the occurrence of flesh browning under South African conditions was inconsistent with the $GDD_{>10\text{ }^{\circ}\text{C}}$ model proposed in Australia. According to the $GDD_{>10\text{ }^{\circ}\text{C}}$ prediction model, fruit harvested from South Africa should have developed radial browning, but local fruit tend to develop diffuse flesh browning instead (Crouch et al., 2014, Majoni et al., 2013).

The purpose of this study was to investigate possible relationships between pre-harvest temperatures in two climatically different apple production regions and the occurrence of flesh browning in fruit tissues of 'Cripps' Pink' apples. This was achieved by recording hourly temperature data from five sites in each climatic region, calculating the $GDD_{>10\text{ }^{\circ}\text{C}}$ and relating the incidence of flesh browning to the Australian flesh browning model and to temperature data.

MATERIALS AND METHODS

Plant material

Twenty 'Cripps' Pink' orchards from five farms in the Koue Bokkeveld region (lat. 33°05'00"S long. 19°25'00"E) and five farms in the Elgin region (lat. 34°8'55"S, long. 19°2'34"E) in South Africa, were chosen as replicates for the trial (Table 1). Multiple harvests of 'Cripps' Pink' apples occur commercially each season depending on blush colour development. 'Cripps' Pink' apples for this trial were harvested at the last commercial harvest. Full bloom dates of the different orchards are summarised in Tables 2 and 3. Management practices followed in

the different orchards were according to commercial practices in the different regions. However, there were differences between orchards regarding soil type, tree age, rootstock and yield as summarised in Table 1.

Sampling procedure

A total of 1200 fruit were harvested across 20 orchards per season (2011/2012 and 2012/2013). A sub-sample of twenty fruit from each orchard was randomly selected for measurement of maturity indices at harvest. Fruit were picked randomly on spurs at approximately shoulder height. Harvest dates differed between farms as fruit matured at different times. Harvesting occurred at the last commercial harvest when fruit had a starch breakdown of > 50 % and was completed within one week. Harvesting at > 50 % starch breakdown levels was done to increase the likelihood of browning to occur as previous work by Majoni (2012) indicated that harvest maturity plays a role. Fruit were harvested and stored in regular atmosphere (RA) at -0.5 °C until all orchards were harvested. With completion of the harvest of all orchards, fruit were moved into controlled atmosphere (CA) (1 % CO₂, 1.5 % O₂, -0.5 °C) storage for 7 months. After storage, fruit were repacked into fruit trays and commercial apple cartons lined with polyethylene bags (37.5 µm thickness) to reduce moisture loss. The packed fruit were then stored for 4 weeks at RA (-0.5 °C) to simulate shipment, followed by a one week shelf-life period (20 °C). After completion of the full storage duration (7 months CA, 4 weeks RA and 1 week shelf-life), fruit were analysed for browning incidence and other quality parameters such as flesh firmness, ground colour, % blush, blush intensity, total soluble solids concentration (TSS), titratable acidity (TA), internal ethylene and CO₂ content of fruit.

Quality and maturity indices

After storage, maturity and quality indexing of fruit was performed on 40 fruit per replicate by ExperiCo (Agri-Research Solutions; Stellenbosch). Flesh firmness (kg) was measured equatorially on two opposite peeled sides of each fruit, using a FTA (Fruit Texture Analyser, Güss Instruments, Strand) fitted with an 11.1 mm plunger. Ground colour of fruit were rated from 0.5 (green) to 5 (yellow), using the South African Industry colour chart for apples and pears (Unifruco Research Service (PTY) Ltd.). Chlorophyll content in the mesophyll layer of the fruit was measured equatorially on two opposite sides of each fruit, first on the blush side and secondly on the opposite side, with a DA-Meter (I_{AD}) (Sintéleia, Bologna, Italy). The percentage blush was determined by estimating the percentage of the fruit surface covered by a red blush. The blush intensity was rated from 1 (dull red blush) to 12 (intense red blush), using colour chart P16 for 'Pink Lady'TM apples. An equatorial slice of each fruit was

used to create a composite sample for analysis of TSS (%) (Atago DBX-30 digital refractometer). TA was determined by titrating a 10 g aliquot of a composite juice sample with 0.1 M NaOH to a pH end point of 8.2 (Metrohm Dosimat titrator AG 605, Herisau, Switzerland). TA was expressed as malic acid equivalents (%). Starch breakdown was expressed as the percentage of breakdown after one half of each fruit's equatorial cut surface was stained with a 0.5 M potassium iodide solution and then left for one minute before evaluation using the Unifruco starch conversion chart (Unifruco research services (PTY) Ltd.).

For determination of internal ethylene and CO₂ concentrations, a 10 L glass desiccator was filled with 8 L of water which was then degassed by applying a vacuum for 15 min. Two apples were placed under a bell jar per given time, ensuring that no air bubbles were formed. A vacuum was applied to the desiccator for 40-60 sec to extract air from the intercellular air spaces and cortex and the gas collected at the top of the bell jar. The vacuum was released and the gas extracted from the fruit was collected using 10 mL gas tight syringes (Baton Rouge, USA). Thereafter it was injected into a gas chromatograph (Model 6890N, Agilent Technologies, Wilmington, USA) with PorapakQ and Molsieve packed columns and flame ionisation and thermal conductivity detectors.

Fruit were divided into two storage samples according to harvest date. After 7 months CA storage (1.5 % O₂ and 0.5 % CO₂ at -0.5 °C), fruit were stored for a further 4 weeks in RA at -0.5 °C, followed by one week under shelf-life conditions. Fruit quality was assessed on the remaining 40 fruit per sample at ExperiCo (Agri-Research Solutions; Stellenbosch) after the 7 months CA, 4 weeks RA and 1 week of shelf-life at 20 °C. Quality assessment included flesh firmness, determination of ground colour of the peel by colour chart, peel colour/maturity by DA-Meter, TSS, TA, internal defects and type, external blemishes (%) and decay (%). Internal quality was determined destructively by cutting fruit equatorially through the stem-end where internal defects and types of browning could be assessed visually and expressed as a percentage affected fruit for each replication. Other than internal browning no other internal defects were detected. Types of browning were identified by the browning pattern of the affected tissue on the freshly cut surface of the apple as described by James (2007). Radial browning was identified by browning of tissue surrounding the vascular bundles and diffuse browning, was identified by browning of the cortex tissue below the fruit peel and between the vascular bundles (Fig. 1 and 2)

Temperature parameters

Hourly temperature data was recorded with automatic weather stations on or near the farms. The following weather stations were used in the Koue Bokkeveld: Nooitgedacht, Tandfontein and De Keur, and in Elgin: Applegarth, Applewaith and Rivera. In addition, during 2012/2013, hourly temperatures were also recorded with Tiny Tag temperature loggers (Tiny Tag Plus 2 – TGP 4017, Gemini Data Loggers (UK) LTD) in one orchard per farm from September until the harvest in April or May. Daily $GDD_{>10^{\circ}\text{C}}$ was calculated as follows:

$$GDD_{>10^{\circ}\text{C}} = \frac{\text{minimum temperature} + \text{maximum temperature}}{2} - 10$$

Average maximum (max), minimum (min) and the average max – min temperatures as well as accumulated $GDD_{>10^{\circ}\text{C}}$ were calculated for growth stages of fruit. Research by Bergh (1990), Lau (1998) and Janssen et al. (2008) led to the identification of different cell division, cell expansion and maturation stages from full bloom until harvest. Although cell division occurs from 0 to between 42 and 49 DAFB under local conditions in ‘Starking’ apples (Bergh, 1990) and 56 DAFB for ‘Royal Gala’ (Greybe, 1997), the period from 0 to 50 DAFB correlated with incidence of diffuse browning in this specific study on ‘Cripps’ Pink’. The growth stages were identified as the cell division stage, from full bloom until 50 DAFB; the early cell expansion stage, from 50 DAFB until 100 DAFB; the late cell expansion stage, from 100 DAFB to 150 DAFB; and two maturation stages, which overlap or not according to the date of full bloom and the length of the season, namely the first maturation stage, from 150 DAFB to 200 DAFB and the last maturation stage, which is the last 60 days before harvest. Only growth stages which showed a correlation with browning incidence are discussed.

Averages of each temperature parameter were calculated for different developmental stages: 0 to 50 (cell division), 50 to 100 (cell expansion), 100 to 150 (starch accumulation), 150 to 200 DAFB and the last 60 days before harvest (starch decline). Accumulated $GDD_{>10^{\circ}\text{C}}$ were also calculated for these periods.

Statistical analysis

An analysis of variance was used to compare the average harvest maturity indexes of 20 fruit per replicate for flesh firmness, ground colour, DA, size, blush intensity, blush % and starch breakdown between seasons and regions. The TA, TSS, CO_2 and ethylene content were measured from a composite sample for each replicate (TSS and TA) or from two representative fruit from each replicate (CO_2 and ethylene) and averages were therefore not calculated for each replicate for these maturity parameters. Pearson’s correlation coefficient was generated and used to establish correlations between browning incidence and maturity indices. The Pearson correlation coefficient was used to investigate possible correlations

between browning incidence and type of browning incidence with average minimum, maximum, average max - min temperatures and the accumulated $GDD_{>10}^{\circ C}$ in each of the growth phases. Data were analysed with SAS Enterprise guide version 5.1 (SAS Institute, 2006, Cary, NC 27513, USA).

RESULTS

Production region and seasonal differences

Days after full bloom

There was a significant interaction between region and season and number of days from full bloom until harvest (Table 4). The number of days from full bloom to harvest (205 DAFB for 2011/12 and 202 DAFB for 2012/2013) was similar for orchards in the Elgin region during both seasons. The number of days from full bloom to harvest in 2011/2012 (218 DAFB) in the Koue Bokkeveld was significantly more than for 2012/2013 (203 DAFB) and significantly more than for Elgin, regardless of season (Table 4). Dates recorded for full bloom of each orchard in the Koue Bokkeveld varied more between orchards in the 2011/2012 season compared to the 2012/2013 season (Table 2 and 3). Harvest dates differed between seasons with a period of one week at the most.

Fruit maturity at harvest

There was a significant interaction at harvest between region and season for starch breakdown, flesh firmness and TA measured (Table 5). Starch breakdown of fruit was more advanced in 2012/2013 (66 % for Elgin and 68 % for the Koue Bokkeveld) compared to 2011/2012 (50 % for Elgin and 65 % for the Koue Bokkeveld). The difference in starch breakdown between seasons was more pronounced for orchards in Elgin and less for orchards in the Koue Bokkeveld. Fruit harvested from Elgin from the 2011/2012 season exhibited significantly less starch breakdown compared to fruit harvested in the 2012/2013 season, and fruit from the Koue Bokkeveld. Fruit firmness did not differ between seasons for the Elgin region, but in 2011/2012 had a significant lower fruit firmness compared to that of 2012/2013 in the Koue Bokkeveld. The TA concentration of fruit did not differ significantly between seasons in the Koue Bokkeveld region. The TA concentration of fruit differed between seasons in the Elgin region and was higher for the harvest of 2011/2012. Fruit size, ground colour and internal CO_2 content of fruit was larger, yellower or higher for fruit harvested in 2011/2012 (Table 6). Ground colour, DA measurement, TSS, and internal CO_2 and C_2H_4 of fruit did not differ significantly between the Elgin and Koue Bokkeveld regions, but percentage blush was higher in the Koue Bokkeveld (Table 6)

Fruit quality after storage

Fruit quality after storage (7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life at 20 °C) did not differ significantly between seasons or regions, except for ground colour, which was significantly more advanced in 2012/2013 compared to 2011/2012 (Table 7). There was a significant interaction between region and season for TA concentration of fruit after storage (Table 8). The TA concentration of fruit was higher for the 2011/2012 season for fruit from both regions, but the difference between seasons was more pronounced in fruit from Elgin compared to fruit from the Koue Bokkeveld.

Temperature parameters for growth periods

Accumulated $GDD_{>10^{\circ}\text{C}}$ differed significantly between seasons for all growth stages except for the late maturation stage (60 days before harvest) (Table 9). More $GDD_{>10^{\circ}\text{C}}$ was accumulated during 2012/2013 compared to 2011/2012. Accumulated $GDD_{>10^{\circ}\text{C}}$ differed significantly between regions during the cell division and cell expansion stages. The Elgin region accumulated higher $GDD_{>10^{\circ}\text{C}}$ for these growth stages compared to the Koue Bokkeveld. Average maximum temperatures differed significantly between seasons during the cell division (0-50 DAFB), cell expansion (100-150 DAFB) and late maturation (60 days before harvest) stages. Average maximum temperature was higher during the 2012/2013 season. Production regions differed significantly in average maximum temperatures during the maturation stage (60 days before harvest). Average maximum temperature during this growth stage was higher in Elgin compared to the Koue Bokkeveld. There was a significant interaction between seasons and regions in the average maximum temperatures during the cell expansion (100-150 DAFB) and maturation (150- 200 DAFB) stages (Table 10). Average minimum temperatures differed significantly between seasons during the early cell expansion (50 to 100 DAFB) and the maturation (150 to 200 DAFB; 60 days before harvest) stages (Table 9). Regions differed significantly according to minimum temperatures during the cell division (0 to 50 DAFB), early cell expansion (50-100 DAFB) and maturation (60 days before harvest) stages. Average minimum temperatures were higher during the early cell expansion stage and the maturation stage (150 to 200 DAFB) and lower during the 60 days before harvest (maturation) stage in 2012/2013 compared to 2011/2012. There was a significant interaction between region and season in average minimum temperature during the late cell expansion stage (100-150 DAFB) (Table 10). The highest average minimum temperature during this stage was found for Elgin during 2012/2013 and the lowest for the Koue Bokkeveld during 2012/2013. Average max - min temperatures differed significantly between regions during all growth stages except the cell division stage (0 to 50 DAFB) (Table 9). Seasons differed in average max – min for the maturation stage (60 days before

harvest). Average max – min temperature differences were larger during the maturation stage (60 days before harvest) during the 2012/2013 season compared to the 2011/2012 season. The Koue Bokkeveld had larger differences in max – min temperatures compared to the Elgin region.

Internal browning incidence

No interaction between season and region was found in the incidence of radial, diffuse, “combination” or total browning (Table 11). Incidence of radial -, “combination” and total incidence of browning differed significantly between seasons, with a higher incidence (11, 19 and 46 %, respectively) for the 2011/2012 compared to the 2012/2013 season (0, 0 and 18 %, respectively) (Table 11). Fruit affected by “combination” browning showed browning patterns similar to diffuse (browning of cortex tissue below fruit peel) and radial browning (browning of tissue surrounding vascular bundles) (Fig. 3). Incidence of diffuse browning did not differ significantly between seasons (16 % for 2011/2012 and 18 % for 2012/2013). There was no significant difference in browning incidence between the regions in any of the browning types.

Internal browning incidence of orchards within a region.

Diffuse flesh browning

The incidence of diffuse flesh browning in the Koue Bokkeveld ranged from 0 % to 56 %, and 0 % to 41 %, for Elgin in 2011/2012 (Table 2). Diffuse browning incidence varied more between orchards in the Elgin region during 2012/2013 (Table 3). In the Elgin region, incidence of diffuse browning differed between 0 % and 100 %, while in the Koue Bokkeveld region, incidence varied between 0 % and 41 % for the 2012/2013 season. The highest incidence of diffuse browning for both seasons was found on Esperanto (56 % for 2011/2012 and 41 % for 2012/2013), Bronaar (34 % for 2011/2012 and 41 % for 2012/2013) and Elgin Orchards (40 % for 2011/2012 and 100 % for 2012/2013).

When diffuse browning was statistically evaluated for one growing region over two seasons, the two seasons within that growing region also did not differ in incidence of diffuse browning (Table 12).

Radial flesh browning

The highest account of radial browning was reported for Fine Farms (41 %) in the Elgin region, followed by Remhoogte (28 %) in the Koue Bokkeveld in the 2011/2012 season (Table 2). No account of radial browning was found in the 2012/2013 season. Fruit from one orchard on each of the following farms: Beaulieu and Applegarth in Elgin and

Bokveldskloof in the Koue Bokkeveld, did not develop radial browning during the 2011/2012 season leading to significant differences for seasons within each region (Table 12).

“Combination” flesh browning

“Combination” browning appeared in both Elgin and the Koue Bokkeveld in the 2011/2012 season, with an incidence of 0 % to 56 % in Elgin orchards and 0 % to 44 % in the Koue Bokkeveld (Table 2). “Combination” browning did not develop after CA storage and shelf-life in the 2012/2013 season and thus, incidence of “combination” browning differed significantly between seasons ($P = 0.006$, Elgin) for both areas ($P = 0.001$, Koue Bokkeveld) (Table 12). An average incidence of 17 % “combination” browning was reported for the Koue Bokkeveld and 20 %, for the Elgin region in 2011/2012 (Table 12).

Total browning incidence

The total browning incidence did not differ between regions but differed between seasons (Table 11). However the total incidence of browning recorded in Elgin did not differ significantly between seasons when seasons were evaluated separately for each region. A significant difference ($P = 0.023$) in total incidence of browning was however, found between seasons in the Koue Bokkeveld (Table 12). Total browning incidence was significantly higher in the 2011/2012 season (47 %) compared to the 2012/2013 (17 %) season in the Koue Bokkeveld region.

Total browning incidence more than doubled in percentage from 2011/2012 to 2012/2013 (Table 11) and varied between orchards in the same region and season (Fig. 4). During 2011/2012, fruit from one orchard on Esperanto had a total browning incidence of 97 %, while another orchard on Bokveldskloof did not develop any browning (Table 2). Both farms are in the Koue Bokkeveld. Similar variations in browning incidence between orchards were noticed for the Elgin region, where fruit from Applegarth developed no browning whereas 90 % of fruit from Dennegeur had internal browning after long term CA storage.

Although browning incidence was lower during 2012/2013, the variation between orchards persisted in both regions. The highest incidence of browning in the Koue Bokkeveld was found in Esperanto (41 %), and Bronaar (41 %) (Table 2). No browning was found in fruit from Bokveldskloof and Remhoogte. In the Elgin region, all fruit from one orchard on Elgin Orchards developed browning. Fruit from Applegarth, Beaulieu, Fine Farms and Dennegeur did not develop browning. The biggest variation in browning incidences between orchards on the same farm was found on Elgin Orchards (100 % vs 50 %). Total browning incidence in the 2011/2012 season was higher compared to the 2012/2013 season for all orchards, except for Applegarth and Elgin Orchards, in the Elgin region.

Correlation between temperature and incidence of browning types during growth stages for the 2011/2012 season

Incidence of diffuse flesh browning in the 2011/2012 season showed a strong negative correlation ($R^2 = -0.79$) with accumulated $GDD_{>10\text{ }^\circ\text{C}}$ during the maturation stage (60 days before harvest) (Table 13). Temperatures during the maturation stage (April and May) were very similar for regions (27 °C maximum and 12.1 °C minimum for Koue Bokkeveld; 26 °C maximum and 14 °C minimum for Elgin during May) (20.9 °C maximum and 8.3 °C minimum for Koue Bokkeveld; 22.4 °C maximum and 11.2 °C minimum for Elgin during April) (Table 14) and even though minimum temperatures were slightly lower for the Koue Bokkeveld the incidence of diffuse browning did not differ significantly between regions during 2011/2012 (Table 12).

Average maximum temperatures during the early cell expansion stage (50-100 DAFB) showed a significant negative correlation (-66 %) with incidence of radial browning (Table 13). Lower average maximum temperatures during the early cell expansion stage (Table 9) led to higher incidence of radial browning. The early cell expansion stage occurs from beginning of November to end of January. During these months, average maximum temperatures varied between 20 °C and 30 °C (Table 14). Average maximum temperatures in the low twenties promoted the susceptibility of fruit towards radial browning. During the early cell expansion stage (50-100 DAFB) in 2011/2012 average maximum temperatures ranged from 20.9 °C to 21.3 °C for November, 23 °C to 24 °C for December and 26 °C to 29 °C for January in Elgin and the Koue Bokkeveld, respectively (Table 14).

Correlation between temperature and incidence of browning types during growth stages for the 2012/2013 season

The average max - min temperatures during the cell division stage (0 to 50 DAFB) showed a negative correlation (-74 %) with incidence of diffuse browning (Table 15). Small differences in max – min temperatures during this stage (end of October to end of November) led to an increase in incidence of diffuse browning (Table 14).

Average maximum temperature during the maturation phase (150 to 200 DAFB) correlated positively (69 %) with incidence of diffuse flesh browning (Table 15). There was also a significant interaction between production region and growing season in the Koue Bokkeveld for average maximum temperature during this time (Table 16). Average maximum temperatures during this stage were 22.3 °C (2011/2012) compared to 24.3 °C (2012/2013) in Elgin and 25.4 °C (2011/2012) compared to 23.5 °C (2012/2013), in the Koue Bokkeveld (Table 16).

The average max – min temperatures during the maturation phase (60 days before harvest) correlated positively (78 %) with incidence of diffuse flesh browning (Table 15). Thus the bigger the difference, the higher the incidence of diffuses flesh browning. The average length of the 2012/2013 season from full bloom until harvest was 203 days (Table 4). Fruit ripening and blush development are affected by the maturation stage from the middle of March until the end of April. The high average maximum temperatures (27 °C) and large differences between max - min temperatures experienced during these months (Table 16) led to an increase in development of diffuse flesh browning during this season.

Comparison of temperature parameters over seasons within a production region.

Accumulated Growing Day Degree

Accumulated $GDD_{>10\text{ }^{\circ}\text{C}}$ differed significantly between growth seasons with 1709 GDD in 2011/2012 and 1832 GDD in 2012/2013 (Table 9). According to the browning prediction model of Jobling and James (2008), both seasons would have been classified not to be prone to either diffuse or radial browning, or perhaps slightly more prone to radial browning in the first season.

Accumulated $GDD_{>10\text{ }^{\circ}\text{C}}$ during all phenological stages, except for the last maturation stage (60 days before harvest), differed significantly between the seasons in the Elgin region (Table 17). Accumulated $GDD_{>10\text{ }^{\circ}\text{C}}$ during the cell division stage and the early cell expansion stage differed significantly between seasons in the Koue Bokkeveld region. In all cases there were more accumulated $GDD_{>10\text{ }^{\circ}\text{C}}$ in 2012/2013 season, compared to 2011/2012.

Average maximum temperatures

Average maximum temperatures during the cell division (0-50 DAFB) (20 °C for 2011/2012; 25.5 °C for 2012/2013), early (24.4 °C for 2011/2012; 28.7 °C for 2012/2013) and late cell expansion (25.3 °C for 2011/2012; 28.1 °C for 2012/2013) and last maturation stage (60 days before harvest) (23.3 °C for 2011/2012; 25.1 °C for 2012/2013) differed significantly between seasons in the Elgin region (Table 16). Average maximum temperatures during these stages were higher during the 2012/2013 season. Seasons for the Koue Bokkeveld differed significantly during the cell division (0-50 DAFB) (20.1 °C for 2011/2012; 25.9 °C for 2012/2013), early cell expansion (50-100 DAFB) (24.5 °C for 2011/2012; 27.9 °C for 2012/2013) and the maturation (150-200 DAFB) stages (25.4 °C for 2011/2012; 23.5 °C for 2012/2013). Average maximum temperatures during these stages were higher during the

2012/2013 season except for temperatures during the maturation stage which was higher in the 2011/2012 season.

Average minimum temperatures

Average minimum temperatures during the maturation stages differed significantly between seasons in the Elgin region (Table 18) (11.6 °C for 2011/2012; 10.1 °C for 2012/2013 (150 to 200 DAFB) (11.9 °C for 2011/2012; 10.8 °C for 2012/2013 (60 days before harvest)). Lower temperatures were experienced during the maturation stages in 2012/2013 compared to 2011/2012. Average minimum temperatures during the cell expansion (10.5 °C for 2011/2012; 12.4 °C for 2012/2013 (early 50-100 DAFB)) (13.6 °C for 2011/2012; 12.1 °C for 2012/2013 (late 100-150 DAFB)) and maturation stages (11.1 °C for 2011/2012; 7.9 °C for 2012/2013 (150-200 DAFB)) (9.8 °C for 2011/2012; 8.3 °C for 2012/2013 (60 days before harvest)) differed significantly between seasons in the Koue Bokkeveld region. Average minimum temperatures were lower during the 2012/2013 season during the late cell division - and maturation stages and higher during the early cell expansion stage.

Average difference between maximum and minimum (max – min) temperatures

Average max - min temperatures differed significantly between seasons in the Elgin region for the early (10.5 °C for 2011/2012; 13.1 °C for 2012/2013) and late (11.5 °C for 2011/2012; 13.1 °C for 2012/2013) cell expansion stages and the last maturation stage (60 days before harvest) (11.4 °C for 2011/2012; 14.3 °C for 2012/2013) (Table 19). Differences were more pronounced during the 2012/2013 season. Average max - min temperatures were significantly larger during the last maturation stage (60 days before harvest) (13.0 °C for 2011/2012; 15.3 °C for 2012/2013) in the Koue Bokkeveld during 2012/2013 compared to the 2011/2012 season.

Harvest maturity

Correlations of harvest maturity with type of browning in the 2011/2012 season

Incidence of radial browning did not correlate significantly with any of the maturity parameters (Table 20). A significant positive correlation (52 %) was recorded for TA concentration and fruit with diffuse browning. The TA concentration of fruit varied between 0.49 % and 0.95 % during the 2011/2012 season (Table 2). Fruit size showed a significant, but weak positive correlation with “combination” browning.

Correlations of harvest maturity with type of browning for the 2012/2013 season

As diffuse flesh browning was the only type of browning to develop in the 2012/2013 season, results and correlations will be given only for diffuse browning incidence.

Diffuse browning incidence showed a significant, moderate positive correlation of 45 % with TSS (Table 22). Fruit that developed diffuse browning had higher TSS concentrations. The TSS concentration of fruit followed the same trend as incidence of diffuse browning, and did not differ significantly between growing seasons or regions (Table 21). The TSS concentration of fruit varied from 12.0 % to 15.1 % in 2012/2013 (Table 3).

Comparison of maturity parameters over seasons within the same region.

Elgin

Fruit diameter (68.7 mm for 2011/2012; 66.1 mm for 2012/2013), ground colour (3.0 for 2011/2012; 2.3 for 2012/2013), TA (0.75 % for 2011/2012; 0.62 for 2012/2013) and blush intensity (5.6 for 2011/2012; 7.7 for 2012/2013) at harvest differed significantly between seasons (Table 21). Fruit were slightly larger, had a more advanced (yellow) ground colour, a higher TA concentration and lower blush intensity for the 2011/2012 season. Incidence of radial and “combination” browning differed significantly between seasons (Table 12), but no correlations could be established between maturity parameters and incidence of radial browning. A very weak, but significant correlation existed between fruit size and “combination” browning (Table 20).

Fruit firmness, DA, % blush, TSS and starch breakdown did not differ significantly between seasons (Table 21) and neither did the incidence of diffuse browning (Table 12). Diffuse browning showed a positive correlation with fruit TSS (Table 22).

Koue Bokkeveld

Fruit firmness (7.7 kg for 2011/2012; 8.4 kg for 2012/2013), ground colour (3.1 for 2011/2012; 2.4 for 2012/2013) and blush intensity (5.8 for 2011/2012; 8.1 for 2012/2013) differed significantly between seasons in the Koue Bokkeveld region (Table 21). Fruit from the 2011/2012 season had lower fruit firmness, a more yellow ground colour and lower blush intensity – all indicating a more advanced maturity. Incidence of diffuse browning did not differ significantly between the seasons (17 % for 2011/2012; 20 % for 2012/2013) (Table 12). None of the above mentioned maturity indices correlated significantly with incidence of diffuse, radial or “combination” browning (Tables 20 and 22), in spite of the significant difference in the incidence of radial and “combination” browning between seasons (Table 12).

The correlation between fruit quality after storage and incidence and type of browning per season.

2011/2012

Incidence of radial browning was weakly, but significantly, correlated with TA (- 50 %) concentration but strongly with fruit size (70 %) (Table 23). Large fruit with low TA concentration were inclined to develop radial browning after long term CA storage. Fruit from the Elgin region had a higher TA compared to the Koue Bokkeveld region after storage and ripening (Table 7). The TA concentration of fruit harvested from Elgin and the Koue Bokkeveld were similar (Table 5).

Incidence of diffuse browning was significantly correlated to fruit TSS (45 %) after storage and ripening (Table 23). Fruit with a high TSS concentration after CA storage may have been slightly more susceptible to the development of diffuse browning. The TSS concentration of fruit did not differ significantly at harvest between seasons or regions (Table 6) and ranged from 12.9 % to 13.7 % after storage (Table 25).

Incidence of “combination” browning was significantly correlated to TA (- 64 %), ethylene (-60 %) and CO₂ (- 46 %) concentration of fruit after storage (7 months of CA storage at - 0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) (Table 23). Fruit diameter showed a significant correlation (45 %) with incidence of “combination” browning after storage. Large fruit with low TA, low internal ethylene and low CO₂ content were likely to develop “combination” browning after storage.

2012/2013

Incidence of diffuse browning showed a weak, but significant negative correlation with fruit firmness (- 45 %) (Table 24). As fruit firmness decreased and fruit became more mature, fruit were more likely to develop diffuse browning. Fruit firmness did not differ significantly between seasons or regions and ranged from 6.1 kg to 6.6 kg (Table 25).

DISCUSSION

Australian researchers Jobling et al. (2004) and James (2007) described the appearance of radial, diffuse and CO₂ internal browning in ‘Cripps’ Pink’ apples and identified production regions in Australia where fruit were more susceptible to developing a certain type of browning based on the climate (temperature) of that particular region. Apples produced in warmer regions (1200 – 1700 GDD_{>10°C}) were prone to develop radial browning and fruit from colder regions, (< 1200 GDD_{>10°C}) were more prone to develop diffuse browning (James, 2007). Elgin and the Koue Bokkeveld were selected as climatically different deciduous fruit growing regions in South Africa, with Elgin being the warmer region and the

Koue Bokkeveld the cooler region (HORTGRO Science, 2013; Midgley and Lötze, 2011). The incidence of browning was compared between Elgin and the Koue Bokkeveld and related to the temperature parameters during phenological stages of the growing season (DAFB), and to fruit maturity and quality parameters at harvest. Both regions accumulated more than 1500 GDD_{>10°C} (Fig. 5) and radial as well as diffuse browning developed. According to the Australian model, the South African growing conditions are not conducive (too warm, GDD_{>10°C} >1100) to the development of diffuse flesh browning. However diffuse browning was the main type of browning found. The results from this study are in agreement with results from a previous South African study (Crouch et al., 2014; Majoni et al., 2012) where diffuse browning was the main and in some trials the only type of browning to develop. These results therefore do not support those from Australian based research on the GDD_{>10°C} prediction model (James, 2007).

The 2011/2012 growing season was significantly longer than that of 2012/2013 for the Koue Bokkeveld and both growing seasons for the Elgin region (Table 4). Fruit maturity was not significantly different, even though the two seasons were different in terms of type of browning incidence (Table 6). Therefore the length of the growing season did not affect fruit maturity at harvest but may have influenced the effect of temperature on physiological growth stages.

Total browning incidence decreased significantly from 2011/2012 to 2012/2013 in both areas (Table 11). Regions did not differ significantly with regard to total browning or type of browning incidence. A decrease in browning incidence from 2011/2012 to 2012/2013 was noticed in all orchards in the Koue Bokkeveld (Fig. 4). Three out of five farms in Elgin showed a decrease in total browning incidence from 2011/2012 to 2012/2013 (Fig. 4), however this difference between seasons was not significant (Table 12). These orchards which did not show a similar decline in browning incidence indicate that seasonal changes in temperature parameters and orchard factors such as tree age or soil type may possibly influence incidence of IFB. Radial and “combination” browning only developed during the 2011/2012 season (Table 11). Incidence of diffuse browning did not differ significantly between season or region, but varied in severity between orchards. This occurrence in the incidence of diffuse browning indicated that fruit are increasingly susceptible to diffuse browning development when stored for long periods under CA, followed by RA and shelf-life conditions. Seasonal variation and not differences between production regions determined the incidence of browning development in this study.

Diffuse flesh browning

The significant positive correlation between TSS and diffuse browning (Table 21) may indicate that diffuse browning develops in more mature fruit as found in studies by Crouch et

al. (2014) and Majoni et al. (2013). Eventhough all fruit were harvested post optimally a variability in fruit maturity will be present between single fruit so that diffuse browning develops in fruit with the highest TSS content. Contrasting to this, diffuse browning also showed a positive correlation with TA which is usually associated with a high storage potential of fruit (Ghafir et al., 2009). High potassium (K) concentrations have a positive effect on acidity of fruit (Johnson, 2000; Wilkinson, 1958). The correlation of diffuse browning with TA may therefore not be direct but indirect because of the enhancing effect which high K content of fruit may have on the acidity of fruit. The influence of mineral content on fruit is investigated in Paper 2.

Diffuse browning incidence showed a strong negative correlation with the average max - min temperatures during the cell division stage (0 to 50 DAFB) in the 2012/2013 season (Table 15), regardless of region. The average length of the 2012/2013 season was 203 days from full bloom until harvest (Table 4) in both regions. Large max – min differences during the cell division stage in both regions (13.2 °C for Elgin, 17.7 °C for Koue Bokkeveld during October; 16.8 °C for Elgin and 18.1 °C for Koue Bokkeveld during November) (Table 14) led to a low incidence of diffuse browning in Elgin and the Koue Bokkeveld (Table 12). Temperature differences (max – min) were higher in Elgin and the Koue Bokkeveld in 2012/2013 compared to 2011/2012 (9.8 °C for Elgin and 12.5 °C for Koue Bokkeveld during October; 10.5 °C for Elgin and 13.8 °C for Koue Bokkeveld during November) (Table 14). The high max – min temperatures during 2012/2013 coincided with high maximum temperatures during the months of October and November. A study by Bergh et al. (1990) showed that high temperatures during the first 42 DAFB led to increased rates of fruit growth and fruit set while low temperature led to lower fruit set (Bergh, 1990). Low temperatures during the first 42 DAFB slows down cell growth in the cortical region (Bergh, 1990) leading to development of fruit with smaller cells and a higher density (Little and Holmes, 2000). Therefore, low temperatures during the cell division stage of 2011/2012 may have led to the development of smaller and denser fruit. These fruit would be more susceptible to storage disorders, such as diffuse browning, as diffusion of gas is altered by increased fruit density (Lau, 1998; Sharples, 1975; Stanley et al., 2000) which is detrimental in a naturally high density apple such as 'Cripps' Pink'. Fruit with higher density have a lower gas diffusion potential which can lead to a build-up of CO₂ resulting in cell damage (Lau, 1998). Low crop load correlated with high incidence of CO₂ damage of 'Braeburn' apples in studies on 'Braeburn' browning disorder (BBD) (Volz et al., 1999). Fruit from light cropping trees have been found to have a smaller Ca concentration and lower storage potential (Ferguson and Watkins, 1992). This illustrates how temperature, fruit set and mineral content of fruit can influence the susceptibility of fruit to a CO₂ induced injury.

Incidence of diffuse browning during the 2011/2012 season showed a strong negative correlation with accumulated $GDD_{>10^{\circ}\text{C}}$ during the maturation stage (60 days before harvest) (Table 13). Lower maximum and minimum temperatures during the maturation stage led to a higher incidence of diffuse browning. Diffuse flesh browning is a disorder mainly induced by chilling injuries (Bramlage et al., 1980; James et al., 2005) and extended periods in cold storage. Shorter storage periods but at lower temperatures, can also lead to the development of chilling injury in fruit (Saltveit and Morris, 1990; Sevillano et al., 2009). In two studies on apples, low temperatures during the growing season predisposed fruit to chilling injury during storage (Johnson and Ridout, 1998; Sharples, 1975). DeEll et al. (2005) proposed cold injury to be related to late season temperatures. Browning disorders after cold temperature are a stress response resulting in an increase in the ratio of saturated to unsaturated fatty acids (Marangoni et al., 1996). An alteration of the lipid ratio caused by low temperature during the growing season decreases the sterols in the membranes (Lindberg et al., 2005) which decreases the fluidity and increases the rigidity of the membrane (Kays, 1991; Lindberg et al., 2005). Increased rigidity of the cell membrane leads to increased susceptibility of membranes toward lipid bi-layer separation and fluid leakage during storage (Kays, 1991; Marangoni et al., 1996). 'Cripps' Pink' fruit that had diffuse browning after long term CA storage at low temperatures had higher ratios of saturated to unsaturated fatty acids compared to that of non-brown fruit (Majoni et al., 2013). Low temperature (low $GDD_{10^{\circ}\text{C}}$) during the last 60 days before harvest could have caused an increase in the ratio of saturated to unsaturated fatty acids leading to increased susceptibility of fruit toward cold injury. Incidence of diffuse browning did not correlate with CO_2 content of fruit which would have been an indication of stress. Further research into the effect of late season temperature on the ratio of saturated to unsaturated fatty acids in the fruit and its relationship with incidence of diffuse browning would be needed to investigate this theory.

Average maximum temperatures during the maturation stage (150-200 DAFB) showed a positive correlation with incidence of diffuse browning in 2012/2013 (Table 15). Average max – min temperatures during the maturation stage (60 days before harvest) also showed a positive correlation with incidence of diffuse browning. Inadequate diurnal temperature variation during the maturation stage delays blush development (Steyn et al., 2004) and consequently delays harvest (James et al., 2007). Fruit maturity is often compromised as growers wait for the development of blush and higher blush intensities in 'Cripps' Pink' apples (Majoni, 2012).

The advanced maturity of fruit could have predisposed fruit to the development of diffuse browning as fruit were harvested at the latest possible commercial harvest. Fruit harvested post-optimally have a lower ascorbic acid level and a higher lipid peroxidation after one month of CA storage at low temperature compared to fruit harvested at optimal maturity and

a higher level of diffuse flesh browning (Majoni et al., 2013). Membrane integrity is lost when degradation processes, exceed membrane repair (Rawlyer et al., 1999). Delayed harvest of 'Cripps' Pink' apples caused by high temperatures or the lack of colder night temperatures during the maturation stage may therefore lead to a predisposition towards diffuse browning development. The differences in the correlation of temperature parameters on diffuse flesh browning development during the maturation stage from negative for $GDD_{10^{\circ}C}$ ($R^2=-79\%$) in 2011/2012 to positive for maximum temperature ($R^2=69\%$) and average max-min ($R^2=0.78$) in 2012/2013 could be because of a different method of inducing diffuse browning in fruit or the influence of an undetermined factor. Maximum and minimum temperatures were higher during the maturation stage in 2011/2012 compared to 2012/2013 (25.4 °C maximum for 2011/2012; 23.5 °C maximum for 2012/2013) (11.1 °C minimum for 2011/2012; 7.9 °C minimum for 2012/2013 (150-200 DAFB)) (9.8 °C minimum for 2011/2012; 8.3 °C minimum for 2012/2013 (60 days before harvest)) (Table 14) which would have resulted in higher $GDD_{10^{\circ}C}$. Incidence of diffuse browning over both seasons and regions ranged between 16 % and 20 %. This is high concerning fruit losses and the possibility of rejections but low when compared to the overall 80 % of non-brown fruit. However, the current export tolerance for internal browning is 1 %. If one affected fruit is found in one bag in one box the whole pallet can be rejected depending on the client and how they calculate the defect percentage (H. Griessel (Tru-Cape Marketing), pers. comm, 2014). High maximum temperatures during the maturation stage in 2011/2012 led to a low incidence of diffuse browning (negative correlation with $GDD_{10^{\circ}C}$) while the lower maximum temperatures during 2012/2013 also led to low incidence of diffuse browning (negative correlation with average maximum). Over maturity of fruit and low temperatures ($GDD_{10^{\circ}C}$) during the maturation stage (2011/2012) may have resulted in changes to membranes which may have predisposed fruit to diffuse browning development. Alternatively, the correlation with $GDD_{10^{\circ}C}$ may have been coincidental, since other parameters making up the GDD did not show any correlation in that season. The extended cold storage period at -0.5 °C under CA conditions, along with the maturation stage and advanced maturity possibly due to high maximum temperatures and high max-min temperatures of fruit, may have contributed to the development of chilling injury in this study. Fruit should be harvested at optimum maturity to avoid development of diffuse browning during long term storage. It is recommended that fruit of post-optimum maturity should not be stored for longer than 4 months under CA conditions at -0.5 °C in seasons with high maximum temperatures during the last 60 DAFB of maturation.

Radial browning

No correlations were found between maturity parameters and incidence of radial browning. Incidence of radial browning varied among orchards within the same season indicating that orchard factors such as crop load, tree age or soil type could influence susceptibility of fruit towards radial browning alongside temperature for certain developmental stages. Tree age and soil type of orchards were noted (Table 1) but not included in the analysis of data and rather left for investigation and discussion in Paper 2. In terms of temperature, radial browning incidence showed a negative correlation with average maximum temperatures during the early cell expansion phase (Table 13). Low maximum temperatures (20 °C) during the early cell expansion period led to a high incidence of radial browning in the 2011/2012 season. No radial browning was recorded in the 2012/2013 season when temperatures during the early cell expansion period was higher compared to 2011/2012 (Table 14).

Warrington et al. (1999) showed that fruit which were subjected to higher temperatures early in the growing season reached physiological maturity earlier than fruit subjected to cooler temperatures at the beginning of the growing season. Radial browning development was associated with senescent breakdown (Wilkinson and Fidler, 1973). The low maximum temperatures (20 °C) in this study recorded during the early cell expansion stage was comparable to the “high temperature” treatment (22 °C) in the studies by Warrington et al. (1999). Fruit harvest maturity in this study did not correlate to incidence of radial browning and could not be related to the difference in incidence between seasons. High temperatures leading to increased maturity at harvest therefore also do not seem to be the cause of radial browning in this study.

James et al. (2010) associated radial browning with fractured cell walls while diffuse browning was associated with cell collapse. When apoplastic pathways are disrupted by leakage from damaged cells into intercellular spaces, diffusion of O₂ and CO₂ slows down which in turn increases susceptibility towards low O₂ and high CO₂ damage during long term storage (Lau, 1998; Schotsmans et al., 2004). This type of leakage is associated with membrane deterioration during senescence of fruit. Exactly why cortex tissue below the fruit peel stays unaffected is not known. It is however possible that the damage caused to the apoplastic pathways may have reduced the diffusion of damaging substances away from the affected area which in turn may have obstructed the flow of antioxidants to the affected areas thus concentrating the damage to cells near the vascular bundles, which would have also affected gas diffusion and transport of assimilates of these tissues specifically.

Temperature as a function of solar radiation determines the rate of photosynthesis and affects transpiration, which in turn, affects fruit growth (Landsberg, 1979). High temperatures during the cell division stage leads to increased fruit set (Kondo et al., 1987).

Low maximum temperatures during the cell expansion stage correlated negatively with incidence of radial browning and are associated with low rates of photosynthesis. Low rates of photosynthesis during exponential cell growth could increase the competition between fruit for photosynthates (Hayashi and Tanabe, 1991). Increased solar radiation associated with higher temperatures during the early stages of cell expansion would supply fruit with the necessary amounts of photosynthates to support physiological activities associated with fruit growth (Blackman and Wilson, 1951). Competition between fruit when photosynthates are low will lead to the development of fruit with deficits depending on the position of the fruit within the canopy and fruit cluster. This could contribute to susceptibility of fruit towards postharvest disorders during long term CA storage. Average maximum temperatures found in this study ranged from 22 °C to 28 °C.

Radial browning has been associated with senescent breakdown aggravated by high CO₂ levels (Lau, 1998). This association between browning disorders and high internal CO₂ and low internal O₂ content of fruit has been found for 'Elstar' (Streif and Saquet, 2003), 'Braeburn' (Clark and Burmeister, 1999) and 'Fuji' (Grant et al., 1996, Volz et al., 1998). In this study internal CO₂ content of fruit at harvest, did not correlate with incidence of radial or any other browning type. Fruit density was not measured in this study. Incidence of radial browning showed a significant negative correlation with decreased ethylene content of fruit after long term storage. This anomaly could be caused by a shutdown of the respiration system of the fruit caused by damage to the respiration systems or depletion of energy to uphold normal cell function of the fruit after such long term cold storage under low O₂ and high CO₂ conditions. Accumulation of ROS in the fruit causes damage to membranes and loss of cell function (Purvis, 2004). The ROS form when fruit are subjected to stress caused by an accumulation of CO₂ (de Castro et al., 2007). The TA of fruit measured after storage showed a significant negative correlation with incidence of radial browning. Fruit which developed radial browning had a low TA concentration after long term storage. TA plays an important role in respiration as these organic acids are intermediates in the citric acid cycle. Changes in TA are related to the rate of metabolism of the fruit (Clarke et al., 2003). Titratable acids decrease with an increase in duration of storage time (Jan and Rab, 2012). During increased respiration, organic acids and the TA concentration of fruit decreases (Ghafir et al. 2009).

Fruit compete for photosynthates and minerals during the growing season. If a shortage in photosynthates exists during the early cell expansion stage because of low solar radiation accompanied by a low reserve status in the tree, fruit could be predisposed towards radial browning development.

Similar to radial browning, water core of apples also affect areas around the vascular bundles while the cortex tissue stays unaffected. Water core is caused by flooding of

intercellular spaces with sorbitol when fruit are at an advanced maturity and night temperatures before harvest are low. Flooding of the intercellular spaces with sorbitol causes a disruption of gas diffusion and a build-up of CO₂ in the affected area (Argenta et al., 2002). This could lead to internal breakdown in advanced cases of water core. Vascular tissue in fruit affected by water core does not have the capacity to convert sorbitol to fructose, which can diffuse to other parts of the fruit (Kollas, 1968). It is proposed that an increase in leaf to fruit ratio increases the incidence of water core as the larger number of leaves feed more sorbitol to the fruit (Kollas, 1968). Water core has been found at harvest or after a period of CA storage. Radial browning was found in fruit after the 7 month CA period (Paper 3). As radial browning and water core have similar affected tissue, the transport of solutes in and out of the fruit should be investigated as factors influencing radial browning. Although fruit affected by water core show similar patterns of affected tissue to fruit affected by radial browning, temperature during the maturation stage rather than the cell expansion stage affects susceptibility of fruit towards water core.

“Combination” browning

A third type of browning was identified as a combination of radial and diffuse browning (Fig. 3). Browning occurs below the fruit peel and around the vascular bundles in accordance with affected areas of diffuse and radial browning. Results from Paper 3 (NIR trial) showed that radial browning is present in fruit directly after seven months of CA storage (0.5 % CO₂ and 1.5 % CO₂) at -0.5 °C. Most diffuse browning developed in fruit during storage at RA (four weeks) and shelf-life (one week) conditions. It is during this period that “combination” browning develops, as diffuse browning develops in fruit already subject to radial browning. This shows that fruit affected by “combination” browning are thus sensitive to factors which affect radial as well as diffuse browning.

“Combination” browning showed a positive correlation with fruit size (Table 23) and a negative correlation with TA, ethylene and CO₂ in fruit after storage. Large fruit with low TA concentration and low ethylene and CO₂ activity were more likely to show “combination” browning pattern after long term storage and ripening possibly due to depletion of reserves for the Krebs cycle and inactivity of ethylene production during the post-climacteric stage of maturity (senescence) after long term storage under low O₂ conditions.

Total browning incidence

The largest difference in incidence of browning on the same farm was found between orchards on Remhoogte in the Koue Bokkeveld (Table 2). Fruit from orchard one developed 75 % browning and 25 % of fruit from orchard two developed browning after 7 months long

term CA storage at $-0.5\text{ }^{\circ}\text{C}$ for the harvest of 2011/2012. These two orchards are adjacent to one another and fruit were harvested on the same day of the same season. Harvest maturity, soil type, rootstock and tree age of fruit from these two orchards are very similar. This variability indicates the influence of other factors like fruit mineral content or tree health on the susceptibility of fruit towards browning. The highest incidence of browning for 2011/2012 was found for orchards on Esperanto (Koue Bokkeveld) which were also the only orchards with young trees. Other farms with a similar incidence of browning as found on Esperanto, such as Dennegeur and Elgin Orchards, had older trees. This indicates how multifaceted the induction of internal flesh browning is. Multivariate analysis was not applied in this study but would be useful to show which orchard differences may be linked to diffuse browning incidence in the same region. It would be helpful to measure and include causal factors of water core; such as leaf:fruit ratio, crop load and girdling,- thinning and pruning-practices; into the multivariate analysis to investigate similarities between this disorder and radial browning, since the tissue affected is similar even though the disorder looks slightly different.

Total browning incidence differed significantly between the two seasons (Table 11). Temperatures which correlated with radial- or “combination” browning should differ between seasons as the incidence of these browning types differed significantly between seasons. Temperatures which correlated with incidence of diffuse browning should not differ between seasons as incidence of diffuse browning did not differ significantly between seasons. Radial, diffuse and “combination” browning appear in fruit as a result of membrane leakage. Susceptibility of membranes to loss of integrity is caused by different factors for radial and diffuse browning. Membrane leakage leading to diffuse browning was possibly caused by storing over mature fruit for a long period under cold CA conditions which impacted on membrane maintenance and all the factors that play a role in curbing its senescence. Low maximum temperatures during the early cell expansion phase (50-100 DAFB) correlated to radial browning which may have caused competition between fruit for photosynthates or minerals and may also have possibly affected the tree reserve status. This may have led to a deficit in the strength of cell structures so that fruit were unable to withstand the onslaught of ROS during long term storage and membrane degradation may have exceeded repair ability. Low maximum temperatures may have also influenced cell expansion and therefore tissue density next to the vascular bundles. This in turn may have changed import and export into and from tissues surrounding the vascular bundles or may have influenced tissue density in already dense fruit during long term storage at low O_2 and high CO_2 levels.

Different types of browning have different causal factors and therefore it is important to be able to distinguish between these types of browning to determine the appropriate management approach in the orchard and/or storage facility to reduce browning incidence.

Browning types and causal factors of the browning types alone should be investigated separately to manage IFB and total incidence should not be used to create management practises.

CONCLUSION

Diffuse browning was found for both seasons and radial browning only for 2011/2012. However, according to the Australian model orchards accumulated $GDD_{>10^{\circ}\text{C}}$ associated with incidence of radial browning ($GDD_{>10^{\circ}\text{C}} > 1200$) for both seasons. Incidence of browning types did not correlate with accumulated $GDD_{>10^{\circ}\text{C}}$ of the entire season and therefore it is concluded that the Australian prediction model cannot be used as an accurate prediction of browning potential under South African conditions. Significant correlations between incidence of browning types and temperature parameters during different growth periods indicated that susceptibility of fruit towards IFB development could be induced at specific growth period. Browning types differed according to temperature conditions and the growth period at which susceptibility towards the type of browning is induced. Significant positive correlations between diffuse browning and TSS at harvest and low maximum temperatures during the maturation stage of fruit development may indicate that over-maturity of fruit associated with low blush development may induce susceptibility of fruit towards diffuse browning development. Incidence of radial browning correlated significantly with low maximum temperatures. Fruit harvested in 2011/2012 were susceptible to radial browning development but not fruit harvested in 2012/2013. The main difference regarding temperature of these seasons would be the lower maximum temperatures during 2011/2012 compared to that of 2012/2013. This suggests that insufficient photosynthesis associated with cloudy weather and low maximum temperatures could lead to susceptibility of fruit towards radial browning. More research spanning over a longer period of time would be necessary to conclude on the cause of radial browning as incidence of radial browning occurred only during 2011/2012.

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Tables:

Table 1: Orchard information in the Elgin and the Koue Bokkeveld production regions.

Area	Farm name	Soil type ^a	Tree age (years)	Root-stock	Yield 2012 (t/ha)	Yield 2013 (t/ha)
Koue Bokkeveld	Donkerbos	S	16	M793	83	85
		S	16	M793	82	59
	Esperanto	S	7	M793	57	82
		S	7	M793	57	82
	Bronaar	LS	18	seedling	89	53
		LS	18	seedling	95	59
	Bokveldskloof	SCL	14	M793	135	106
		C	15	M793	126	108
	Remhoogte	SCL	16	M793	87	78
		SCL	16	M793	63	78
Elgin	Elgin Orchards	S	16	M793	47	47
		L	16	M793	45	88
	Applegarth	SCL	18	M109	89	134
		SCL	17	M25	58	107
	Beaulieu	L	16	M25	84	143
		L	16	M25	102	155
	Fine Farms	L	17	M25	54	106
		C	15	M793	64	103
	Dennegeur	C	11	M793	51	-
		C	15	seedling	67	-

^a S = Sand, LS = Loam Sand, SCL = Sand Clay Loam, C = Clay

Table 2: Full bloom and harvest dates, maturity information at harvest and browning incidence (after 7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) of ‘Cripps’ Pink’ apples harvested from each orchard in the 2011/2012 season in the Western Cape, South Africa.

Area	Farm name	Yield 2012 (t/ha) ^a	Full bloom	Harvest	DAFB ^a	Maturity indices measured at harvest					Browning incidence			
						Starch break-down (%)	Diameter (mm)	Firmness (kg)	TSS (%)	TA (%)	Total (%)	Radial (%)	Diffuse (%)	Combination (%)
Koue	Donkerbos	83	23-Sep	07-May	226	66.3	64.5	7.6	12.6	0.49	41	13	3	25
		Bokkeveld				82	55.6	66.5	7.1	11.4	0.59	3	3	0
	Esperanto	57	04-Oct	02-May	210	67.8	71.4	7.6	14.4	0.90	84	0	56	28
		57				57.2	69.8	7.9	15.0	0.95	97	13	41	44
	Bronaar	89	01-Oct	07-May	218	72.7	69.6	8.1	11.6	0.59	72	9	34	28
		95				74.3	73.1	8.4	12.4	0.66	53	19	6	28
	Bokveldskloof	135	26-Sep	02-May	218	58.1	66.3	7.4	12.5	0.70	0	0	0	0
		126				49.4	65.0	7.4	12.1	0.81	22	16	6	0
	Remhoogte	87	02-Oct	07-May	217	-	66.2	7.6	13.3	0.62	75	28	6	41
		63				82.7	70.1	7.6	12.7	0.73	25	13	3	9
Elgin	Elgin Orchards	47	10-Oct	07-May	209	46.9	67.6	7.8	13.4	0.88	72	22	28	22
		45				55.6	67.2	7.8	12.9	0.70	78	19	41	19
	Applegarth	89	10-Oct	24-Apr	196	55.0	66.8	7.7	13.3	0.70	0	0	0	0
		58				55.0	67.3	7.7	13.2	0.71	9	3	3	3
	Beaulieu	84	08-Oct	02-May	206	54.4	67.3	8.7	12.6	0.83	6	0	6	0
		102				41.9	67.4	8.3	12.7	0.84	38	3	28	6
	Fine Farms	54	05-Oct	07-May	214	31.9	72.6	7.6	13.1	0.72	50	13	13	25
		64				-	70.0	7.9	11.8	0.67	56	41	6	9
	Dennegeur	51	18-Oct	07-May	201	63.2	70.5	7.7	11.3	0.77	53	9	16	28
		67				44.5	70.1	8.3	11.7	0.72	91	6	28	56

^aDays after full bloom

Table 3: Full bloom and harvest dates, maturity information at harvest and browning incidence (after 7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) of ‘Cripps’ Pink’ apples harvested from each orchard in the 2012/2013 season in the Western Cape, South Africa.

Area	Farm name	Yield 2013 (t/ha)	Full Bloom	Harvest Date	DAFB ^a	Maturity indices measured at harvest					Browning incidence			
						Starch break-down (%)	Diameter (mm)	Firmness (kg)	TSS (%)	TA (%)	Total (%)	Radial (%)	Diffuse (%)	Combination (%)
Koue	Donkerbos	85	12-Oct	06-May	206	73.8	65.8	7.9	13.1	0.56	13	0	13	0
	Bokkeveld	59				56.6	67.3	7.6	12.1	0.65	3	0	3	0
	Esperanto	82	12-Oct	29-Apr	199	75.6	69.6	9.1	15.1	0.75	41	0	41	0
		82				72.7	67.4	9.5	13.4	0.70	38	0	38	0
	Bronaar	53	11-Oct	06-May	207	61.6	66.9	9.0	14.2	0.68	41	0	41	0
		59				58.1	69.1	8.5	13.1	0.67	28	0	28	0
	Bokvelds-kloof	106	11-Oct	29-Apr	200	70.6	69.0	8.3	12.6	0.70	0	0	0	0
		108				65.0	72.1	8.3	12.0	0.67	0	0	0	0
	Remhoogte	78	13-Oct	06-May	205	71.9	67.8	8.1	13.4	0.80	3	0	3	0
		78				69.4	66.7	7.9	13.8	0.79	0	0	0	0
Elgin	Elgin	47	12-Oct	07-May	207	66.4	65.9	8.3	14.1	0.64	50	0	50	0
	Orchards	88				76.9	66.3	8.0	13.2	0.48	100	0	100	0
	Applegarth	134	17-Oct	01-May	196	61.6	66.5	8.2	12.5	0.64	0	0	0	0
		107				63.1	66.6	7.9	12.4	0.68	13	0	13	0
	Beaulieu	143	13-Oct	07-May	206	73.1	65.1	7.9	12.0	0.58	3	0	3	0
		155				80.6	67.2	7.8	12.0	0.64	0	0	0	0
	Fine Farms	106	20-Oct	07-May	199	59.4	65.1	7.6	13.6	0.60	9	0	9	0
		103				76.0	65.3	8.0	13.3	0.57	0	0	0	0
	Dennegeur	-	13-Oct	07-May	206	51.3	67.4	8.4	13.4	0.76	0	0	0	0
					55.6	65.6	7.9	13.0	0.56	28	0	28	0	

^aDays after full bloom

Table 4: Total number of days from full bloom (October) until harvest (April/May) of 'Cripps' Pink' apples for the growing seasons of 2011/2012 and 2012/2013 and the two production regions (Koue Bokkeveld and Elgin) in the Western Cape, South Africa.

Production region	Season	Days after full bloom
Elgin	2011/2012	205b
	2012/2013	202b
Koue Bokkeveld	2011/2012	218a
	2012/2013	203b
<i>Source of variation:</i>		<i>Pr>F</i>
<i>Season</i>		<i>0.003</i>
<i>Region</i>		<i>0.016</i>
<i>Season*Region</i>		<i>0.026</i>

Table 5: Interaction between production region (Elgin and Koue Bokkeveld) and growing season (2011/2012; 2012/2013) for maturity indices (starch breakdown, firmness and titratable acidity(TA)) measured after harvest, of 'Cripps' Pink' apples in the Western Cape, South Africa.

Production region	Season	Starch breakdown (chart index)	Firmness (kg)	TA (%)
Elgin	2011/2012	49.8b	8.0ab	0.75a
	2012/2013	66.4a	8.0ab	0.62b
Koue Bokkeveld	2011/2012	64.9a	7.7b	0.70ab
	2012/2013	67.5a	8.4a	0.70ab
<i>Source of variation</i>		<i>Pr>F</i>		
<i>Season</i>		<i><0.01</i>	<i><0.01</i>	<i>0.022</i>
<i>Region</i>		<i>0.01</i>	<i>0.57</i>	<i>0.603</i>
<i>Season*Region</i>		<i>0.03</i>	<i>0.01</i>	<i>0.037</i>

Table 6: Maturity indices (size, ground colour, DA, % blush, blush intensity, TSS, internal C₂H₄ and internal CO₂) of ‘Cripps’ Pink’ apples measured after harvest for harvest seasons 2011/2012 and 2012/2013 in production regions (Elgin, Koue Bokkeveld) of the Western Cape, South Africa.

Treatment	Fruit diameter (mm)	Ground colour (chart index)	DA (I _{AD}) ³	Percentage Blush (chart index)	Blush intensity (chart index)	TSS (%)	Internal C ₂ H ₄ (μL·L ⁻¹)	Internal CO ₂ (%)
Season:								
2011/2012	68.5a	3.0a	0.9	55.0	5.7b	12.7	1.8	1.2a
2012/2013	67.1b	2.4b	0.9	55.4	7.9a	13.1	2.0	0.7b
Region:								
Elgin	67.4	2.6	0.9	54.1a	6.6	12.8	2.1	0.9
Koue Bokkeveld	68.2	2.8	0.8	56.9b	6.9	13.0	1.7	0.9
<i>Source of variation</i>				<i>Pr>F</i>				
<i>Season</i>	<i>0.045</i>	<i><0.0001</i>	<i>0.945</i>	<i>0.694</i>	<i><0.0001</i>	<i>0.154</i>	<i>0.886</i>	<i>0.006</i>
<i>Region</i>	<i>0.216</i>	<i>0.156</i>	<i>0.433</i>	<i>0.032</i>	<i>0.467</i>	<i>0.359</i>	<i>0.696</i>	<i>0.812</i>
<i>Season*Region</i>	<i>0.058</i>	<i>0.945</i>	<i>0.435</i>	<i>0.171</i>	<i>0.738</i>	<i>0.821</i>	<i>0.869</i>	<i>0.095</i>

Table 7: Fruit quality indices (firmness, ground colour, DA, TSS, size, blush %, blush intensity) measured after storage and ripening (7 months CA at -0.5 °C + 4 weeks RA at -0.5 °C + 7 days shelf-life) of 'Cripps' Pink' apples for different harvest seasons (2011/2012; 2012/2013) and production regions (Elgin, Koue Bokkeveld) in the Western Cape, South Africa.

Treatment	Firmness (kg)	Diameter (mm)	Ground colour chart index	DA (I _{AD}) ³	TSS (%)	Percentage Blush	Blush intensity index
<u>Season:</u>							
2011/2012	6.2	70.1	2.76b	0.62	13.3	52.9	6.9
2012/2013	6.4	68.9	3.00a	0.53	13.3	52.6	7.2
<u>Region:</u>							
Elgin	6.2	69.3	2.79	0.61	13.1	50.9	6.7
Koue Bokkeveld	6.4	69.8	2.97	0.47	13.5	54.7	7.4
<i>Source of variation</i>				<i>Pr>F</i>			
<i>Season</i>	<i>0.431</i>	<i>0.057</i>	<i>0.040</i>	<i>0.192</i>	<i>0.822</i>	<i>0.929</i>	<i>0.553</i>
<i>Region</i>	<i>0.227</i>	<i>0.406</i>	<i>0.106</i>	<i>0.074</i>	<i>0.120</i>	<i>0.277</i>	<i>0.153</i>
<i>Season*Region</i>	<i>0.179</i>	<i>0.281</i>	<i>0.667</i>	<i>0.282</i>	<i>0.094</i>	<i>0.216</i>	<i>0.646</i>

Table 8: The interaction between main effects, season (2011/2012 and 2012/2013) and region (Elgin and Koue Bokkeveld), found for TA concentration of fruit measured after storage and ripening (7 months CA at -0.5 °C + 4 weeks RA at -0.5 °C + 7 days shelf-life)

Production region	Season	TA (%)
Elgin	2011/2012	0.58a
	2012/2013	0.32c
Koue Bokkeveld	2011/2012	0.46b
	2012/2013	0.38bc
<i>Source of variation:</i>		<i>Pr>F</i>
<i>Season</i>		<i><.0001</i>
<i>Region</i>		<i>0.350</i>
<i>Season*Region</i>		<i>0.005</i>

Table 9: Temperature parameters (accumulated GDD_{>10°C}, average maximum, average minimum, average max - min) of two seasons (2011/2012; 2012/2013) and two growing regions (Elgin, Koue Bokkeveld) in the Western Cape, South Africa during various growth stages (cell division, cell expansion and maturation) of 'Cripps' Pink' apples.

	Accumulated GDD _{>10 °C}						Average maximum (°C)			Average minimum (°C)				Max - Min				
	0-50 ^a	50-100 ^b	100-150 ^c	150-200 ^d	last 60 ^e	Sep-Apr	0-50 ^a	50-100 ^b	last 60 ^e	0-50 ^a	50-100 ^b	150-200 ^d	last 60 ^e	0-50 ^a	50-100 ^b	100-150 ^c	150-200 ^d	last 60 ^e
	DAFB ^f						DAFB			DAFB				DAFB				
<u>Season:</u>																		
2011/2012	223b	639.9b	1153b	1492b	389	1709b	20.1b	24.4b	23.1b	8.6	12.3b	12.5b	10.9a	11.4	12.3	13.1	12.5	12.2b
2012/2013	381a	984.8a	1444a	1957a	500	1832a	25.7a	28.3a	24.4a	9.3	13.5a	13.9a	9.6b	14.3	13.5	14.1	13.9	14.8a
<u>Region:</u>																		
Elgin	327a	905.5a	1364a	1867	474	1881a	22.8	26.5	24.2a	17.7a	11.8b	11.5b	18.5a	11.8	11.8b	12.3b	11.5b	12.8b
Koue Bokkeveld	277b	719.1b	1234b	1582	414	1661b	23.0	26.2	23.3b	16.7b	14.0a	15.0a	16.8b	13.9	14.0a	14.9a	15.0a	14.2a
<u>Source of variation</u>	<i>Pr>F</i>																	
Season	<.001	<0.001	<.001	0.038	0.41	0.016	<.001	<0.001	0.003	0.084	0.034	<.001	<.001	0.062	0.11	0.07	0.108	<0.001
Region	0.003	0.015	0.018	0.184	0.652	<0.001	0.456	0.68	0.025	<.001	<0.01	0.003	<0.001	0.158	0.011	<0.001	0.001	0.016
S*R	0.725	0.459	0.217	0.096	0.496	0.122	0.661	0.595	0.212	0.766	0.444	0.059	0.493	0.813	0.103	0.303	0.891	0.575

^a cell division
^b early cell expansion
^c late cell expansion
^d maturation
^e maturation
^f Days after full bloom

Table 10: Interaction between production region (Elgin and Koue Bokkeveld) and growing season (2011/2012; 2012/2013) for various growth stages (cell division, cell expansion and maturation) of ‘Cripps’ Pink’ apples in the Western Cape, South Africa.

Region	Season	Average Maximum		Average minimum
		100-150 ^c	150-200 ^d	100-150 ^c
		DAFB ^e		DAFB
Elgin	2011/2012	25.3b	22.3b	13.8a
	2012/2013	28.1a	24.3ab	13.9a
Koue Bokkeveld	2011/2012	28.3a	25.4a	13.6a
	2012/2013	27.2ab	23.5ab	12.1b
<i>Source of variation</i>		<i>Pr>F</i>		
<i>Season</i>		<i>0.094</i>	<i>0.975</i>	<i>0.051</i>
<i>Region</i>		<i>0.056</i>	<i>0.097</i>	<i>0.013</i>
<i>Season*Region</i>		<i>0.001</i>	<i>0.010</i>	<i>0.045</i>

^c late cell expansion

^d maturation

^eDays after full bloom

Table 11: Total browning and type of browning (diffuse, radial and combination) incidence of ‘Cripps’ Pink’ apples between two seasons (2011/2012; 2012/2013) and two growing regions (Elgin, Koue Bokkeveld) in the Western Cape, South Africa.

Treatment:	Radial browning (%)	Diffuse browning (%)	Combination browning (%)	Total browning (%)
<u>Season:</u>				
2011/2012	11.4a	16.3	18.6a	46.3a
2012/2013	0.0b	18.4	0.0b	18.4b
<u>Region:</u>				
Elgin	5.8	18.6	8.4	32.8
Koue Bokkeveld	5.6	16.1	10.2	31.9
<i>Source of variation</i>		<i>Pr>F</i>		
<i>Season</i>	<i>0.0011</i>	<i>0.8232</i>	<i>0.0014</i>	<i>0.0455</i>
<i>Region</i>	<i>0.9571</i>	<i>0.7985</i>	<i>0.7273</i>	<i>0.9426</i>
<i>Season*Region</i>	<i>0.9571</i>	<i>0.8983</i>	<i>0.7273</i>	<i>0.8292</i>

Table 12: Incidence of total browning and types of browning (radial, diffuse and combination) compared between seasons (2011/2012; 2012/2013) for each production region (Elgin and Koue Bokkeveld) of ‘Cripps’ Pink’ apples in the Western Cape, South Africa.

Treatment	Radial browning (%)	Diffuse browning (%)	Combination browning (%)	Total browning (%)
<u>Elgin:</u>				
2011/2012	12a	17	17a	45
2012/2013	0b	20	0b	20
<i>Pr>F</i>	<i>0.010</i>	<i>0.760</i>	<i>0.006</i>	<i>0.097</i>
<u>Koue Bokkeveld:</u>				
2011/2012	11.3a	15.6	20.3a	47.2a
2012/2013	0.0b	16.6	0.0b	16.6b
<i>Pr>F</i>	<i>0.001</i>	<i>0.914</i>	<i>0.001</i>	<i>0.023</i>

Table 13: Correlation between incidence of browning types (radial, diffuse and combination) after 7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life with temperature parameters (calculated per growth stage) for the season of 2011/2012 in the Western Cape, South Africa.. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Temperature parameter	Total browning and browning type	Cell division		Early cell expansion		Late cell expansion		Maturation	
		(0-50 DAFB)		(50-100 DAFB)		(100-150 DAFB)		(60 days before harvest)	
		r ^a	p-value	r ^a	p-value	r ^a	p-value	r ^a	p-value
GDD _{>10 °C} ^b	Radial browning	-0.41	0.24	-0.33	0.35	-0.57	0.09	0.00	0.99
	Diffuse browning	0.30	0.39	0.34	0.33	0.07	0.84	-0.79	0.01
	Combination browning	0.35	0.32	0.23	0.52	-0.09	0.81	-0.44	0.21
Average max (°C)	Radial browning	-0.16	0.65	-0.66	0.04	-0.25	0.48	-0.61	0.06
	Diffuse browning	0.18	0.62	0.36	0.30	0.05	0.90	-0.03	0.93
	Combination browning	0.07	0.85	0.32	0.37	-0.04	0.92	-0.59	0.07
Average minimum (°C)	Radial browning	-0.06	0.88	-0.33	0.35	0.47	0.17	-0.03	0.92
	Diffuse browning	-0.01	0.98	0.22	0.53	0.08	0.82	0.10	0.78
	Combination browning	0.11	0.77	0.11	0.76	-0.17	0.64	-0.09	0.81
Max-Min** (°C)	Radial browning	-0.33	0.35	-0.28	0.43	-0.37	0.30	-0.35	0.32
	Diffuse browning	0.31	0.39	0.11	0.76	0.02	0.96	-0.12	0.73
	Combination browning	0.25	0.48	0.19	0.60	0.01	0.97	-0.29	0.42

^a Pearson's correlation coefficient

^b $GDD_{>10 °C} = \frac{max+min}{2} - 10°C$

**Max-Min= Average (daily maximum – daily minimum)

Table 14: Average maximum, minimum and average max - min temperatures for each month from September to April of the two growth seasons (2011/2012; 2012/2013) for Elgin and the Koue Bokkeveld as production regions of 'Cripps' Pink' apples in the Western Cape, South Africa.

Temp	Area	Season	Sep	Oct	Nov	Dec	Jan	Feb	March	April
Average maximum (°C)	Elgin	2011/2012	17.4	19.6	20.9	23.5	26.6	25.8	26.0	22.4
		2012/2013	19.5	22.3	26.7	29	28.0	27.8	26.9	23.9
	Koue Bokkeveld	2011/2012	18.6	19.9	21.3	24.2	29.2	27.4	27.0	20.9
		2012/2013	18.3	23.8	26.6	28.4	28.3	27.5	26.5	21.9
Average minimum (°C)	Elgin	2011/2012	7.3	9.8	10.5	13.3	15.8	14.6	14.0	11.2
		2012/2013	7.9	9.1	10.0	15.4	14.1	13.7	13.1	8.5
	Koue Bokkeveld	2011/2012	5.5	7.8	7.6	10.3	14.5	12.2	12.1	8.3
		2012/2013	6.3	6.1	8.6	13.2	11.8	11.6	10.5	5.8
Max – Min** (°C)	Elgin	2011/2012	10.1	9.8	10.5	10.2	10.9	11.1	11.9	11.5
		2012/2013	11.9	13.2	16.8	14.2	14.3	14.1	13.2	15.4
	Koue Bokkeveld	2011/2012	12.5	12.5	13.8	13.9	14.7	15.2	15.0	12.5
		2012/2013	14.0	17.7	18.1	15.2	16.4	15.8	15.9	16.1

**Max-Min= Average (daily maximum – daily minimum)

Table 15: Correlation between diffuse browning incidence (after 7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) and temperature parameters (calculated per growth stage) for the season of 2012/2013. No other browning type developed for this season. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Temperature parameter	Cell division (0-50 DAFB)		Early cell expansion (50-100 DAFB)		Late cell division (100-150 DAFB)		Maturation (150-200 DAFB)		Maturation (60 days before harvest)	
	r ^a	p-value	r ^a	p-value	r ^a	p-value	r ^a	p-value	r ^a	p-value
* GDD _{>10 °C}	-0.22	0.54	-0.07	0.84	-0.15	0.68	0.31	0.38	-0.22	0.54
Average max	-0.06	0.87	0.32	0.37	0.22	0.54	0.69	0.03	0.53	0.12
Average minimum	-0.25	0.49	-0.29	0.41	-0.46	0.19	-0.38	0.28	-0.34	0.34
Max-Min** (°C)	-0.74	0.01	-0.53	0.11	-0.45	0.19	-0.38	0.28	0.78	0.01

* $GDD_{>10\text{ }^{\circ}\text{C}} = \frac{\text{max} + \text{min}}{2} - 10^{\circ}\text{C}$

**Max-Min= Average (daily maximum – daily minimum)

^a Pearson's correlation coefficient

Table 16: Average maximum temperature (°C) of growth stages (cell division, cell expansion and maturation) compared between seasons (2011/2012; 2012/2013) for each production region (Elgin and Koue Bokkeveld) of ‘Cripps’ Pink’ apples in the Western Cape, South Africa.

Treatment	Physiological growth stages				
	Cell division (0-50 DAFB)	Early cell expansion (50-100 DAFB)	Late cell expansion (100-150 DAFB)	Maturation (150-200)	Maturation (60 days before harvest)
<u>Elgin:</u>					
2011/2012	20.0b	24.4b	25.3b	22.3	23.3b
2012/2013	25.5a	28.7a	28.1a	24.3	25.1a
<i>Pr>F</i>	<0.0001	0.016	<0.001	0.106	0.016
<u>Koue Bokkeveld:</u>					
2011/2012	20.1b	24.5b	28.3	25.4a	22.8
2012/2013	25.9a	27.9a	27.2	23.5b	23.7
<i>Pr>F</i>	<0.0001	0.001	0.278	0.042	0.100

Table 17: Accumulated growing day degrees above 10 °C (GDD_{>10 °C}) of growth stages (cell division, cell expansion and maturation) compared between seasons (2011/2012; 2012/2013) for each production region (Elgin and Koue Bokkeveld) of ‘Cripps’ Pink’ apples in the Western Cape, South Africa.

Treatment	Physiological growth stages				
	Cell division (0-50 DAFB)	Early cell expansion (50-100 DAFB)	Late cell expansion (100-150 DAFB)	Maturation (150-200)	Maturation (60 days before harvest)
<u>Elgin:</u>					
2011/2012	213.0b	623.7b	1143.1b	1494.0b	425.4
2012/2013	403.0a	1103.9a	1540.9a	1881.7a	484.2
<i>Pr>F</i>	<i><.0001</i>	<i>0.003</i>	<i><.0001</i>	<i><.0001</i>	<i>0.089</i>
<u>Koue Bokkeveld:</u>					
2011/2012	233.7b	656.0b	1163.4	1489.9	352.7
2012/2013	358.5a	865.7a	1348.6	1633.1	515.1
<i>Pr>F</i>	<i>0.002</i>	<i>0.036</i>	<i>0.071</i>	<i>0.068</i>	<i>0.555</i>

Table 18: Average minimum temperature (°C) of growth stages (cell division, cell expansion and maturation) compared between seasons (2011/2012; 2012/2013) for each production region (Elgin and Koue Bokkeveld) of 'Cripps' Pink' apples in the Western Cape, South Africa.

Treatment	Physiological growth stages				
	Cell division (0-50 DAFB)	Early cell expansion (50-100 DAFB)	Late cell expansion (100-150 DAFB)	Maturation (150-200)	Maturation (60 days before harvest)
<u>Elgin:</u>					
2011/2012	9.8	13.9	13.8	11.6a	11.9a
2012/2013	10.4	14.8	13.9	10.1b	10.8b
<i>Pr>F</i>	<i>0.322</i>	<i>0.415</i>	<i>0.964</i>	<i>0.042</i>	<i>0.034</i>
<u>Koue Bokkeveld:</u>					
2011/2012	7.5	10.5a	13.6a	11.1a	9.8a
2012/2013	8.2	12.4b	12.1b	7.9b	8.3b
<i>Pr>F</i>	<i>0.156</i>	<i>0.010</i>	<i>0.018</i>	<i><0.001</i>	<i>0.004</i>

Table 19: Average max - min temperatures (°C) of growth stages (cell division, cell expansion and maturation) compared between seasons (2011/2012; 2012/2013) for each production region (Elgin and Koue Bokkeveld) of 'Cripps' Pink' apples in the Western Cape, South Africa.

Treatment	Physiological growth stages				
	Cell division (0-50 DAFB)	Early cell expansion (50-100 DAFB)	Late cell expansion (100-150 DAFB)	Maturation (150-200 DAFB)	Maturation (60 days before harvest)
<u>Elgin:</u>					
2011/2012	10.2	10.5b	11.5b	10.7	11.4b
2012/2013	13.4	13.1a	13.1a	12.2	14.3a
<i>Pr>F</i>	<i>0.066</i>	<i>0.004</i>	<i>0.030</i>	<i>0.268</i>	<i>0.007</i>
<u>Koue Bokkeveld:</u>					
2011/2012	12.7	14.0	14.6	14.3	13.0b
2012/2013	15.2	14.0	15.1	15.6	15.3a
<i>Pr>F</i>	<i>0.330</i>	<i>0.984</i>	<i>0.609</i>	<i>0.252</i>	<i>0.004</i>

Table 20: Correlation of incidence of total browning and browning types (radial, diffuse and combination), after 7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life, with maturity parameters (starch breakdown, firmness, fruit diameter, ground colour, % blush, TSS and TA) of 'Cripps' Pink' apples at harvest for the season of 2011/2012 (pooled over regions) in the Western Cape, South Africa. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

	Radial browning		Diffuse browning		Combination browning	
	r ^a	p-value	r ^a	p-value	r ^a	p-value
Starch breakdown (chart)	0.06	0.80	-0.04	0.88	0.03	0.92
Firmness (kg)	-0.01	0.96	0.25	0.30	0.24	0.31
Fruit diameter (mm)	0.11	0.64	0.35	0.14	0.44	0.05
Ground colour (chart)	-0.08	0.74	0.41	0.07	0.31	0.18
% Blush (%)	0.28	0.24	0.21	0.38	0.21	0.38
TSS (%)	-0.08	0.74	0.42	0.06	0.19	0.42
TA (%)	-0.18	0.45	0.52	0.02	0.04	0.86

^a Pearson's correlation coefficient

Table 21: Maturity indices (starch breakdown, firmness, diameter, ground colour, DA, % blush, blush intensity, TSS and TA) of 'Cripps' Pink' apples measured after harvest compared between seasons (2011/2012 and 2012/2013) for each production region (Elgin and Koue Bokkeveld) of the Western Cape, South Africa.

Treatment	Starch breakdown (chart index)	Firmness (kg)	Fruit diameter (mm)	Ground colour (chart index)	DA (I_{AD}) ³	% Blush	Blush intensity (chart index)	TSS (%)	TA (%)
<u>Elgin:</u>									
2011/2012	49.8	8.0	68.7a	3.0a	0.9	55.6	5.6b	12.6	0.75a
2012/2013	66.4	8.0	66.1b	2.3b	1.0	52.7	7.7a	13.0	0.62b
<i>Pr>F</i>	<i>0.528</i>	<i>0.834</i>	<i>0.001</i>	<i><.0001</i>	<i>0.128</i>	<i>0.437</i>	<i>0.001</i>	<i>0.294</i>	<i>0.001</i>
<u>Koue Bokkeveld:</u>									
2011/2012	64.9	7.7b	68.2	3.1a	0.8	55.7	5.8b	12.8	0.70
2012/2013	67.5	8.4a	68.2	2.4b	0.8	58.2	8.1a	13.3	0.70
<i>Pr>F</i>	<i>0.528</i>	<i>0.003</i>	<i>0.946</i>	<i><.0001</i>	<i>0.553</i>	<i>0.674</i>	<i>0.002</i>	<i>0.320</i>	<i>0.893</i>

Table 22: Correlation of incidence of diffuse browning (after 7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) with maturity parameters of ‘Cripps’ Pink’ apples at harvest for the season of 2012/2013 (pooled over and regions) in the Western Cape, South Africa. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Maturity Parameter	r ^a	p-value
Starch breakdown (%)	0.18	0.45
Firmness (kg)	0.36	0.12
Fruit diameter (mm)	-0.10	0.68
Ground colour (chart)	0.06	0.80
DA (I _{AD}) ³	-0.21	0.38
% Blush	0.35	0.14
Blush intensity (chart)	0.32	0.17
TSS (°Brix)	0.45	0.05
TA (%)	-0.39	0.09
C ₂ H ₄ (μL·L ⁻¹)	-0.05	0.82
CO ₂ (%)	0.03	0.91

^a Pearson's correlation coefficient

Table 23: Correlation of incidence of total browning and browning types (radial, diffuse and combination), after 7 months of CA storage at -0.5 °C + RA storage at -0.5 °C + 7 days shelf-life, with quality parameters (firmness, fruit diameter, ground colour, DA, % blush, blush intensity, TSS and TA) of 'Cripps' Pink' apples after storage (7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) as variables for the season of 2011/2012 (regions pooled). The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Maturity parameters	Radial Browning		Diffuse browning		Combination browning	
	r ^a	p-value	r ^a	p-value	r ^a	p-value
Firmness (kg)	-0.26	0.26	-0.36	0.12	-0.39	0.09
Fruit diameter (mm)	0.70	<0.01	-0.01	0.97	0.45	0.05
Ground colour (index)	0.31	0.19	-0.11	0.65	0.07	0.76
DA (I _{AD}) ³	-0.04	0.87	0.21	0.38	0.19	0.41
Blush %	0.06	0.81	0.20	0.41	0.28	0.24
Blush intensity	0.05	0.85	0.06	0.80	0.34	0.15
TSS (%)	0.17	0.48	0.45	0.05	0.41	0.07
TA (%)	-0.50	0.03	-0.32	0.16	-0.64	<0.01
C ₂ H ₄ (μL·L ⁻¹)	-0.70	0.01	-0.27	0.25	-0.60	0.01
CO ₂ (%)	-0.40	0.08	-0.23	0.34	-0.46	0.04

^a Pearson's correlation coefficient

Table 24: Correlation between incidence of diffuse browning and quality parameters (after 7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) of 'Cripps' Pink' apples as variables for the season of 2012/2013 (regions pooled) in the Western Cape, South Africa. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Maturity parameter	r ^a	p-value
Firmness (kg)	-0.45	0.05
Diameter (mm)	0.05	0.84
Ground colour (chart)	0.32	0.18
DA (I _{AD}) ³	-0.29	0.23
Blush %	0.30	0.21
Blush Intensity (chart)	0.18	0.44
TSS (%)	0.33	0.15
TA (%)	-0.38	0.10

^a Pearson's correlation coefficient

Table 25: Fruit quality of ‘Cripps’ Pink’ apples measured after storage (7 months CA at -0.5 °C + 4 weeks RA at -0.5 °C + 7 days shelf-life) compared between seasons (2011/2012 and 2012/2013) for each production region (Elgin and Koue Bokkeveld) of ‘Cripps’ Pink’ apples.

Treatment:	Firmness (kg)	Ground colour (chart index)	DA (I _{AD}) ³	TSS (%)	TA (%)	Diameter (mm)	Blush %	Blush intensity (chart index)
<u>Elgin:</u>								
2011/2012	6.2	2.6	0.6	13.3	0.6a	70.2a	53.2	6.6
2012/2013	6.1	2.9	0.6	12.9	0.3b	68.3b	48.6	6.7
<i>Pr>F</i>	<i>0.717</i>	<i>0.083</i>	<i>0.864</i>	<i>0.293</i>	<i><0.001</i>	<i>0.001</i>	<i>0.270</i>	<i>0.928</i>
<u>Koue Bokkeveld:</u>								
2011/2012	6.2	2.9	0.6	13.2	0.5	70.0	52.7	7.1
2012/2013	6.6	3.1	0.5	13.7	0.4	69.5	56.7	7.7
<i>Pr>F</i>	<i>0.100</i>	<i>0.252</i>	<i>0.112</i>	<i>0.197</i>	<i>0.080</i>	<i>0.640</i>	<i>0.476</i>	<i>0.439</i>

Figures:



Figure 1: Radial browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life) in a 'Cripps' Pink' apple harvested in 2011/2012 in the Western Cape, South Africa.



Figure 2: Diffuse browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life) in a 'Cripps' Pink' apple harvested in 2011/2012 in the Western Cape, South Africa.



Figure 3: "Combination" browning found after storage (7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) in a 'Cripps' Pink' apple harvested in 2011/2012 in the Western Cape, South Africa.

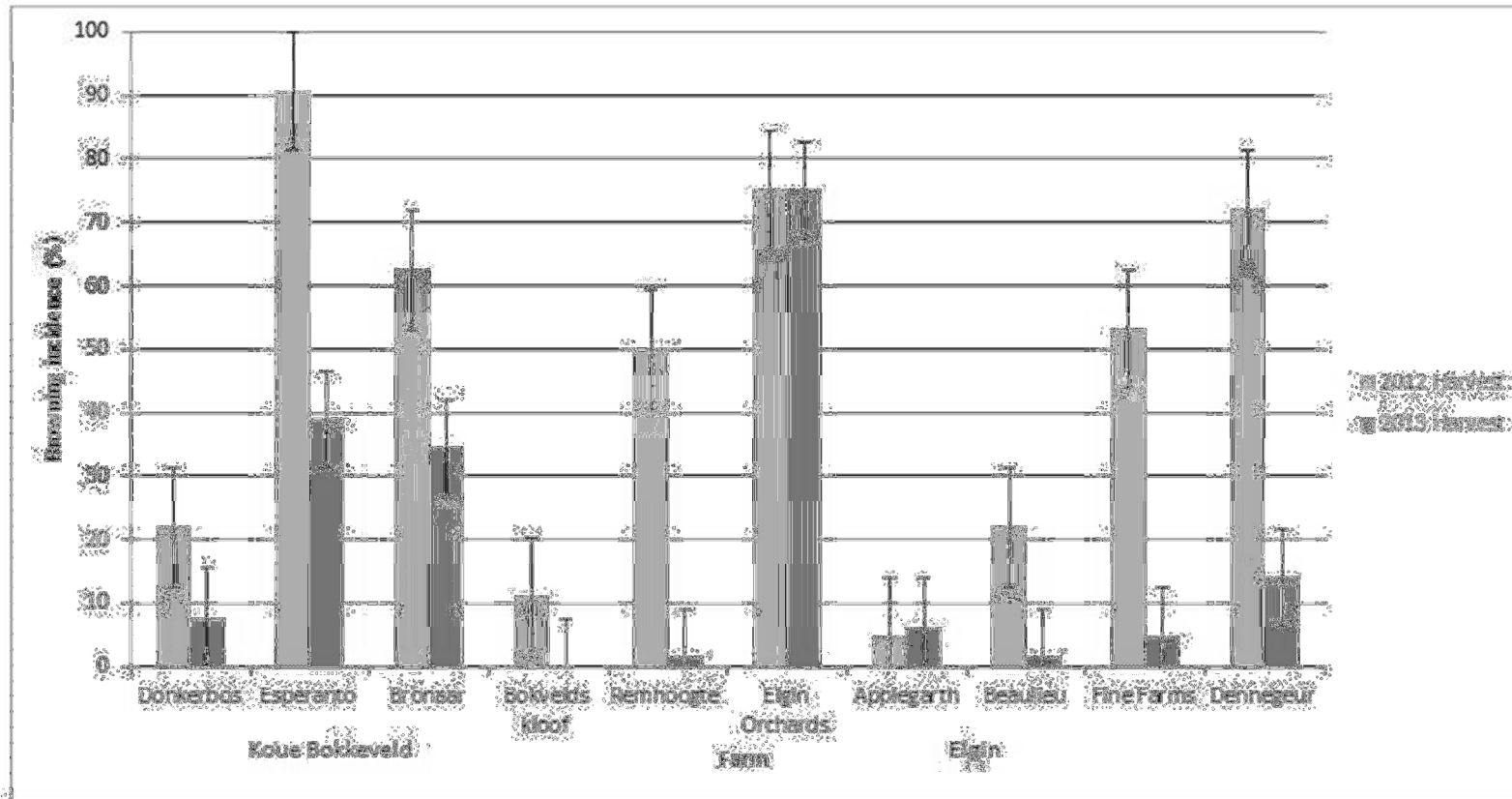


Figure 4: The difference in browning incidence per farm for each harvest year (with standard error bars).

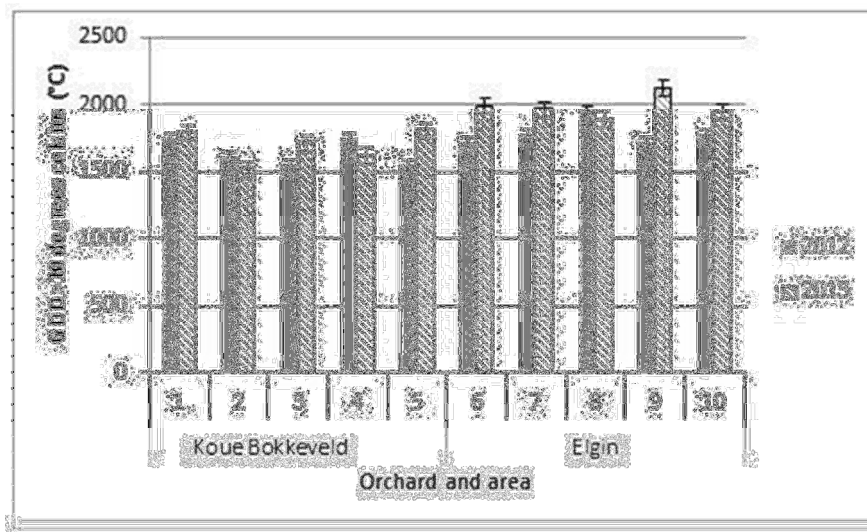


Figure 5: Accumulated growing day degrees above 10 °C (GDD_{>10 °C}) for the period of 1 September to harvest for each orchard in different regions (Koue Bokkeveld, Elgin) for different harvest seasons (2012, 2013) of 'Cripps' Pink' apples in the Western Cape, South Africa.

PAPER 2

THE INFLUENCE OF TREE AGE, SOIL TYPE AND MINERAL COMPOSITION OF FRUIT ON INTERNAL FLESH BROWNING OF 'CRIPPS' PINK' APPLES

ABSTRACT

The market value of 'Cripps' Pink' apples is reduced by internal browning found after extended controlled atmosphere (CA) storage and a regular atmosphere (RA) shipment period. The aim of this study was to investigate the influence of tree age, soil type and mineral composition of fruit on the incidence of internal browning in an attempt to explore possible pre-harvest factors affecting browning development. Fruit were harvested at post-optimum maturity (starch breakdown > 50 %) from six farms in the Koue Bokkeveld region in the 2011/2012 and 2012/2013 seasons. Fruit were examined after 7 months controlled atmosphere (CA) at -0.5 °C + 4 weeks regular atmosphere (RA) storage at -0.5 °C + 1 week shelf-life at ambient. Incidence of the total browning and type of browning, fruit maturity and mineral concentration of fruit were measured after the storage period and compared between orchards with young and old trees and between orchards with clay and sandy soils. Mineral analysis of 25 brown and 25 non-brown fruit was done. Radial browning was seasonal and highest during 2011/2012, which influenced the occurrence of higher "combination" and total browning for that season. Diffuse browning did not differ between seasons in this trial. The highest incidence of total, diffuse and "combination" browning was found in orchards with young trees. Incidence of radial browning did not differ between orchards with different tree ages or soil types. The potassium (K), calcium (Ca) and magnesium (Mg) concentration of fruit and the ratio of nitrogen (N):Ca, phosphorus (P):K, K:Mg, K:Ca, Ca:P and Ca:Mg differed significantly between brown and non-brown fruit. The P and Mg concentration and ratio of P:K, K:Mg, Ca:P and Ca:Mg correlated with the percentage of non-brown or brown fruit. Non-brown fruit had higher K concentrations and K:Mg, K:Ca, N:Ca, Ca:P and P:K ratios. Brown fruit had high concentrations of Ca and Mg and high P:K, Ca:Mg and Ca:P ratios. Higher K concentration and K:Mg ratios were observed in both seasons for non-brown fruit and the K:Mg ratio had a strong positive correlation with non-brown fruit and a negative correlation with browning incidence. The mineral concentration of especially K and ratio of K:Mg does seem to be involved in browning development, however these results should be confirmed over more seasons.

Keywords: *Malus domestica*, 'Pink Lady'TM, diffuse browning, radial browning, "combination" browning, potassium, potassium to magnesium ratio.

INTRODUCTION

The Cripps' Pink cultivar fruited for the first time in 1979 after a cross was made between the cultivars Lady Williams and Golden Delicious in 1973 (Nicholas and Vermeij, 1998). The fruit were described as uniquely flavoured, yellow-green with a partial red blush and a thin peel which does not crack, thus having a high tolerance to sunburn (Cripps et al., 1993). The fruit can be stored for periods of up to six months in cold storage and has a fine texture which does not brown easily after being cut and exposed to air. A major problem for 'Cripps' Pink' growers is internal flesh browning of fruit which becomes apparent after long term storage under controlled atmosphere (CA) conditions (James et al., 2005; Jobling et al., 2004). 'Cripps' Pink' is sold at a premium as 'Pink Lady'TM when the blush colour covers more than 40 % of the total surface area (Hurdall and Fourie, 2003). Internal browning has therefore also become a problem for the Pink LadyTM trademark. Three different types of internal flesh browning (IFB) have been identified. These types of browning differ according to affected tissue and the temperature conditions under which they mainly occur (James, 2007). Diffuse flesh browning (DFB) is related to chilling injury (Bramlage et al., 1980; James et al., 2005) and fruit maturity (Majoni et al., 2013) which leads to collapse of the fruit cortex. Incidence of diffuse flesh browning is influenced by temperature during the cell division and maturation stage of fruit development (Paper 1). Radial flesh browning (RFB) shows symptoms of senescent breakdown (Wilkinson and Fidler, 1973) and occurs in tissue adjacent to the vascular cells (Fig. 1), mostly in the stem end of the fruit and lessens towards the calyx end (James, 2007). Low maximum temperatures during the cell expansion phase also seem to play a role (Paper 1) and according to Australian research radial browning is induced between 1100 and 1700 growing day degrees above 10 °C (GDD_{10°C}) (Jobling and James, 2008). A third type of browning that is CO₂ induced occurs in all climatic areas and consists of cell membrane damage which forms cavities or pits inside the fruit. This type of browning is related to controlled atmosphere storage associated with high CO₂ and low O₂ levels (Lau, 1998). The mineral composition of fruit protects it against physiological disorders by affecting fruit quality at harvest (Bramlage et al., 1980; Sharples, 1980). Fruit quality is affected by fruit size, the state of the membranes at harvest and the mineral and photosynthate concentration of fruit at harvest (Ferguson and Watkins, 1989). Enlargement of fruit by increasing potassium (K) and nitrogen (N) uptake reduces the concentration of fruit flesh nutrients and photosynthate concentration by dilution (Fallahi et al., 1985; Faust, 1989). Increased phosphorus (P) concentration of fruit stabilize membranes (Neilsen et al., 2008). Calcium (Ca) affects the rigidity of cell walls and a deficit alters the cell wall structure so that it becomes less ridged (Huber, 1983). Cell membranes are affected by calcium. A deficit

leads to an increase in micro viscosity of membranes and an increase in permeability which can lead to a loss of compartmentalization (Marinos, 1962). Although Ca, for example, has a clear role in cell wall and cell membrane protection and strengthening (Rossignol et al., 1977) a single nutrient cannot be seen as the sole cause or lack of a physiological disorder occurrence. Minerals such as Ca, magnesium (Mg), K, N, P and micro nutrients such as boron (B), zinc (Zn) and manganese (Mn) are known to correlate with postharvest quality of fruit. De Castro et al. (2008) found a relationship between high Mg, Ca and B and low K, with a low incidence of IFB. High concentrations of N, Mn and Zn and lower concentrations of K and Mg in fruit were correlated with a higher incidence of CO₂ injury (de Castro et al., 2008). The effect of mineral concentration of fruit on postharvest quality is most pronounced when a mineral is in excess or deficient, or a mineral combination is not in balance (Bramlage, 1993). It is important to establish threshold values for different minerals and their influence on browning development.

Factors such as tree vigour, soil type and crop load are known to influence the mineral composition of fruit. Cultural practices which enhance tree vigour and fruit size adversely affects fruit quality (Bramlage, 1993). Differences in soil texture leads to differences in cation holding capacity, water retention and pH (Rengasamy and Churchman, 1999). The largest differences occur between sandy and clay soils with clay soils having a larger cation holding capacity, a better ability to hold water and a finer texture. Sandy soils, with their coarse texture, have a lower cation exchange capacity and lose water much quicker leading to leaching of minerals under flooding conditions (Su et al., 2004). Crop load was found to influence the 'Braeburn' browning disorder (Percy, 1997; Tough et al., 1998). Heavy crop loads lead to a lower IFB incidence in this cultivar. The vigorous growth of light cropping trees led to the transport of minerals to the stronger vegetative sink (shoots) leaving fruit as weak competitors for minerals and thus receiving less (Hanger, 1979).

The combined effects of high and low temperature during the growing season, fruit maturity and the mineral composition of fruit as affected by soil type and tree age may contribute to the development of postharvest internal browning of 'Cripps' Pink' apples.

The objective of this study was to determine whether mineral nutrients related to fruit quality differ for fruit that become brown and to identify whether soil type and tree age play a role in mineral composition and incidence of IFB of 'Cripps' Pink' apples.

MATERIALS AND METHODS

Plant material and orchard information

Six orchards from three farms in the Koue Bokkeveld (lat. 33°05'00"S long.19°25'00"E) region in South Africa were chosen based on tree age and soil type. Three orchards with old trees and 3 orchards with young trees were selected (Tables 1 and 2). Of the same 6 orchards, 3 orchards were planted in sandy soils and 3 of the orchards were planted in clay soils. Each 'Cripps' Pink' orchard is typically harvested 2 to 3 times depending on the crop load and the blush colour development of fruit throughout the canopy. 'Cripps' Pink' apples were harvested as close to the last commercial harvest as possible in order to increase the chances of flesh browning to develop (Majoni, 2012).

All trees were planted on M793 rootstock. Data are summarised in Table 1. All cultural orchard practices were in agreement with commercial norms for the region.

Sampling procedure

Five replicates of 60 fruit each were harvested per orchard for both seasons (2011/12 and 2012/13). A subsample of 20 fruit from each of the 5 replicates was randomly selected for measurement of maturity indices at harvest. Fruit with the 'Pink Lady™' standard according to blush percentage were selected for harvest (> 40 % blush). Harvesting of fruit from the different farms took place over one week. For both seasons, the fruit were harvested into plastic lug boxes which were stored in RA at -0.5 °C for one week during the accumulation phase after harvest. Fruit were divided into two storage groups according to harvest date (Table 3). Fruit were then stored in CA (1 % CO₂, 1.5 % O₂ at -0.5 °C) for 7 months. After storage, fruit were packed into commercially used fruit trays and cartons lined with polypropylene bags (37.5 micron). The packed fruit were then stored for 4 weeks at RA (-0.5 °C) to simulate shipment followed by a one week period at shelf-life conditions. After completion of the storage period (7 months CA, 4 weeks RA and 1 week shelf-life), fruit were analysed for browning incidence and other quality parameters such as flesh firmness, ground colour, % blush, blush intensity, total soluble solids concentration (TSS), titratable acid (TA), ethylene (C₂H₄) and CO₂ concentration of fruit. Due to gas chromatographic equipment failure at the time of evaluation, the latter two indices are only reported for the 2011/2012 season.

Quality and maturity indices

After harvest, maturity indexing of fruit was performed on 20 fruit per orchard by ExperiCo (Agri-Research Solutions, P O Box 4022, Idas Valley, Stellenbosch, 7609, South Africa). Flesh firmness (kg) was measured equatorially on two opposite pared sides of the fruit using a FTA (Fruit Texture Analyser, Güss Instruments, Strand) fitted with an 11.1 mm plunger. Ground colour of fruit were rated from 0.5 (green) to 5 (yellow), using the South African Industry colour chart for apples and pears (Unifruco Research service (Pty) LTD). Blush intensity was determined using the industry colour chart P16 for Pink Lady™ apples, where 0 indicates fruit with no red blush colour and 12 indicates fruit with an intense red blush colour. A slice of each fruit was used to create a composite sample for TSS (%) measurement with a digital refractometer (Atago DBX-30, Japan). TA was determined by titrating a 10 g aliquot of a composite juice sample per replicate with 0.1 M NaOH to a pH end point of 8.2, using a Dosimat titrator (Metrohm 605, Herisau, Switzerland). TA was expressed as malic acid equivalents (%).

Starch breakdown was expressed as percentage breakdown after colouration of fruit with iodine. One half of the fruit's equatorial cut surface was stained with a 0.5 M potassium iodide solution and then left for one minute before evaluation using the Unifruco starch conversion chart (Unifruco research services (PTY) Ltd.).

Fruit quality was assessed on the remaining 40 fruit per sample at ExperiCo (Agri-Research Solutions, P O Box 4022, Idas Valley, Stellenbosch, 7609, South Africa) after the 7 months CA, 4 weeks RA and 1 week under shelf-life conditions. Quality assessment included flesh firmness, determination of Ground colour of the peel by colour chart, peel colour/maturity by DA-Meter, TSS, TA, internal defects and type (%), external blemishes (%) and decay (%). Measurements of flesh firmness and ground peel colour were performed on single fruit while TSS and TA concentration of fruit were measured from composite juice samples.

Internal quality was determined destructively by cutting fruit horizontally between the equatorial region and the stem-end region where internal defects and types of browning could be assessed visually and were expressed as a percentage affected fruit for each replication. Other than internal browning no other internal defects were detected. Types of browning were identified by the browning pattern of the affected tissue on the freshly cut surface of the apple described by James (2007). Radial browning was identified by browning of tissue surrounding the vascular bundles (Fig 1.) and diffuse browning (Fig. 2), was identified by browning of the cortex tissue below the fruit peel and between the vascular bundles.

Twenty five brown and 25 non-brown apples were selected for mineral analysis from each orchard, as far as browning incidence allowed. Mineral analysis was conducted at Bemlab

(PO Box 684, Strand, Somerset Mall, 7137, South Africa) as single fruit samples. Samples were analysed via the standard method using the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) procedure together with a nitrogen analyser.

Statistical analysis

An analysis of variance (ANOVA) was used to compare maturity of fruit which were harvested from sandy soil orchards to fruit harvested from clay soil orchards. Maturity of fruit harvested from orchards with young trees was compared to fruit harvested from orchards with old trees using an ANOVA. The Pearson's correlation coefficient was generated and used to find correlations of browning incidence of each orchard with maturity indices measured pre-storage and mineral analysis measured after long term storage.

An ANOVA was used to find if a single mineral or a relationship between minerals such as N:Ca, N:Mg, P:K, K:Mg, K:Ca, Ca:P and Ca:Mg differed in brown versus non-brown fruit. This comparison of mineral composition was repeated for brown versus non-brown fruit harvested from orchards with different soil types (sand and clay), from orchards with different tree ages (old and young) and fruit harvested from different seasons (2011/2012 and 2012/2013).

Average mineral concentration of fruit was calculated separately for brown and non-brown fruit found for each replicate of each orchard. The average of each mineral for brown or non-brown fruit was then correlated to the percentage of brown or non-brown fruit for that replicate. Thus, the mineral concentration of non-brown fruit was correlated to the percentage of non-brown fruit for the specific replication within the specific orchard. The same was done for brown fruit brown fruit.

RESULTS

Incidence of browning types and total browning

Incidence of radial browning differed significantly between seasons with a higher incidence in the 2011/2012 (13 %) season compared to the 2012/2013 season (1 %) (Table 3). "Combination" browning can only develop if both radial and diffuse browning are present in fruit (Paper 3), therefore the incidence of "combination" browning also differed significantly between the 2011/2012 season (24 %) and 2012/2013 season (4 %), due to the difference in radial browning between seasons. Incidence of diffuse browning did not differ significantly between seasons. Seventeen percent of fruit harvested in the 2011/2012 season and 21 % of fruit harvested in the 2012/2013 season developed diffuse flesh browning after long term CA storage at -0.5 °C. Total incidence of browning differed significantly between seasons

and was much higher for the 2011/2012 season (55 %) compared to the 2012/2013 season (26 %).

Total incidence of browning varied between orchards. The highest total incidence of browning for the 2011/2012 season (80 %) was found in orchard 23 on Esperanto (young trees, clay soil) and the lowest incidence (9 %) was found in orchard 26 on Bokveldskloof (old trees, clay soils) (Table 1). The highest incidence of total browning for the 2012/2013 season (47 %) was found in orchard 24 on Esperanto (young trees, sandy soils) and the lowest incidence (4 %) was found in orchard 21 on Donkerbos (old trees, sandy soils) (Table 2). No obvious trend between browning incidence and specific orchards could be identified.

Browning type and incidence, and fruit maturity harvested in the 2011/2012 season for orchards with different soil types and tree ages.

Incidence of radial browning did not differ significantly between fruit harvested from orchards with different soil types or tree ages (Table 4). Incidence of diffuse, “combination” and total browning differed significantly between fruit harvested from orchards on sandy and clay soils and fruit harvested from orchards with old trees and orchards with young trees. Fruit harvested from orchards with sandy soils were more susceptible to development of diffuse browning (24 %) compared to fruit harvested from orchards with clay soils (10 %). Fruit harvested from orchards with young trees showed a higher incidence of diffuse browning (31 %) compared to fruit harvested from orchards with old trees (3 %). Incidence of “combination” browning was higher in fruit harvested from orchards with sandy soils (27 %) and young trees (34 %) compared to clay (20 %) soils and old trees (13 %). The total incidence of browning was significantly higher for orchards with sandy soils (62 %) or young trees (78 %) compared to orchards with clay soils (48 %) or old trees (32 %).

Maturity of fruit differed significantly between orchards with different tree ages. Starch breakdown was further developed in fruit harvested from orchards with young trees and orchards with sandy soils (Table 4) even though 2 out of 3 orchards with young trees were harvested a week before fruit from orchards with old trees (Tables 1 and 2). Ground colour of fruit harvested from young trees differed significantly from fruit harvested from orchards with old trees. Fruit harvested from young trees had a more yellow ground and larger fruit compared to fruit harvested from orchards with old trees. The blush percentage, blush intensity, TSS and TA concentration of fruit were significantly higher in fruit harvested from orchards with young trees. The CO₂ concentration of fruit was significantly higher for fruit from sandy soil orchards compared to fruit from clay soil orchards. Fruit harvested from orchards with young trees were significantly riper than fruit harvested from orchards with old trees.

Browning type and incidence, and fruit maturity harvested in the 2012/2013 season for orchards with different soil types and tree ages.

Incidence of radial browning was very low in this season. Radial browning however still differed significantly between fruit harvested from orchards which differed in soil type as well as tree age (Table 5). Fruit from young trees and fruit from sandy soils had a slightly higher incidence of radial browning. Incidence of diffuse browning differed significantly between fruit from young and old orchards. Fruit harvested from orchards with young trees (33 %) were more susceptible to the development of diffuse browning compared to fruit harvested from orchards with old trees (8 %). “Combination” browning was slightly higher compared to radial browning but only differed significantly for tree age. Fruit harvested from young orchards had higher levels of “combination” browning.

Fruit maturity indices firmness, blush percentage, blush intensity, TA and TSS (Table 5), differed significantly between fruit from young and old orchards. These maturity parameters were all higher for fruit harvested from young orchards even though fruit from 2 out of 3 orchards with young trees were harvested a week earlier than fruit from old orchards. Fruit harvested from young orchards were more mature compared to fruit harvested from old orchards judging by the starch breakdown even though firmness was also higher for these fruit. Fruit diameter and TA differed significantly between fruit harvested from orchards with different soil types. Fruit harvested from clay soil orchards were larger, had a lower TA and starch breakdown levels were lower. Fruit firmness and ground colour were not significantly different for orchards on different soil types.

Fruit maturity differences for seasons

Fruit maturity parameters were measured at harvest for the seasons of 2011/2012 and 2012/2013. Starch breakdown, ground colour, size and blush percentage of fruit did not differ significantly between seasons (Table 6). Fruit firmness, blush intensity and TSS concentration of fruit were significantly higher for fruit harvested during the 2012/2013 season. The TA concentration of fruit in the 2012/2013 season was significantly lower compared to the 2011/2012 season fruit.

Correlations of fruit maturity measured at harvest with incidence and type of browning

Incidence of radial browning showed a significant but weak negative correlation with TA of fruit ($R^2 = -0.38$) harvested during the 2011/2012 season (Table 7). Fruit with a lower TA at

harvest were more susceptible to development of radial browning compared to fruit with a higher TA. Incidence of radial browning showed a significant positive correlation with internal CO₂ of fruit ($R^2 = 0.46$). Orchards with riper fruit had a higher incidence of radial browning (Table 7).

Incidence of diffuse browning in 2011/2012 correlated positively to ground colour ($R^2 = 0.54$), starch breakdown ($R^2 = 0.41$), fruit diameter ($R^2 = 0.70$), blush percentage ($R^2 = 0.39$), blush intensity ($R^2 = 0.56$), TSS ($R^2 = 0.70$) and TA ($R^2 = 0.70$) of fruit. Large fruit with a more advanced starch breakdown, yellow ground colour, high percentage of blush coverage and blush intensity and high TSS and high TA concentration (thus, more mature) were susceptible to development of diffuse browning after long term CA storage at -0.5 °C, shipping simulation (RA for 4 weeks at -0.5°C) and ripening for 7 days at ambient temperature.

Incidence of “combination” browning in 2011/2012 showed a positive correlation with fruit diameter ($R^2 = 0.56$), blush intensity ($R^2 = 0.39$), internal CO₂ ($R^2 = 0.43$) and TSS ($R^2 = 0.57$) of fruit. Large fruit with higher blush intensity, TSS and internal CO₂ were more susceptible to development of “combination” browning after long term storage and ripening. Incidence of radial, diffuse and “combination” browning showed a weak but positive correlation with firmness and TA of fruit harvested during the 2012/2013 season (Table 8). No other correlations were found for this season.

Mineral concentration of fruit compared between two seasons

All mineral concentrations of fruit differed significantly between seasons (Table 9). Fruit in the 2011/2012 season had a significantly lower N, P and Ca concentration and a significantly higher K and Mg concentration compared to fruit in 2012/2013. The ratios of N:Ca, N:Mg, P:K, K:Mg, Ca:P and Ca:Mg were higher in fruit in 2012/2013 season compared to fruit of the 2011/2012 season. The ratios of K:N and K:Ca were higher in fruit harvested in 2011/2012 compared to fruit harvested in 2012/2013.

Mineral concentration of brown and non-brown fruit

Brown and non-brown fruit harvested during the 2011/2012 season differed significantly according to K concentration, and the ratios of P:K, K:N and K:Mg (Table 10). Non-brown fruit had a higher K concentration and ratios of K:N and K:Mg. Brown fruit had a higher ratio of P:K. Brown and non-brown fruit harvested during the 2012/2013 season differed significantly according to K, Ca and Mg concentration and ratios of N:Ca, K:N, K:Mg, K:Ca, Ca:P and Ca:Mg (Table 11). Brown fruit had higher Ca and Mg levels and higher ratios of Ca:P and Ca:Mg compared to that of non-brown fruit. Non-brown fruit had a higher

concentration of K and higher ratios of N:Ca, K:N, K:Mg and K:Ca. The trend of higher K concentrations and K:Mg ratio in non-brown fruit found in the 2011/2012 season (Table 10) repeated itself in 2012/2013 (Table 11).

Mineral concentration of fruit harvested from orchards that differ in soil type and tree age

Fruit harvested from young orchards during the 2011/2012 season had a significantly higher N, P, K, Ca, Mg and Ca:P ratio compared to fruit harvested from orchards with old trees. Significantly higher ratios of P:K, K:N, K:Mg, K:Ca and a lower K:N ratio were found in fruit harvested from orchards with older trees (Table 12). Higher levels of K and K:Mg were found in fruit harvested from orchards planted in a clay soil compared to fruit harvested from orchards with a sandy soil. A higher Ca:P ratio was found for fruit harvested from sandy soils. Fruit harvested from orchards with sandy soils were more prone to diffuse- and “combination” browning development during storage (Table 5).

Fruit harvested in 2012/2013 from young orchards had a significantly higher N and Ca concentration and N:Ca, N:Mg, Ca:P and Ca:Mg ratios and significantly lower P and K concentration, and P:K, K:N, K:Mg, and K:Ca ratios compared to fruit harvested from old orchards (Table 13). Fruit harvested from clay soil orchards had a significantly higher N, P, K, N:Ca, N:Mg, K:Mg and K:Ca ratios and a significantly lower Ca concentration and K:N, Ca:P and Ca:Mg ratios. Incidence of browning or browning types did not differ between sand and clay soil orchards for this season.

Correlations between mineral concentration of fruit and classification according to the percentage of brown and non-brown fruit

The P concentration ($R^2 = 0.43$) as well as the ratio of P:K ($R^2 = 0.42$), K:Mg ($R^2 = 0.60$) and Ca:Mg ($R^2 = 0.27$) of fruit correlated positively with the incidence of non-brown fruit (Table 14). Percentage of non-brown fruit correlated negatively with Mg ($R^2 = -0.45$) and the ratio of Ca:P ($R^2 = -0.34$). Fruit with a high P concentration, a high ratio of P:K, K:Mg and Ca:Mg; and a low concentration of Mg and ratio of Ca:P were not likely to develop internal browning during long term storage (Table 14). All correlations were, however, weak except for the K:Mg positive correlation to non-brown fruit ($R^2=60$). Incidence of browning correlated positively with Mg concentration of fruit ($R^2 = 0.50$) and negatively with the ratio of K:Mg ($R^2 = -0.46$) and Ca:Mg ($R^2 = -0.35$). Fruit with high Mg concentration and low ratios of K:Mg and Ca:Mg were more susceptible to development of internal browning.

DISCUSSION

Brown fruit in the 2011/2012 season had a lower K concentration, K:N and K:Mg ratios and a higher P:K ratio compared to non-brown fruit (Table 10). For the 2012/2013 season brown fruit again had a lower K concentration, and K:N and K:Mg ratios but also a higher Mg, Ca concentration and Ca:P, Ca:Mg ratios, and a lower K:Ca ratio (Table 11). These results do not support findings by de Castro et al (2008) where high Mg, Ca and low K concentration of 'Cripps' Pink' were related to low incidence of internal flesh browning. The K and Mg content of fruit for both seasons was below the lower benchmark for mineral content of 'Cripps' Pink' apples associated with good storage potential (Table 15). The P and Ca content of fruit were within the range of fruit mineral content associated with good storage potential (Bemlab, PO Box 684, Strand, Somerset Mall, 7137, South Africa) (Table 15). Low levels of fruit K can be found in high density production systems, coarse textured soils or in the presence of K-fixing clay minerals. In the absence of adequate K supply trees are unable to support the area of foliage produced and this deficiency is exacerbated with an increase in N (Gildehaus, 1931). The K content of fruit is important for protection of cell against chilling injury. Potassium regulates water and osmotic potential and reduces electrolyte leakage caused by chilling injury (Beringer and Troldenier, 1980; Singer et al., 1996). As the N concentration of fruit did not differ between brown and non-brown fruit and did not correlate with the percentage of brown or non-brown fruit, it can be hypothesised that the low K:N ratio found in brown fruit for both seasons (Tables 10 and 11) may be due to a lack of K and not an increase in N concentration of fruit.

The higher Mg and Ca content of brown fruit in 2012/2013 could be due to an influx of these minerals caused by the lower K concentration of brown fruit. Interactions between Ca and K or K and Mg are important for Ca related storage disorders and sometimes more so than for Ca alone (Holland, 1980; Waller, 1980). K, Ca and Mg are strongly antagonistic during nutrient uptake and can result in a deficiency of the depressed nutrient (Voogt, 1998). The K concentration of trees change the distribution of Mg, and low K concentration of leaves diverts a large part of Mg to fruit or other storage organs (Lüdders et. al, 1973) which may lead to an increase in susceptibility toward browning as the ratio of K:Mg in fruit decreases. When K is deficient, Ca and Mg uptake is also increased (Kirkby and Mengel, 1976). As both Ca and Mg were higher in brown fruit it is difficult to determine which mineral in the ratio of Ca:Mg influences browning. The attributes of Ca to increased storage quality of fruit (Poovaiah, 1986) and the significant negative correlation of Ca:Mg with non-brown fruit may

indicate an antagonistic effect of Mg towards Ca which may influence diffuse browning susceptibility.

Brown fruit also had higher Ca:P and Ca:Mg ratios compared to non-brown fruit in the 2012/2013 season but not in the 2011/2012 season. Brown fruit had a higher Mg and Ca concentration compared to that of non-brown fruit. As the P concentration of brown and non-brown fruit did not differ significantly the effect of higher Ca:P ratios on browning may rather be initiated by the higher Ca concentration found in brown fruit than a difference in P concentration. However, the main focus should be on mineral nutrients and ratio's that were related to browning over both seasons and K and K:Mg may therefore play a bigger role compared to mineral changes only related to browning in one season.

Incidence of diffuse-, "combination"- and the total incidence of browning were higher in young orchards compared to old orchards during both seasons (Table 4 and 5) and higher in orchards with sandy soil compared to clay soil orchards during 2011/2012. Maturity of fruit harvested from young orchards was more advanced compared to that of fruit harvested from old orchards during the 2011/2012 season but also according to starch breakdown in the 2012/2013 season. This may therefore also have played a role in diffuse and combination browning development, and should be considered on an orchard level when harvesting and storing for an extended period.

Radial browning and "combination" browning found for the 2011/2012 season and diffuse browning correlated positively to maturity parameters associated with increased maturity of fruit such as high CO₂ concentration, ground colour and starch breakdown (Table 7). Majoni (2012) found that fruit with an advanced maturity at harvest developed higher levels of diffuse browning during long term CA storage at -0.5 °C followed by storage in RA at -0.5 °C and ripening at ambient. It should be kept in mind that unlike the study of Majoni (2012) where fruit were harvested at two maturities (< 40 % starch breakdown and > 50 % starch breakdown) the fruit from this study were harvested at the last commercial harvest (one maturity). However, it seemed that the variation in maturity within post-optimally harvested fruit was large enough to correlate with browning or non-browning incidence. Ideally Majoni (2012) should have also correlated maturity and browning incidence to confirm these indices when harvested at an optimum maturity.

Young trees are vigorous and fruit from young trees are generally high in N, low in Ca, are large and have poor quality retention after harvest and storage (de Villiers, 1961; Sharples, 1973). This is in agreement with our findings where fruit from young orchards were larger and more susceptible to diffuse- and "combination" browning development compared to that of fruit from old orchards. However, fruit from young trees had a higher Ca, Mg and N concentration compared to fruit from old trees which were less prone to develop diffuse

browning in both seasons. Old trees also had higher ratios of P:K, K:N, K:Mg, K:Ca and K:N. Fruit from young trees had a higher Ca concentration and a lower K:Mg ratio and also had more diffuse and combination browning. Old trees in turn had lower levels of diffuse browning and had a higher concentration of K:Mg. These similarities show that the mineral composition along with the maturity of fruit from young orchards could have predisposed fruit to internal browning development.

Sandy soils caused higher levels of diffuse, “combination” and total browning in the 2011/2012 season but this effect was not significant in the 2012/2013 season. Mineral concentration of sandy soils is known to vary throughout the season and from one season to the next due to the ease with which it changes (Rengasamy and Churchman, 1999).

CONCLUSION

Mineral content of brown and non-brown fruit differed significantly according to the K, Mg and the ratio of K:Mg content of fruit for both seasons. Brown fruit had a lower K content and a higher Mg content and correlated negatively with the ratio of K:Mg. Browning was related to the low K content of fruit which could have predisposed fruit to chilling injury as it is known that K plays an important role in protection of cells against chilling injury (Singer et al., 1996). Fruit with differences in mineral concentrations could be explained by orchard differences in soil type and tree age. Fruit harvested from orchards with young trees were more susceptible to development of diffuse browning and “combination” browning but particularly diffuse browning. In some seasons it appears that sandy soils may influence particularly diffuse browning. Incidence of radial browning in the seasons studied seems to be less affected by soil type or tree age. Fruit harvested from young trees in both seasons or from sandy soil orchards in 2011/2013 were riper and may therefore have been more susceptible to development of diffuse and “combination” browning. The implication of lower K concentration and K:Mg ratio on browning development and the fact that K fruit levels were low throughout the trial should be further investigated. Leaf analysis should be included to aid in diagnosis of a possible deficiency and addressed through mineral nutrition trials. A multivariate analysis which could correlate incidence of browning for each orchard with the mineral concentration of fruit, soil type and tree age of each orchard could explain how mineral interactions influence browning from one year to the following. Research should ideally also be conducted on more orchard combinations and over more than two seasons in order to confirm results and have a clearer indication of interactions between various factors and the role of soil type and mineral composition on the development of flesh browning of ‘Cripps’ Pink’ apples after long term CA storage. The effect of harvest maturity on susceptibility of fruit towards browning and different browning types should be investigated to

assess the effect of fruit maturity as affected by orchard factors over time on incidence of browning.

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Tables:

Table 1: Orchard information in the Koue Bokkeveld production region, as well as maturity information at harvest and browning incidence after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for ‘Cripps’ Pink’ apples harvested during the 2011/2012 season in the Western Cape, South Africa

Orchard information						Harvest maturity indices						Post-storage incidence of internal browning (%)			
Farm	Orchard	Soil type	Planting year	Crop load (t/ha) 2011	Crop load (t/ha) 2012	DAFB	Harvest date	Starch break-down (chart index) (%)	Firmness (kg)	TSS (%)	Diameter (mm)	Radial browning	Diffuse browning	Combination browning	Total browning
Donkerbos	21	sandy	1997 (old)	63	83	226	07-May	62	7.6	12.5	65.7	11	4	18	34
Donkerbos	22	clay	1997 (old)	51	82	226	07-May	69	8.0	13.4	68.5	33	0	20	53
Esperanto	23	clay	2005 (young)	49	57	210	02-May	65	7.8	14.2	69.7	8	26	39	73
Esperanto	24	sandy	2006 (young)	-	57	210	02-May	66	7.6	15.0	70.1	6	43	27	76
Esperanto	25	sandy	2006 (young)	98	97	210	02-May	86	7.2	13.0	68.7	16	23	35	74
Bokveldskloof	26	clay	1998 (old)	66	135	218	02-May	65	7.4	11.8	65.4	3	4	1	9

Table 3: Orchard information in the Koue Bokkeveld production region, as well as maturity information at harvest and browning incidence after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for ‘Cripps’ Pink’ apples harvested during the 2012/2013 season in the Western Cape, South Africa

Farm	Orchard information					Harvest maturity indices						Post-storage incidence of internal browning (%)			
	Orchard	Soil type	Tree age	Crop load (t/ha) 2012	Crop load (t/ha) 2013	Harvest date	DAFB	Starch break-down (chart index) (%)	Firmness (kg)	TSS (%)	Diameter (mm)	Radial browning	Diffuse browning	Combination browning	Total browning
Donkerbos	21	sandy	1997 (old)	83	85	06-May	206	74	8.0	13.3	67.2	0	3	0	4
Donkerbos	22	clay	1997 (old)	82	59	06-May	206	64	7.8	13.2	68.2	0	8	0	8
Esperanto	23	clay	2005 (young)	57	82	29-Apr	199	74	9.0	15.1	69.7	1	34	3	38
Esperanto	24	sandy	2006 (young)	57	140	29-Apr	199	71	9.4	15.1	67.3	3	33	10	47
Esperanto	25	sandy	2006 (young)	97	110	29-Apr	199	71	8.3	12.9	70.0	3	32	6	40
Bokveldskloof	26	clay	1998 (old)	135	106	29-Apr	200	61	8.3	12.6	70.5	0	14	3	18

Table 3: Incidence of internal browning types of ‘Cripps’ Pink’ apples harvested in the Koue Bokkeveld (2011/2012 and 2012/2013) after storage and ripening (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life at 20 °C) from orchards in the Koue Bokkeveld region in the Western Cape, South Africa.

Season	Incidence of internal browning (%)			
	Radial browning	Diffuse browning	Combination browning	Total browning
2011/2012	13a	17	23a	54a
2012/2013	1b	20	3b	25b
<i>Source of variation</i>			<i>Pr>F</i>	
<i>Season</i>	<i><.0001</i>	<i>0.408</i>	<i><.0001</i>	<i>0.0004</i>

Table 4: Incidence of internal browning types of ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life at 20 °C) and maturity indices measured at harvest (starch breakdown, firmness, ground colour, DA, diameter, blush %, blush intensity, TA, TSS, C₂H₄ and CO₂) from orchards in the Koue Bokkeveld region with different soil types (sand and clay) and tree ages (young and old) for the season of 2011/2012 in the Western Cape, South Africa .

Treatment:	Maturity indices											Internal Browning incidence (%)			
	Starch break-down (chart index)	Firmness (kg)	Ground colour (chart index)	DA (I _{AD}) ³	Diameter (mm)	Blush %	Blush intensity (chart index)	TA (%)	TSS (%)	C ₂ H ₄ (μL·L ⁻¹)	CO ₂ (%)	Radial browning	Diffuse browning	Combination browning	Total browning
<u>Soil type:</u>															
Clay	62b	7.5	2.8	0.69b	67.3	54.8	6.7	0.79	12.8	1.49	1.06b	15	10b	20b	48b
Sand	71a	7.5	2.7	0.81a	68.1	49.2	6.2	0.75	13.1	1.26	1.24a	11	24a	27a	62a
<u>Tree age:</u>															
Old	62b	7.4	2.6b	0.79a	66.0b	47.0b	5.3b	0.68b	12.2b	1.03	1.12	16	3b	13b	32b
Young	72a	7.5	2.9a	0.71b	69.5a	57.0a	7.6a	0.86a	13.8a	1.72	1.18	10	31a	34a	78a
<i>Source of variation</i>								<i>Pr>F</i>							
<i>Soil type</i>	0.023	0.796	0.76	0.008	0.244	0.176	0.382	0.18	0.27	0.999	0.05	0.878	<0.01	0.023	0.049
<i>Tree age</i>	0.038	0.297	0.005	0.003	<0.0001	0.004	<0.0001	<0.0001	<0.0001	0.363	0.52	0.615	<.0001	<0.01	<0.01

Table 5: Incidence of internal browning types in ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) and maturity parameters measured at harvest (starch breakdown, firmness, ground colour, DA, diameter, blush %, blush intensity, TA and TSS) from orchards in the Koue Bokkeveld region with different soil types (sand and clay) and tree ages (young and old) for the season of 2012/2013 in the Western Cape, South Africa.

Treatment:	Harvest maturity indices									Internal browning incidence (%)			
	Starch breakdown (chart index)	Firmness (kg)	Ground colour (chart index)	DA (IAD) ^a	Diameter (mm)	Blush %	Blush intensity (Chart index)	TA (%)	TSS (%)	Radial browning	Diffuse browning	Combination browning	Total browning
<u>Soil type:</u>													
Clay	67	8.5	2.5	0.76	69.5a	54.8	6.8	0.68	12.8	0b	19	2	21
Sand	71	8.4	2.8	0.69	68.2b	49.2	6.2	0.69	13.1	2a	23	5	30
<u>Tree age:</u>													
Old	67	8.0b	2.7	0.71	68.6	47.0b	7.4b	0.61b	12.2b	0b	8b	1b	10b
Young	71	8.9a	2.6	0.74	69	57.0a	15.2a	0.76a	13.8a	2a	33a	6a	42a
<i>Source of variation</i>									<i>Pr>F</i>				
<i>Soil type</i>	<i>0.101</i>	<i>0.739</i>	<i>0.104</i>	<i>0.437</i>	<i>0.03</i>	<i>0.236</i>	<i>0.52</i>	<i>0.75</i>	<i>0.478</i>	<i>0.03</i>	<i>0.318</i>	<i>0.109</i>	<i>0.129</i>
<i>Tree age</i>	<i>0.109</i>	<i><0.0001</i>	<i>0.204</i>	<i>0.572</i>	<i>0.15</i>	<i>0.028</i>	<i><0.0001</i>	<i><0.001</i>	<i><0.0001</i>	<i>0.022</i>	<i><0.001</i>	<i>0.011</i>	<i>0.002</i>

Table 6: Maturity indices (starch breakdown, firmness, diameter, ground colour, DA, blush %, blush intensity, TA and TSS) of ‘Cripps’ Pink’ apples measured after harvest for seasons 2011/2012 and 2012/2013 (pooled across orchards) in the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment:	Starch breakdown (chart index)	Firmness (kg)	Ground colour (chart index)	Diameter (mm)	Blush %	Blush intensity (chart index)	TA (%)	TSS (%)
2011/2012	66.8	7.5b	2.8	67.7	52.0	6.5b	0.77a	12.9b
2012/2013	69.1	8.5a	2.7	68.8	56.7	7.9a	0.69b	13.7a
<i>Source of variation</i>				<i>Pr>F</i>				
<i>Season</i>	0.376	<.0001	0.321	0.588	0.181	0.010	0.007	0.009

Table 7: Correlation of browning types (radial, diffuse and “combination”) after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) with maturity parameters of ‘Cripps’ Pink’ apples at harvest as variables for the season of 2011/2012 (pooled across orchards). The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

	Radial Browning		Diffuse browning		Combination browning	
	r	p-value	r	p-value	r	p-value
Firmness (kg)	-0.29	0.12	0.32	0.08	0.25	0.19
Ground colour (chart index)	-0.35	0.06	0.54	<0.01	0.11	0.57
Starch breakdown (chart index)	0.04	0.85	0.41	0.02	0.16	0.40
Diameter (mm)	-0.06	0.75	0.70	<0.0001	0.56	<0.01
Blush %	-0.17	0.37	0.39	0.03	0.28	0.14
Blush intensity (chart index)	-0.15	0.42	0.56	<0.01	0.39	0.03
TSS (%)	-0.05	0.81	0.70	<0.0001	0.57	<0.01
TA (%)	-0.38	0.04	0.70	<0.0001	0.16	0.42
C ₂ H ₄ (μL·L ⁻¹)	0.03	0.85	0.22	0.25	0.27	0.89
CO ₂ (%)	0.46	0.01	0.21	0.26	0.43	0.02

Table 8: Correlation of browning types (radial, diffuse and “combination”) after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) with maturity parameters of ‘Cripps’ Pink’ apples at harvest as variables for the season of 2012/2013 (pooled across orchards). The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

	Radial Browning		Diffuse browning		Combination browning	
	r	p-value	r	p-value	r	p-value
Firmness (kg)	0.51	<0.01	0.50	<0.01	0.52	<0.01
Ground colour (chart index)	<0.01	0.98	-0.12	0.52	0.13	0.49
Starch breakdown (chart index)	0.08	0.66	0.10	0.58	0.20	0.30
Diameter (mm)	0.05	0.78	0.13	0.50	-0.03	0.87
Blush %	-0.05	0.80	-0.18	0.34	-0.11	0.57
Blush intensity (chart index)	0.04	0.85	-0.08	0.66	0.06	0.77
TSS (%)	0.28	0.14	0.30	0.10	0.30	0.10
TA (%)	0.46	0.01	0.45	0.01	0.45	0.01

Table 9: Mineral concentration (mg / 100g FW) and ratios of different minerals found in ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for the season of 2011/2012 and 2012/2013 in the Koue Bokkeveld production region of the Western Cape, South Africa.

	N	P	K	Ca	Mg	N:Ca	N:Mg	P:K	K:N	K:Mg	K:Ca	Ca:P	Ca:Mg
Treatment	mg / 100g FW					Ratio							
2011/2012	34.10b	8.47b	107.44a	5.51b	4.84a	6.64b	7.12b	0.08b	5.23a	22.44b	20.94a	0.68b	1.13b
2012/2013	43.85a	8.92a	103.86b	6.25a	4.43b	7.59a	10.01a	0.09a	2.47b	23.78a	18.02b	0.77a	1.41a
<i>Source of variation</i>	<i>Pr>F</i>												
<i>Season</i>	<0.0001	0.026	0.040	<0.0001	<0.0001	0.004	<.0001	<0.001	<0.001	<0.001	<0.0001	0.002	<0.0001

Table 10: Mineral concentration (mg / 100g FW) and ratios of different minerals found in brown and non-brown ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for the 2011/2012 season in the Koue Bokkeveld production region of the Western Cape, South Africa.

	N	P	K	Ca	Mg	N:Ca	N:Mg	P:K	K:N	K:Mg	K:Ca	Ca:P	Ca:Mg
	mg / 100g FW					Ratio							
Brown	36.34	8.72	104.73b	5.49	4.83	7.05	7.48	0.084a	4.43	21.84b	20.45	0.67	1.13
Non-Brown	32.08	8.25	109.89a	5.54	4.85	6.27	6.80	0.076b	6.05	22.98a	21.39	0.70	1.14
<i>Source of variation</i>	<i>Pr>F</i>												
<i>Classification</i>	0.055	0.052	0.038	0.802	0.872	0.122	0.142	<0.001	0.052	0.007	0.231	0.309	0.944

Table 11: Mineral concentration (mg / 100g FW) and ratios of different minerals found in brown and non-brown ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for the 2012/2013 season in the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment	N	P	K	Ca	Mg	N:Ca	N:Mg	P:K	K:N	K:Mg	K:Ca	Ca:P	Ca:Mg
	mg / 100g FW					Ratio							
Brown	45.1	8.8	99.3b	6.8a	4.62a	7.06b	9.81	0.09	2.31b	21.71b	15.54b	0.85a	1.48a
Non-Brown	43.0	9.0	107.1a	5.8b	4.29b	7.97a	10.16	0.08	2.58a	25.26a	19.78a	0.71b	1.36b
<i>Source of variation</i>	<i>Pr>F</i>												
<i>Classification</i>	0.164	0.388	0.001	<.0001	<.0001	0.023	0.287	0.069	0.002	<.0001	<.0001	0.003	0.002

Table 12: Mineral concentration (mg / 100g FW) and ratios of different minerals found in ‘Cripps’ Pink’ apples after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for different tree ages (young and old) and soil types (sand and clay) for the 2011/2012 season in the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment	N	P	K	Ca	Mg	N:Ca	N:Mg	P:K	K:N	K:Mg	K:Ca	Ca:P	Ca:Mg
	mg / 100g FW					Ratio							
<u>Tree age:</u>													
Old	28.70b	8.12b	103.42b	4.87b	4.30b	6.55	6.92	0.086a	6.83a	24.08a	22.88a	0.58b	1.23
Young	39.58a	8.22a	111.53a	6.16a	5.39a	6.73	7.32	0.073b	3.48b	20.78b	18.98b	0.79a	1.14
<u>Soil type:</u>													
Clay	32.81	8.46	109.90a	5.39	4.71	6.62	7.08	0.078	5.69	23.62a	22.09	0.65b	1.14
Sand	35.35	8.49	105.06b	5.63	4.97	6.66	7.15	0.082	4.62	21.30b	19.84	0.72a	1.13
<i>Source of variation</i>						<i>Pr>F</i>							
<i>Tree age</i>	<i><.0001</i>	<i>0.045</i>	<i>0.001</i>	<i><.0001</i>	<i><.0001</i>	<i>0.727</i>	<i>0.397</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.619</i>
<i>Soil type</i>	<i>0.796</i>	<i>0.988</i>	<i>0.002</i>	<i>0.498</i>	<i>0.601</i>	<i>0.981</i>	<i>0.927</i>	<i>0.060</i>	<i>0.191</i>	<i><0.0001</i>	<i>0.108</i>	<i>0.025</i>	<i>0.829</i>

Table 13: Mineral concentration (mg / 100g FW) and ratios of different minerals found in ‘Cripps’ Pink’ apples measured after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) for different tree ages (young and old) and soil types (sand and clay) for the 2012/2013 season in the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment	N	P	K	Ca	Mg	N:Ca	N:Mg	P:K	K:N	K:Mg	K:Ca	Ca:P	Ca:Mg
	mg/100g FW					Ratio							
<u>Tree age:</u>													
Old	37.88b	9.82a	110.39a	5.80b	4.38	6.99b	8.75b	0.089a	3.06a	25.48a	20.33a	0.63b	1.33b
Young	47.25a	8.41b	100.16b	6.50a	4.46	7.93a	10.73a	0.084b	2.12b	22.82b	16.71b	0.85a	1.46a
<u>Soil type:</u>													
Clay	46.71a	9.65a	109.59a	5.62b	4.46	8.71a	10.49a	0.088	2.42b	24.87a	20.52a	0.61b	1.26b
Sand	41.37b	8.25b	98.88b	6.79a	4.40	6.61b	9.60b	0.084	2.76a	22.84b	15.84b	0.90a	1.54a
<i>Source of variation</i>						<i>Pr>F</i>							
<i>Tree age</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.001</i>	<i>0.396</i>	<i>0.023</i>	<i><0.0001</i>	<i>0.029</i>	<i><0.0001</i>	<i><.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.002</i>
<i>Soil type</i>	<i><0.001</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.449</i>	<i><0.0001</i>	<i><0.0001</i>	<i>0.053</i>	<i><0.0001</i>	<i>0.050</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>

Table 14: Correlation of classification of fruit in percentage of brown and non-brown fruit after storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) with mineral concentration (mg / 100g FW) of fruit measured after storage of 'Cripps' Pink' apples (pooled over seasons). The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

	Classification Brown		Classification Non-brown	
	r	p-value	r	p-value
N	0.06	0.65	0.00	0.99
P	-0.01	0.95	0.43	<0.01
K	0.05	0.70	0.11	0.39
Ca	-0.04	0.77	-0.11	0.41
Mg	0.50	<.0001	-0.45	<0.01
N:Ca	0.02	0.89	0.07	0.59
N:Mg	-0.12	0.39	0.21	0.11
P:K	-0.05	0.73	0.42	<0.01
K:N	0.03	0.85	-0.12	0.36
K:Mg	-0.46	<0.01	0.60	<.0001
K:Ca	0.02	0.87	0.16	0.23
Ca:P	-0.05	0.73	-0.34	0.01
Ca:Mg	-0.35	0.01	0.27	0.04

Table 15: Mineral concentration of ‘Cripps’ Pink’ apples of good quality at harvest collected from 2010 to 2012 by Bemlab (PO Box 684, Strand, Somerset Mall, 7137, South Africa), Western Cape, South Africa. Mineral concentration of fruit below or above the “low” or “high” thresholds may be detrimental to storage quality. ‘High’ indicates the highest threshold for a specific mineral found in ‘Cripps’ Pink’ with acceptable quality after cold storage and ‘low’ indicates the lowest concentration of a specific mineral in ‘Cripps’ Pink’ apples with acceptable quality after cold storage.

	P	K	Ca	Mg
	(mg / 100g FW)			
Low	7.91	112.03	5.42	5.23
Medium	9.69	128.52	6.48	5.97
High	12.80	153.03	9.34	7.65

Figures:



Figure 1: Radial browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life) for the harvest of 2011/2012 in a 'Cripps' Pink' apple in the Western Cape, South Africa.



Figure 2: Diffuse browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life) for the harvest of 2011/2012 in a 'Cripps' Pink' apple in the Western Cape, South Africa.



Figure 3: “Combination” browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life) for the harvest of 2011/2012 in a ‘Cripps’ Pink’ apple in the Western Cape, South Africa.

PAPER 3**NEAR INFRARED REFLECTANCE SPECTROSCOPY AS A NON-DESTRUCTIVE TOOL FOR DETECTING INTERNAL BROWNING OF 'CRIPPS' PINK' APPLES**

ABSTRACT

The aim of this study was to evaluate near infrared reflectance (NIR) spectroscopy (NIRs) as a non-destructive method for detecting internal browning of 'Cripps' Pink' apples. 'Cripps' Pink' fruit were harvested from a commercial farm in the Koue Bokkeveld region in the Western Cape Province of South Africa, at starch breakdown levels of >50 % as more physiological mature fruit are more susceptible to diffuse flesh browning. Fruit were stored at -0.5 °C for 7 months in controlled atmosphere (CA) at 1.5 % O₂, 1 % CO₂. At the end of this CA storage period, fruit were divided randomly into two groups and subjected to NIRs analysis. NIR spectra were obtained by measuring diffuse reflectance with a spectral range of 800nm-2500nm. The first group was scanned immediately after CA storage (7M) and the second group after 7 months CA storage at -0.5 °C, 4 weeks at regular atmosphere (RA) (-0.5 °C) and 1 week at shelf-life conditions (7M4W7D). The total accuracy with which brown and non-brown fruit were identified, with spectral models built from spectra generated from the different storage groups, was tested. Destructive analysis of browning incidence and measurement of maturity indices took place after both storage periods (7M and 7M4W7D). Principal component analysis (PCA) was used to identify spectral clusters in PCA score plots. PCA separated storage time, 7M and 7M4W7D, as two discernible clusters in PCA score plots. Spectral clusters separating brown and non-brown fruit could not be found in PCA score plots. Partial least squares with discriminant analyses (PLS-DA) showed that browning can be identified by NIR spectra as brown and non-brown fruit were successfully identified after the 7M and 7M4W7D storage periods. The total accuracy of identification of brown or non-brown fruit was dependent on the ratio of brown to non-brown fruit, with increasing accuracy of identification of brown fruit with increasing incidence of browning. NIRs calibration and validation models showed that NIRs can predict total soluble solids (TSS) concentration of 'Cripps' Pink' apples after 7 months CA storage when spectra and TSS were collected from the blush side or the green side opposite to the blush side and the two additional green cheeks of the fruit (to the left and right from the blushed cheek). Further studies should investigate the relationship between TSS and incidence of diffuse browning over time to establish if prediction of TSS with NIRs can be used to predict browning potential of fruit after CA storage.

Keywords: *Malus domestica* L., diffuse browning, radial internal browning, “combination” browning, ‘Pink Lady’TM apples, PLS-DA, NIR spectra.

INTRODUCTION

Some ‘Cripps’ Pink’ apples grown in South Africa are prone to the development of internal flesh browning (IFB) after prolonged controlled atmosphere (CA) storage at -0.5 °C. The ability of ‘Cripps’ Pink’ apples to be kept in storage for extended periods stood out as one of the exceptional qualities of this cultivar as it opened a sales window for South African growers. South African ‘Cripps’ Pink’ apples are stored for up to 7 months in the pursuit of reaching a sales window to markets and thereby earning a higher income. Crouch et al., (2014) showed that it was this extended storage period in CA of fruit harvested at >50 % starch breakdown which induced diffuse browning of internal tissue. Internal browning of ‘Cripps’ Pink’ apples is a postharvest disorder which is not easily detected and leads to losses and claims after shipment and distribution due to the product not conforming to the client’s specifications. ‘Cripps’ Pink’ is sold at a premium as ‘Pink Lady’TM when the blush colour covers more than 40 % of the total surface area (Hurndall and Fourie, 2003). Internal browning has therefore also become a problem for the ‘Pink Lady’TM trademark. These losses create an uncertainty with consumers on the trustworthiness of the ‘Pink Lady’TM brand. As browning develops after an extended period of cold storage and shelf-life, the growers, packhouses and exporters experience huge losses when damaged fruit reach export destinations.

‘Cripps’ Pink’ internal browning has been divided into four different types of browning based on appearance, affected tissue and cause of browning. Radial browning forms a star shaped pattern where the tissue adjacent to the vascular cells is damaged leading to the formation of brown pigments in the affected tissue (James et al., 2010). Diffuse browning is found in the cortical tissue nearest the peel where it forms a brown ring with brown finger-like patterns reaching towards the core of the apple (James et al., 2010) with tissue adjacent to the vascular bundles unaffected. A combination of diffuse and radial browning was identified in the 2012 and 2013 season during this study. In these fruit, browning affected cortical tissue directly below the peel and cortical tissue surrounding the vascular bundles (Paper 4). The fourth type of browning has been identified as CO₂ damage which forms circular brown areas which develops into piths and cavities under severe cases of CO₂ damage and is related to high CO₂ exposure of storage atmosphere (Jobling and James, 2008).

Near infrared reflectance (NIR) spectroscopy (NIRs) is a process where the reflectance, transmittance and absorption of light in the near-infrared range display some of the internal chemical and physical parameters of the fruit (Nicolai et al., 2007). With the help of

analytical tools such as partial least mean squares (PLS) models can be built to find correlations between light scattering caused by internal compounds of fruit and certain physiological characteristics of the fruit (Wold, 1982). Relationships between fruit maturity or quality parameters and NIR spectra have been identified for a broad range of fruit. Apple fruit quality parameters such as acidity, sugar concentration, pH and texture parameters have been correlated with NIR spectra by Moons et al. (1997). Prediction models for other physiological disorders such as bitterpit, watercore and bruises have been built by use of NIR spectra reference data (for example total soluble solids concentration (TSS), titratable acids concentration (TA) or firmness) and PLS (Birth and Olsen, 1964; Brown et al., 1994; Nicolai et al., 2006). Reference data should be a measurable attribute of fruit quality which affects the movement of light through the fruit and is thus detectable by NIRs (Esbensen, 2006). For internal composition or reference data of a fruit to be successfully correlated with NIR spectra, the penetration of light into the fruit should be sufficient. Penetration depths for unpeeled apple discs varied between 0 and 7 mm and the penetration depth for 'Jonagold' was 5.5 mm (Hother et al., 1995).

The reflectance patterns gathered by NIRs recognise the internal state of the fruit. When cells are damaged as with bruising, a reduction in NIR spectra will result as an increase in scattering of radiation in the tissue (Brown et al., 1994). As the different types of browning affect different areas of the fruit, it is unsure if the appearance of browning can be detected and identified with NIR spectra. Diffuse browning may be found within the first 5.5 mm of fruit tissue as it spreads from beneath the fruit peel and develops with the characteristic pattern of cortical cells toward the fruit core. Radial browning does not reach the outer 5.5 mm of fruit tissue uniformly. Diffuse browning is the main type of browning found in South Africa with the risk of incidence increasing when fruit are harvested over mature (>50 % starch breakdown) and/ or stored for longer than 3 months at -0.5 °C under CA conditions (Majoni, 2012; Paper 1 and 2). It is therefore crucial to identify whether NIRs can successfully be used to separate these brown vs. non-brown fruit non-destructively. It has been established that browning shows a significant correlation with the total soluble solid (TSS) concentration, titratable acidity (TA) and firmness of fruit (Paper 2). Multivariate data analysis techniques such as partial least squares (PLS) could possibly be used to link NIR spectra with browning in fruit as NIRs has been shown to interact with molecular groups associated with TSS and TA and these parameters show correlations with browning.

As browning development increases with time of storage, it is unsure if browning and/or the potential for browning development might be correctly identified non-destructively by NIRs before shipment. Following non-destructive identification via NIRs internal brown fruit could be removed, thus ensuring that affected fruit do not reach the market. The objectives of this

paper were to determine whether a correlation could be found between NIR spectra of brown and non-brown fruit.

MATERIALS AND METHODS

Plant material

'Cripps' Pink' apple fruit were harvested from Esperanto, a commercial farm in the Koue Bokkeveld region (33°05'00"S 19°25'00" E), Western Cape Province of South Africa. The trees on M793 rootstock were planted in 2006 at a tree spacing of 4.75 m x 2 m on an east facing slope with North – South row direction. Free standing trees were trained to a modified Solax–ystem. Fruit were harvested post-optimally at the end of the commercial picking or strip pick time at an average starch breakdown of 69 %. This was done to induce diffuse browning, since Majoni (2012) showed that over maturity of fruit at harvest increases diffuse browning development in fruit that are stored under CA for longer than 4 months at -0.5 °C. In the second season (2012/2013) 'Cripps' Pink' apples were harvested from the same orchard at an average starch breakdown of 77 %. Fruit of similar size located on spurs at approximately 1.5 m above the orchard floor, between the inside and outside of the canopy were selected. All cultural orchard practices were in agreement with commercial norms for the region.

Experimental design

A total of 420 fruit were harvested across a single orchard for each season (2011/2012, 2012/2013 and 2013/2014) with five replicates of 40 fruit for each of the two storage periods. A sub-sample of twenty fruit was randomly selected for measurement of maturity indices at harvest. The 400 fruit were harvested into crates which were stored in regular atmosphere (RA) at -0.5 °C for one week during which harvesting of fruit from orchards used for Paper 2 and Paper 3 took place. With completion of the harvest of all orchards, fruit were moved into controlled atmosphere (CA) (1 % CO₂, 1.5 % O₂, -0.5 °C) storage for 7 months. After storage, fruit were divided into two groups to form two storage groups namely, 7M (7 months CA storage) and 7M4W7D (7 months CA + 4 weeks RA at -0.5 °C + 1 week shelf life at 20 °C). Fruit to be stored for the additional 4 weeks RA were repacked into fruit trays and commercial apple cartons lined with polyethylene bags (37.5 µm thickness) to reduce moisture loss. After the 4 weeks at RA (-0.5 °C) to simulate shipment, the 7M4W7D fruit were subjected to a one week shelf-life period (20 °C). Fruit were scanned with NIRs after each group's respective storage period. After scanning of fruit with NIRs, fruit were analysed for browning incidence and other quality parameters such as flesh firmness, ground colour,

% blush, blush intensity, total soluble solids (TSS), titratable acidity (TA), internal ethylene and CO₂ concentration of fruit.

Before NIRs scanning commenced, fruit were left over night (16h) at room temperature (20 °C) for fruit to warm up to that temperature.

Quality and maturity indices

Twenty fruit were used for maturity and quality measurements made at harvest which were conducted at ExperiCo (Agri-Research Solutions, P O Box 4022, Idas Valley, Stellenbosch, 7609, South Africa). Maturity and quality indices included starch breakdown, fruit firmness, size, weight, blush percentage, blush intensity (Industry colour chart P16 for 'Pink Lady'TM apples, 0 indicating no red colour and 12 indicating an intense red blush colour), DA (chlorophyll content), TSS, TA, O₂, internal CO₂ and ethylene content of fruit as described in Paper 1. Maturity measurements which were made at harvest included starch breakdown and seed colour which is not included as quality indices measured after storage.

Quality and maturity indexing was done on the 420 fruit at ExperiCo (Agri-Research Solutions, P O Box 4022, Idas Valley, Stellenbosch, 7609, South Africa). Quality measurements included external and internal quality (%) and decay evaluation. Internal quality (%) was determined destructively by cutting fruit through the top quarter where both types of browning could be visually assessed. Destructive measurements were taken within 36 hours after NIRs scanning was performed.

Measurement of TSS as a reference parameter for PLS regression was made for each area of fruit scanned with NIRs. This was done by taking a slice of apple from the scanned area (blush side, green side opposite the blush and cheek sides to the left and right of the blush), and squeezing out the juice from the apple slice and dripping it onto the digital refractometer (Atago DBX-30, Japan).

Near infrared spectrum collection

A multi-purpose analyser (MPA) spectrometer (Bruker Optics, Ettlingen, Germany) fitted with a solid probe fiber optics module containing a high sensitivity, thermoelectrically cooled InGaAs detector with a tungsten lamp as the NIR source was used to scan fruit at four quadrants of the fruit. The probe was placed in the top quarter of the fruit for scans as both diffuse and radial browning have been noted to be present in this part of the fruit (James, 2007). As differences between blushed and non-blushed sides of fruit have been noted (Lammertyn et al., 2000) one scan was done on the blushed section of the fruit and another at the opposite green side. The blushed area would denote the visual dividing of the apple

into quarters so that the fruit cheeks, to the right and left side of the blush were also scanned. The four scans of each fruit were averaged for analysis with PLS-DA or analysed as spectra from the blush side, spectra from the green side opposite of blush and the two cheek sides of the fruit (right and left of blush) by PLS regression. The probe of the instrument (5 mm diameter with about 100 optic fibers) was directed onto the peel of the fruit. Conditions for the scanning of fruit consisted of an 8 cm^{-1} resolution, 10 kHz scanner velocity, background with air, 16 scans, 16 background scans over a spectral range of 800 to 2500 nm. Each storage time represented a data set. Spectra collected of intact fruit (before destructive quality measurements) were used to build the 7M and 7M4W7D data sets which were available for statistical analysis.

Statistical and Chemometric data analysis

To determine if it is possible to identify internal brown fruit and if browning could be identified before the shipment, stock rolling and shelf-life period the following analyses were done on NIR spectral data.

SIMCA version 13.0.3 (Umetrics AB, country) chemometric software was used to identify and remove outliers by identification using Hotelling's T. Spectral pre-processing was used to improve the accuracy of spectra and remove spectral noise. Multiplicative scatter correction (MSC) and first derivatives (calculated using a quadratic polynomial order and a section size of 15 points) were used as pre-processing methods.

Principal component analyses (PCA) was obtained using Statistica version 12. PCA scatter plots of spectra generated from brown and non-brown fruit (average of 4 scans per fruit) at different storage times for each season (2011/2012 and 2012/2013) were generated to indicate if browning and/or storage time is discernible. Spectra of brown and non-brown fruit were grouped by storage period (7M or 7M4W7D) and season (2011/2012 or 2012/2013) and used to calculate the optimum number of principal components to use in the models.

Original data (no-pre-processing), MSC and first derivatives were generated for each storage period (7M and 7M4W7D) of each season (2011/2012; 2012/2013) and used for construction of partial least squares discriminant analyses (PLS-DA). Construction of partial least squares discriminant analyses (PLS-DA) was achieved using Statistica version 12. Data sets for each storage time and pre-processing method were divided into training sets (70 % of the samples which contained brown and non-brown 'Cripps' Pink' apples) and test sets (30 % of the samples which contained brown and non-brown 'Cripps' Pink' apples) to classify the browning status of the fruit based on their spectra.

Correlation between incidence of browning types and total incidence of browning (at 7M4W7D) with TSS (measured at 7M) and the mean, standard deviation and range of TSS

as a possible chemical predictor were tested with Statistical Analysis System (SAS) computer program (SAS Enterprise Guide 4.0; SAS Institute, 2006, Cary, NC, USA Enterprise Guide). Partial least squares (PLS) regression obtained from OPUS chemometric software (OPUS version 6.1, Bruker Optics, Ettlingen, Germany) was used to establish mathematical relationships between spectral data and TSS as chemical reference data. TSS was measured from each of the scanned sides of a fruit directly after scanning by pressing juice from an apple slice cut from the scanned area. The “Quant 2 methods” option of the OPUS software was used to select spectral parameters by investigating common wavelength frequency regions in combination with pre-processing methods (Louw and Theron, 2010). Two equal subsets were created by randomly splitting of data into a calibration or validation group. Prediction residuals were calculated by applying the calibration set to the validation set. The calibration model was assessed in terms of coefficient of determination (R^2), root mean square error of estimation (RMSEE) and residual predictive deviation (RPD), which is defined as the ratio of standard deviation of the response variable to the RMSEE. The performance of the model was described by the coefficient of calibration (R^2), which gave the variation in the reference (true) values reproduced in the prediction, and having a maximum value of 1.0 for a perfect prediction (Liew and Lau, 2012). The validation model was assessed in terms of the root mean square error of prediction (RMSEP), which indicates the prediction error, and the RPD which is the ratio of the standard deviation of the response variable to the RMSEP was used. A RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable, a value between 2 and 2.5 indicates that coarse quantitative predictions are possible and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively (Nicolai et al., 2007). Bias, which shows the average differences between actual value and NIRs predicted value (Liew and Lau, 2012), was also used to assess the performance of the model.

RESULTS AND DISCUSSION

Internal browning and quality aspects

For the 2011/2012 season 68 % of fruit developed internal browning (Table 1). For the 2012/2013 season browning incidence was 39 % of which 10 % of fruit developed radial browning and 6 % developed “combination” browning. Incidence of radial browning did not differ significantly between storage durations or between seasons. The percentage of fruit affected by “combination” browning did not differ between storage periods but differed significantly between seasons. Incidence of “combination” browning was higher in the

2011/2012 season (39 %) compared to the 2012/2013 season (6 %). Total browning incidence differed significantly between storage durations with a higher incidence measured at 7M4W7D (68 %) after storage and ripening compared to incidence measured at 7M (40 %) directly after CA storage (Table 1). There was a significant interaction between season and storage duration for the percentage of fruit affected by diffuse browning (Table 2). Incidence of diffuse browning increased from the 7M storage period to the 7M4W7D storage period (Table 2). The extent of the increase in diffuse browning incidence was dependent on the harvest year (Table 2). The incidence of diffuse browning was higher in the 2012/2013 season compared to the 2011/2012 season with a higher incidence at 7M4W7D compared to incidence measured at 7M (Table 2).

Radial browning does not disappear after shipment and shelf-life, and diffuse browning may develop in fruit already subjected to radial browning presenting itself as “combination” browning containing affected tissues of both disorders in one fruit. Radial browning in turn was found to be related to temperature during the cell enlargement stage (50-100 DAFB) (Paper 1) and was not present at harvest but only after CA storage. Diffuse browning was also present after CA storage; however incidence increased with further RA storage at -0.5 °C plus ripening at 20 °C. Diffuse browning is therefore a more typical chilling injury disorder that gets worse during shelf-life (Bramlage et al., 1980; James et al., 2005) and is influenced by senescence and storage time (Majoni et al., 2013).

Incidence of diffuse browning measured after shelf-life (7M4W7D) showed a significant positive correlation ($r^2 = 0.62$) with TSS of fruit measured after CA storage (7M) (Table 3). TSS is an indication of fruit quality and the rate of change in TSS is an indication of fruit maturity. With increased ripening of the fruit at harvest the TSS concentration of fruit increases, primarily through the breakdown of starch to sugar (Little and Holmes, 2000). Diffuse browning has been found to be related to post-optimal harvest maturity combined with long term CA storage (Majoni, 2012). This correlation between diffuse browning and TSS may therefore be a result of increased fruit maturity at harvest and their susceptibility towards diffuse browning development.

Fruit NIR Spectra

Spectra in the wavelength range of 800 nm to 2500 nm were applied for brown and non-brown fruit. A typical spectral curve collected with the FT-NIR spectrometer of brown and non-brown fruit is shown in (Fig. 1). These spectra are similar to the ‘Fuji’ apple profiles presented by Lui and Ying (2005). Spectra in the 800 to 980 nm as well as spectra in the region of 2300 to 2500 nm vary more than other regions and are an indication of noise. Two peaks are found in the spectra with the first in the area of 1400 to 1550 nm and the second

in the area of 1850 to 2200 nm. These peaks are associated with the NIR absorbance bands of water as found by Rambla et al. (1997). Smaller peaks at 980 to 1180 nm are found due to C-H overtone regions associated with sugar solutions (Osborne et al., 1993).

Principal component analysis (PCA) analysis

Principal component analysis was used in an attempt to link fruit characteristics to spectral characteristics. PCA score plots are used to explain how observations are linked to components in such a way that observations with similar characteristics would be grouped together on a score plot (Hanssens, 2011). The four spectra generated per fruit were averaged in an attempt to build better PLS-DA and PCA models than when individual spectra were used. The models built from averaged spectra performed better and therefore only these results will be discussed. PCA plots were built in an attempt to discern between brown and non-brown fruit and the two storage periods, 7M and 7M4W7D.

Analysis of spectra by PCA plots could not discern between spectra from brown and non-brown fruit (Fig. 2). Different storage periods were identified with component one explaining 49 % of the variation and component two explaining 31 % of the variation for fruit harvested in 2012 (Fig. 2). Successful identification of storage period was achieved for fruit harvested in 2013 as well with component one explaining 74 % of the variation and component two explaining 19 % of the variation (Fig. 3).

Classification of browning in apples based on spectra obtained at the 7M stage and the 7M4W7D stage with PLS-DA

2011/2012

Near infrared scans of fruit from the 7M group were obtained, pre-processed and used for statistical analysis in Statistica (version 12) to indicate if internal browning could be identified after 7 months CA storage (7M). Prediction models were obtained by random selection of calibration and validation sets with a 70 % to 30 % selection of training set to test set. The test and training set contained spectra of both known brown and non-brown fruit.

Line plots of adapted accuracy were used to identify the optimum number of PCA components used to construct the training and test sets. The optimum number of principal components used to construct training and test sets created from spectra collected from the 2012 harvest at the 7M stage were three for the derivatives and MSC data sets and four for the original data set (Fig. 4). For the 7M4W7D group two components were used for the original and derivatives data set (Fig. 5) while three components were used for the MSC data set.

In Table 4 results for training and test data sets of each of the storage periods (7M and 7M4W7D) are presented. For spectra collected from the 7M storage period the original data set (no pre-processing) as well as the derivatives data set gave the best results for the training data. The total accuracy for these data sets were 100 %, thus, all brown and non-brown fruit were classified correctly. For the 7M4W7D models the derivatives data set showed the best results for training sets with 100 % correct classification of brown and non-brown fruit. The MSC data set followed with a total accuracy of 93 % and lastly the original data set with a total accuracy of 89 %.

Test set results for the 7M group showed 95 % of brown fruit were identified correctly and 58 % of non-brown fruit were identified correctly by the derivatives data set. This led to the total accuracy of 82 % for the derivatives data set. For the test models of 7M4W7D, the MSC data set showed the best results with a total accuracy of 76 % followed by the original data set with a total accuracy of 75 %. Although the derivatives data set gave the best results for the training set models it showed a total accuracy of only 59 % for the test set models. The test model built with the MSC data set showed an 89 % correct identification of brown fruit and a 47 % correct identification of non-brown fruit.

2012/2013

Spectra collected were averaged for each fruit. Data sets built from these spectra underwent pre-processing or were kept in their original form. The original data set and data sets which were subjected to different pre-processing methods were used to identify brown and non-brown fruit. Results found from the analysis of the original and pre-processed data sets were then compared to each other.

For training and test sets created from spectra collected from the 7M storage period, two components were calculated for the original and derivatives data sets and three components were calculated for the MSC data set (Fig. 6). For the data sets of 7M4W7D, five components were used for the MSC data set (Fig. 7), ten components were used for the derivatives data set and three components were used for the original data set.

Table 5 shows results for training and test data sets of each of the storage periods (7M and 7M4W7D). For spectra collected from the 7M group, the original data set and the derivatives data set showed the best results for the training data. The total accuracy of these datasets was 90 %. Seventy-six percent of brown fruit and 94 % of non-brown fruit were correctly identified for the original data set. Sixty-seven percent of brown fruit and 97 % of non-brown fruit were identified correctly for the derivatives data set. For the 7M4W7D models the derivatives data set had the highest total accuracy with a 100 % correct identification of

brown and non-brown fruit. This was followed by the MSC data set which showed an 80 % total accuracy and the original data set which showed a 77 % total accuracy.

For spectra collected from the 7M group the MSC data set showed the best results for the test sets with a total accuracy of 71 %, followed by the derivatives set and the original data set with total accuracies of 68 % and 63 %, respectively. For the test models of the 7M4W7D group the original and MSC data sets showed a total accuracy of 71 % while the derivatives data set achieved a total accuracy of 69 %. For the original data set 75 % of brown fruit were identified correctly and 65 % of non-brown fruit were identified correctly. For the MSC data set 79 % of brown fruit were identified correctly and 60 % of non-brown fruit were identified correctly.

Total accuracy of identification of brown fruit at the 7M storage stage differed between years. Brown fruit were easily identified by the models built from 2011/2012 data sets with up to 95 % correct identification. Compared to 2012 results for identification of brown fruit, models built from 2012/2013 data sets performed poorly as the highest correct browning identification was only 22 %. The amount of fruit with radial browning was similar at this stage between the harvest years but “combination” browning incidence was much greater for 2011/2012 compared to 2012/2013 (Table 6). This indicates that “combination” browning is better detected by NIRs compared to radial browning. The star shaped pattern of radial browning makes it difficult to detect in areas where the penetration depth of the NIRs is not deep enough. In fruit affected by “combination” browning, the cortical tissue just below the peel of the fruit is affected, as with diffuse browning (Paper 4). Penetration depth varies between wavelength and variety of fruit with a maximum of 7 mm (Hother et al., 1995). Diffuse browning affects the cortical cells below the fruit peel (James, 2007) and falls within the 7 mm penetration depth of the NIRs.

Non-brown fruit were better identified in fruit from the 2013 harvest than fruit from the 2012 harvest due to the larger ratio (and therefore fruit number) of non-brown to brown fruit. Data sets built from spectra collected at the 7M4W7D stage showed an increase in accuracy of browning identification and a decrease in the accuracy with which non-brown fruit are identified from results found at the 7M stage. This occurs simultaneously with an increase in the number of fruit affected by diffuse and “combination” browning and a decrease in the number of non-brown fruit from 7M to 7M4W7D. Therefore the accuracy of the model relies strongly on the ratio of brown to non-brown fruit with which training and test models are built. In seasons when models are biased towards identification of brown fruit (higher number of brown fruit compared to non-brown fruit) non-brown fruit may be misclassified as brown which would lead to commercial losses.

Incidence of browning increased from 7M to 7M4W7D. This indicates that some fruit had the potential for browning at 7M but were still classified as non-brown during destructive analysis of browning. The potential for browning cannot be seen with the eye but could possibly be detected by changes in physiological characteristics of the fruit and measured by non-destructive means. If the potential for browning could be identified by NIRs before it is seen with the eye a larger percentage of non-brown fruit would have been identified “incorrectly” in the test sets. Non-brown fruit were identified with a total accuracy of 93 % at 7M for the 2012/2013 season. This indicates that the potential for browning development measured by NIRs cannot be identified by PLS-DA models before shipment. However, spectra of fruit taken at 7M and then further stored and evaluated for browning at 7M4W7D could be used to construct and test the accuracy of such a PLS-DA model. This would therefore assume that browning potential and physiological changes occur at 7M before browning is visible that will have a spectra different to fruit that do not have the potential to brown after ripening. This however also indicates that at this 7M stage due to the lower risk of browning, a non-brown sorting model would predict the internal state of fruit with a higher accuracy. Fruit numbers in this trial were relatively small (Table 6) compared to fruit sorted over a commercial line. Fruit numbers clearly had a large influence on the accuracy and therefore this model could increase in accuracy on commercial lines and should be evaluated commercially.

PLS prediction models

Quality attributes measured from a subsample of fruit used for the collection of spectra indicated that the fruit were of a representative quality for ‘Cripps’ Pink’ apples after 7 months CA storage (Table 7). Spectral pre-processing was used in an attempt to increase the accuracy of the prediction models. Accuracy of prediction models was increased by use of multiplicative scatter correction (MSC) or straight line subtraction as pre-processing methods. Prediction models were obtained by using the blush side of the apple for the first prediction, the green side of the apple on the opposite side of the blush for the second prediction, both green cheeks of the apple to the right and left side of the blush for the third prediction and all scans per fruit together for the fourth prediction of TSS concentration of the fruit. The range of TSS values used for each calibration and validation set are shown in Table 8. The TSS values which represent spectra used for calibration and validation sets overlapped for each model (based on fruit side scanned) indicating that both calibration and validation sets contained spectra representative of the total spectra used for the specific model. A summary of statistics for calibration and validation results for the prediction of TSS with PLS models for the 2013/2014 season are shown in Table 9. High validation R^2 and

RPD (> 2) values for prediction models from the blush ($R^2 = 81.77$; RPD = 2.42), cheeks ($R^2 = 93.98$; RPD = 4.08) and green side ($R^2 = 90.7$; RPD = 3.29) of the fruit indicated that TSS could be predicted successfully with NIR spectra from these positions on the fruit for 'Cripps' Pink' apples after long term storage. TSS of fruit was best predicted by models built on spectra and TSS collected from the cheek sides of the fruit. Prediction models for TSS using spectra combined for all sides of the fruit were not as reliable with a lower validation predictive R^2 ($R^2 = 63.87$) and a RPD level just above 1.5 which indicates that the model can only discriminate between very high or very low values (Nicolai et al., 2007).

Studies by Bobylen et al. (2010) demonstrate the important influence of cultivar in the formulation of prediction sets for firmness and TSS of fruit. In their studies 'Pink Lady'TM apples had the lowest R^2 values for calibration and validation sets compared to those created from spectra of 'Golden Delicious', 'Braeburn', 'Fuji', 'Royal Gala' and 'Jonagold' apples and accurate prediction models for firmness and TSS of fruit could not be built for 'Pink Lady'TM apples (Bobylen et al., 2010). The high predictive validation R^2 found for our trial confirms that NIRs can predict TSS (Fig. 8) of 'Cripps' Pink' apples as it does for other apple cultivars such as 'Braeburn' and 'Fuji', among others (Bobylen et al., 2010). The results found for this study indicates the importance of measuring TSS directly from the area of scanning and the importance of measuring TSS and gathering spectra from similar sides of the fruit to insure that prediction models and calibration models are large enough and made up of spectra with similar properties.

TSS measurements made at the 7M stage showed a significant positive correlation ($r^2 = 0.69$) with incidence of diffuse browning found at the 7M4W7D stage for the season of 2011/2012 (Table 3). The relationship between susceptibility of fruit towards diffuse browning development and the TSS concentration of fruit after CA storage should be investigated to confirm that there is a direct link between incidence of diffuse browning and TSS concentration of fruit and that the correlation is not coincidental. The wavelength at which covalent bonds of most inorganic molecules absorb NIR can be found in spectral libraries (Pavia et al., 2009). Differences in the chemical structure found between brown and non-brown fruit could be identified by absorbance of reflection of certain compounds in brown fruit or the lack thereof in non-brown fruit (Xing and Guyer, 2008). If the relationship between TSS concentration of fruit after long term storage and the incidence of diffuse browning is not coincidental, prediction models of TSS could possibly be used to predict the potential of diffuse browning development in fruit before shipment.

CONCLUSION

Discriminant analysis using PLS successfully identified browning in fruit at the 7M and the 7M4W7D stage for the 2011/2012 season. Identification of non-brown fruit was not as successful for that season due to fewer affected fruit. Fruit in the 2012/2013 season which were affected by internal browning could be successfully identified (71 %) at the 7M4W7D stage but not at the 7M stage due to low levels of browning. Identification of non-brown fruit at the 7M as well as at the 7M4W7D stage was successful for this year (93 % and 65 %, respectively). Total accuracy of non-brown fruit identification decreased as browning incidence increased from the 2011/2012 season to the 2012/2013 season and from 7M to 7M4W7D storage periods. This indicates that successful identification of brown or non-brown fruit by calibration using PLS-DA is subject to sample size of spectra of affected and unaffected fruit, and should be tested on a larger sample size at a semi-commercial level. Accuracy of browning identification increased with the amount of fruit affected by diffuse and “combination” browning. This was attributed to the shallow penetration depth of NIRs and the characteristic damaged cortical tissue found in fruit affected by diffuse and “combination” browning.

Browning incidence increased from the 7M to the 7M4W7D storage period. Fruit with a high potential to develop internal browning that were not afflicted with diffuse or radial browning at the 7M stage would develop diffuse browning rather than radial browning during RA and shelf-life. The increase of diffuse browning incidence from the 7M stage to the 7M4W7D stage and how this increase is linked to changing atmospheric conditions and temperature, from CA to shelf-life and $-0.5\text{ }^{\circ}\text{C}$ during storage to room temperature during shelf-life, confirms that diffuse browning is a progressive defect. Therefore, if diffuse browning is detected at the 7M stage a higher incidence of diffuse browning will develop during shipment and stock rolling, whereas the incidence of radial browning would remain fairly constant.

Prediction of browning incidence using the correlation between browning incidence and TSS which could be linked to NIR spectra by PLS analysis was successful. A strong positive correlation of TSS (measured at 7M) with incidence of diffuse browning (found at 7M4W7D) for the season of 2011/2012 shows potential for prediction of diffuse browning incidence at shelf-life before shipment by using TSS predictive models built on NIR spectra generated from fruit at the 7M stage. The relationship between TSS of fruit at 7M and incidence of diffuse browning at 7M4W7D should be tested over more seasons and investigated on a physiological level to confirm that the correlation is valid and not coincidental.

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Tables:

Table 1: Total browning and type of browning (radial and “combination”) incidence found for two seasons (2011/2012; 2012/2013) and two storage periods (7M = 7 months CA at -0.5 °C; 7M4W7D = 7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life at 20 °C) of ‘Cripps’ Pink’ apples harvested from the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment:	Radial browning %	Combination browning %	Total browning %
Season:			
2011/12	12	39a	68a
2012/13	10	6b	39b

Storage:			
7M	14	20	40b
7M4W7D	8	25	68a
<i>Source of variation</i>		<i>Pr>F</i>	
<i>Season</i>	<i>0.498</i>	<i><.0001</i>	<i><.0001</i>
<i>Storage</i>	<i>0.0713</i>	<i>0.2019</i>	<i>0.0002</i>
<i>Season*Storage</i>	<i>0.6278</i>	<i>0.4749</i>	<i>0.0614</i>

Table 2: Diffuse browning incidence of ‘Cripps’ Pink’ apples found for two seasons (2011/2012; 2012/2013) and two storage periods (7M = 7 months CA at -0.5 °C; 7M4W7D = 7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life at 20 °C) which interacted (Season*Storage). Fruit were harvested in the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment	Diffuse browning incidence (%)
2011/2012 7M	9c
2011/2012 7M4W7D	26b
2012/2013 7M	4c
2012/2013 7M4W7D	43a
<i>Source of variation</i>	<i>Pr>F</i>
<i>Season</i>	0.0729
<i>Storage</i>	<.0001
<i>Season*Storage</i>	0.0007

Table 3: Correlation between incidence of browning types (radial, diffuse and “combination” browning) and the total incidence of browning after the 7M4W7D stage (7 months of CA storage at -0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) and TSS measurements made from a subsample at the 7M stage (7 months of CA storage at -0.5 °C) of ‘Cripps’ Pink’ apples harvested during the 2012/2012 season from the Koue Bokkeveld production region of the Western Cape, South Africa. The Pearson r-values and corresponding p-values indicate the correlation and significance, respectively, between browning incidence and the different variables measured.

Treatment	TSS (%)	
	r ^a	p-value
Radial browning	-0.20	0.58
Diffuse browning	0.69	0.03
Combination browning	0.24	0.50
Total browning	0.40	0.25

^a Pearson’s correlation coefficient

Table 4: Training sets and test sets for different pre-processing methods of data collected at the 7M stage (7 months CA at 0.5 °C) and at the 7M4W7D stage (-0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) for classification of brown versus non-brown ‘Cripps’ Pink’ apples harvested during 2011/2012 from the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment:	Training set						Test set					
	7M			7M4W7D			7M			7M4W7D		
	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown
<u>No pre-processing</u>												
Brown	100	64	0	97	85	3	72	23	9	91	30	3
Non-Brown	100	0	45	63	9	15	53	7	8	40	9	6
Total Accuracy	100			89			66			75		
<u>Derivatives</u>												
Brown	100	74	0	100	78	0	95	21	1	65	28	15
Non-Brown	100	0	48	100	0	21	58	5	7	44	10	8
Total Accuracy	100			100			82			59		
<u>Multiplicative scatter correction</u>												
Brown	89	59	7	98	84	2	89	31	4	89	31	4
Non-Brown	87	6	39	75	6	18	47	8	7	47	8	7
Total Accuracy	88			93			76			76		

Table 5: Training sets and test sets for different pre-processing methods of data collected at the 7M stage (7 months CA at 0.5 °C) and at the 7M4W7D stage (-0.5 °C + 4 weeks RA storage at -0.5 °C + 7 days shelf-life) for classification of brown versus non-brown ‘Cripps’ Pink’ apples harvested during 2012/2013 from the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment:	Training set						Test set					
	7M			7M4W7D			7M			7M4W7D		
	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown	% correct	Brown	Non-Brown
<u>No pre-processing</u>												
Brown	76	16	5	85	55	10	11	1	8	75	21	7
Non-Brown	94	4	63	66	16	31	79	6	23	65	7	13
Total Accuracy	90			77			63			71		
<u>Derivatives</u>												
Brown	67	14	7	100	65	0	22	2	7	79	22	6
Non-Brown	97	2	65	100	0	47	83	5	24	55	9	11
Total Accuracy	90			100			68			69		
<u>Multiplicative scatter correction</u>												
Brown	19	4	17	83	54	11	0	0	9	79	22	6
Non-Brown	99	1	6	77	11	36	93	2	27	60	8	12
Total Accuracy	80			80			71			71		

Table 6: Incidence, standard deviation of incidence and amount of fruit used (n) of total browning and types of browning (radial, diffuse and “combination”) for different seasons (2011/2012; 2012/2013) and storage periods (7M = 7 months CA at -0.5 °C ; 7M4W7D = 7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) of ‘Cripps’ Pink’ apples harvested from the Koue Bokkeveld production region of the Western Cape, South Africa.

Storage	Radial browning (%)	n	Std dev.	Diffuse browning (%)	n	Std dev.	Combination browning (%)	n	Std dev.	Total browning (%)	n	Std dev.
Season 2011/2012												
7M	14	22	16.9	9	14	7.9	38	56	19.8	61	92	28.1
7M4W7D	10	17	6.7	26	40	9.5	40	59	15.9	76	116	22.7
Season 2012/2013												
7M	14	21	6.5	4	6	4.4	2	3	3.0	19	30	9.5
7M4W7D	6	8	5.9	43	62	14.1	11	19	9.8	59	89	19.2

Table 7: Quality attributes (fruit weight, firmness, diameter, ground colour, percentage blush, blush intensity and TSS) measured at the 7M stage (7 months of CA storage at -0.5 °C) of 'Cripps' Pink' apples harvested during the 2013/2014 season from the Koue Bokkeveld production region of the Western Cape, South Africa.

Variable	Mean	Std Dev	Minimum	Maximum	Range	N
Weight (g)	145.3	18.13	122	201	79	20
Firmness (kg)	6.8	1.27	5.1	7.3	5.2	20
Diameter (mm)	72.1	2.8	67	76	9	20
Ground colour (chart index)	3.4	0.33	3	4	1	19
Percentage Blush	49.5	15.38	25	80	55	20
Blush intensity (chart index)	7.1	2.75	2	11	9	20
TSS (%)	14.4	0.73	13.1	15.8	2.7	20

Table 8: The range of TSS found for different prediction models (blush side, green side, cheek sides and all sides) for each of the calibration and validation sets for 'Cripps' Pink' apples harvested in 2014 from the Koue Bokkeveld production region of the Western Cape, South Africa.

Treatment:	TSS (%)	
	Calibration	Validation
Blush side	13.0 – 16.1	12.8 – 15.9
Green side	12.5 – 15.0	12.5 – 15.0
Cheek sides	12.8 – 15.0	12.7 – 14.9
All sides	12.6 – 16.9	12.5 – 16.3

Table 9: The predictive relationship/models (calibration and validation) of NIRs and TSS as a fruit quality parameter measured after 7 months CA storage by scanned fruit position (blush, green opposite blush, right side from blush, left side from blush) of 'Cripps' Pink' apples harvested during 2013/2014 from the Koue Bokkeveld production region of the Western Cape, South Africa.

Fruit position scanned	Interactive NIR region (nm)	Data pre-processing method	Calibration			Validation			
			R ²	RMSEE	RPD	R ²	RMSEP	RPD	Bias
Blush side	1310-1820.3 800.2-1140.3	Multiplicative scattering correction	91.94	0.223	3.52	81.77	0.304	2.42	-0.0752
Green side	1310-1820.3 800.2-1140.3	Straight line subtraction	91.16	0.190	3.36	90.7	0.185	3.29	-0.0121
Cheek sides	1650.2-1820.3 1310-1480.2 800.2-1140.3	Multiplicative scattering correction	96.47	0.105	5.32	93.98	0.137	4.08	0.0030
All sides	970.1-1820.3	Multiplicative scattering correction	65.37	0.429	1.70	63.87	0.46	1.72	-0.1210

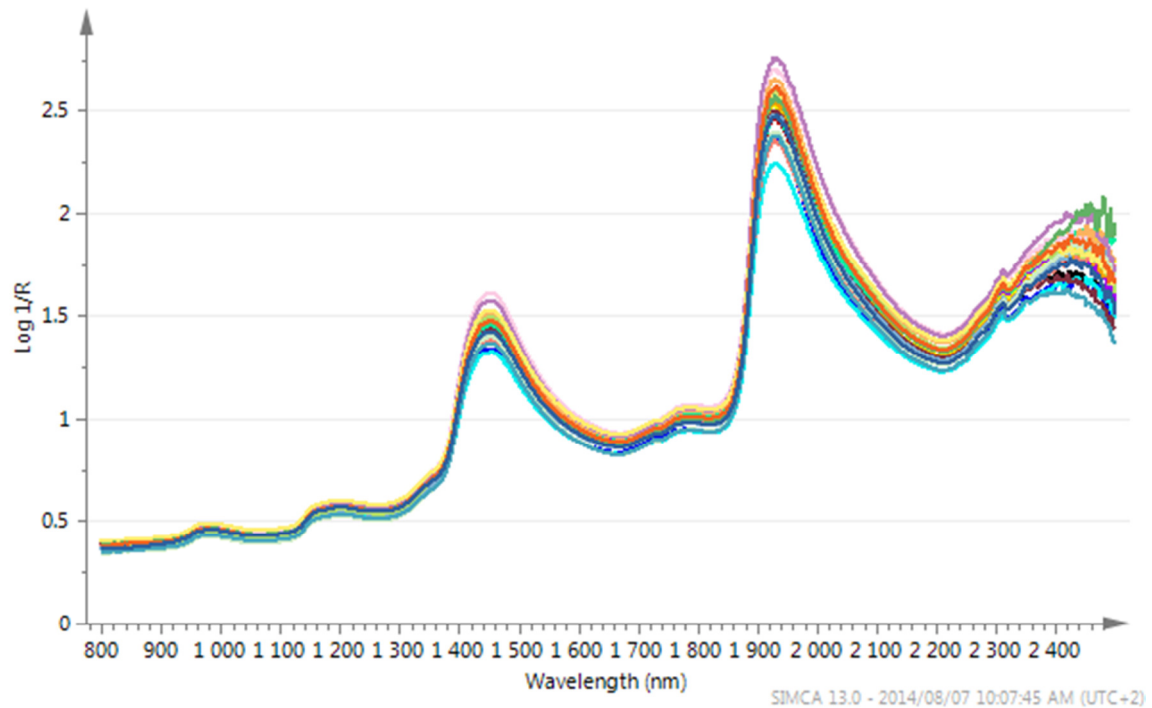
Figures:

Figure 1: Spectra collected from 'Cripps' Pink' apples, after 7M storage, harvested in the season of 2013 from the Koue Bokkeveld production region of the Western Cape, South Africa. Spectra are measured in wavelength (nm) and includes spectra of brown and non-brown fruit.

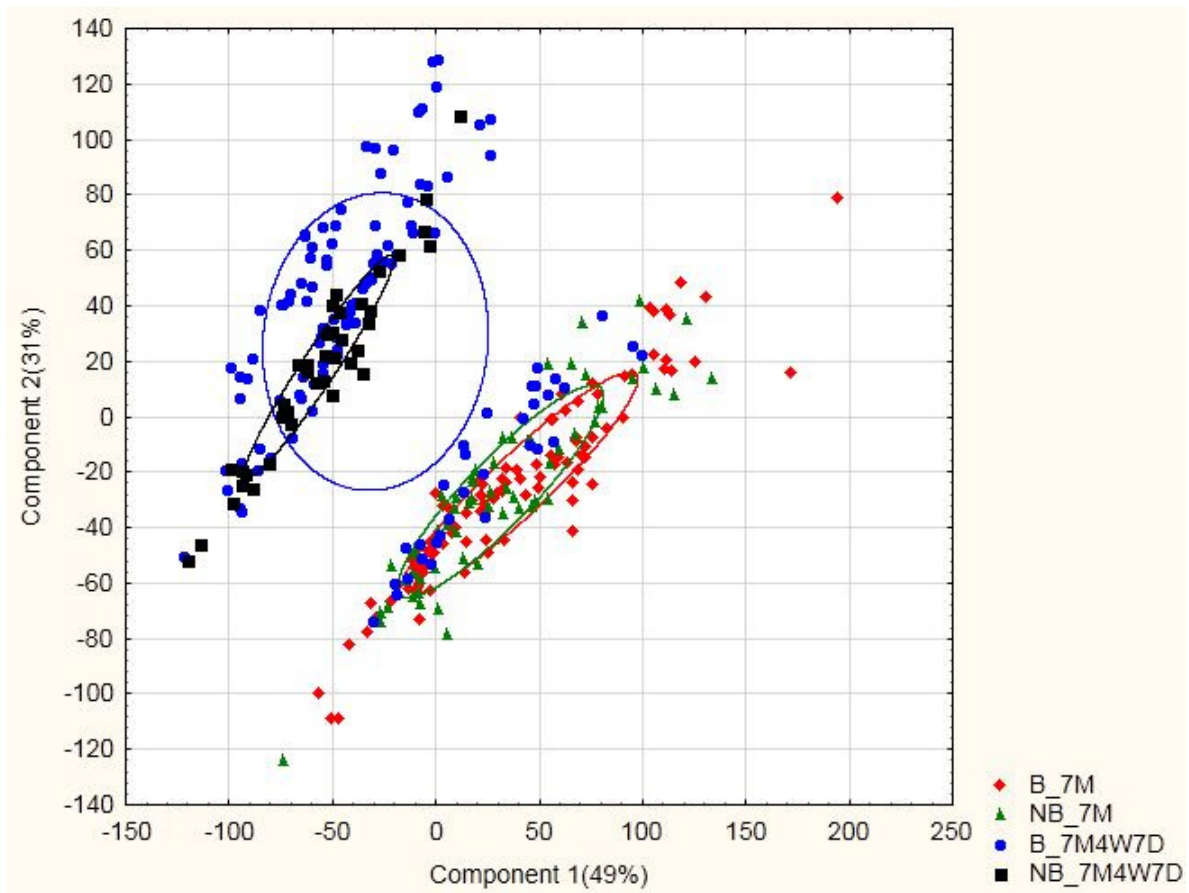


Figure 2: PCA plot identifying brown and non-brown 'Cripps' Pink' apple fruit stored for the periods of 7M and 7M4W7D for the season of 2011/2012 determined by spectral data.

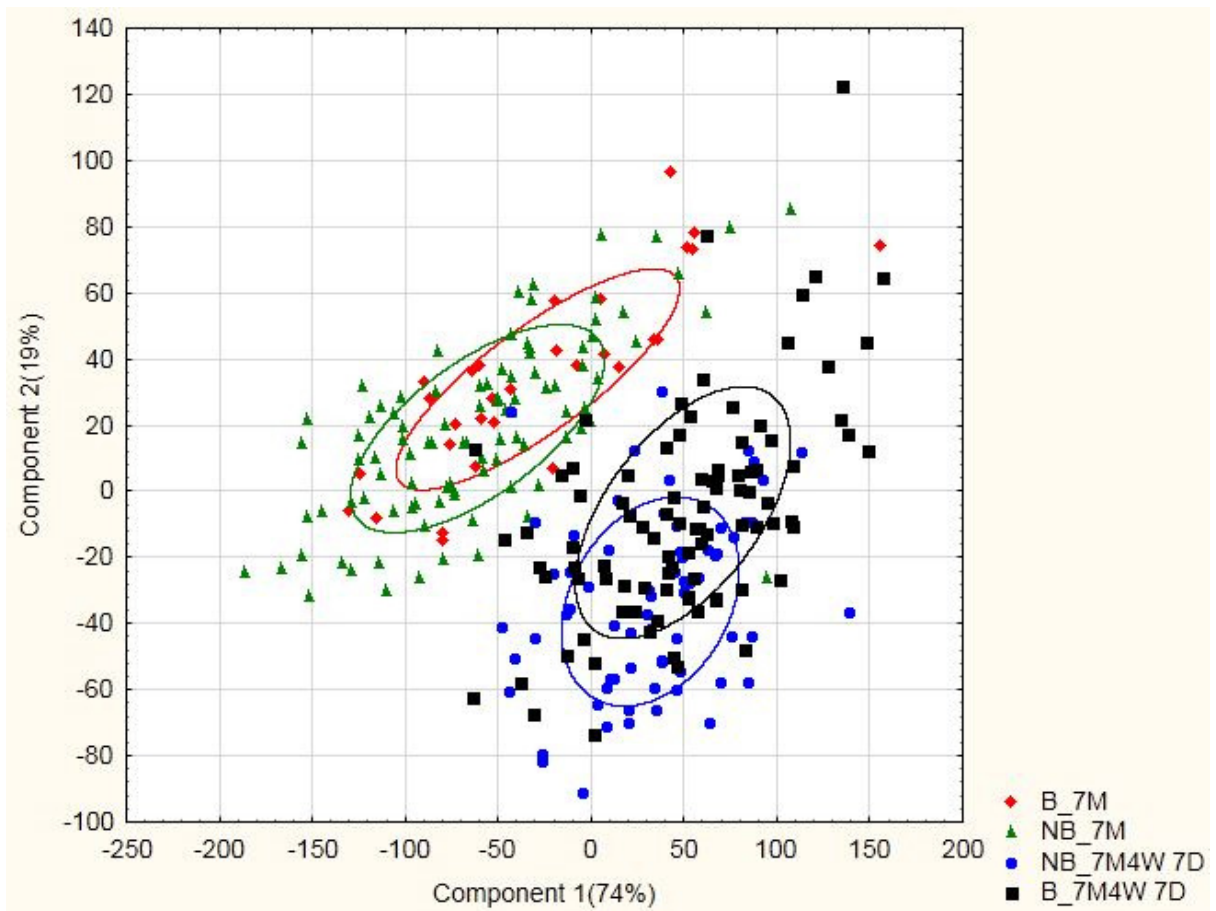


Figure 3: PCA plot identifying brown and non-brown 'Cripps' Pink' apple fruit stored for the periods of 7M and 7M4W7D for the season of 2012/2013 determined by spectral data.

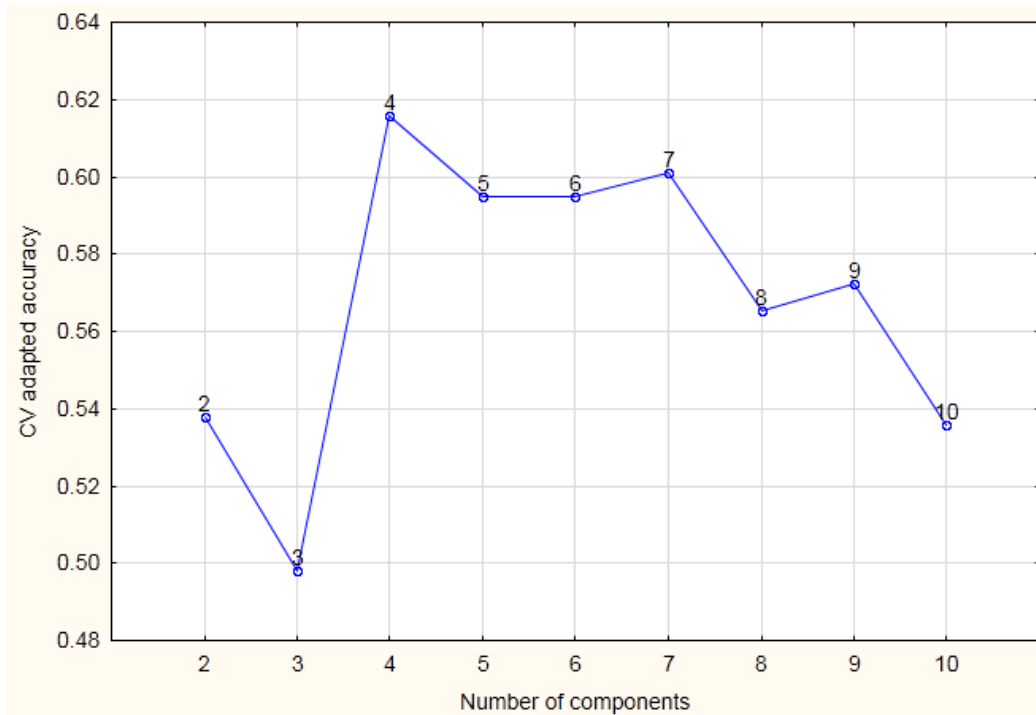


Figure 4: PCA components calculated for the after CA cold storage (7M) data set which had undergone no pre-processing, season of 2011/2012.

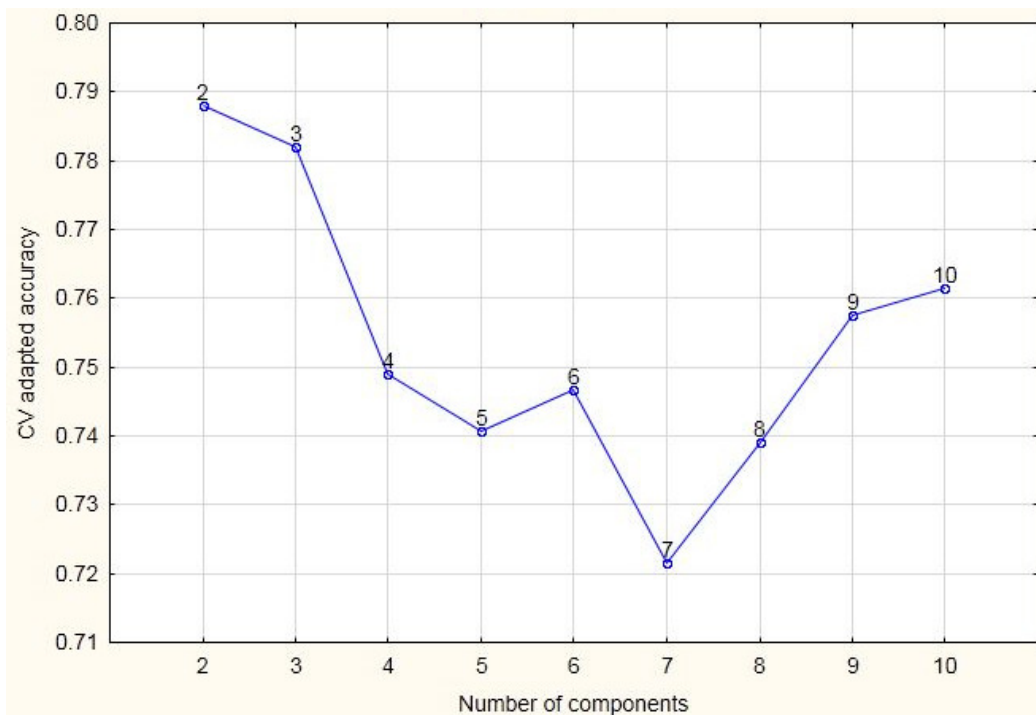


Figure 5: PCA components calculated for the after shipment and shelf-life (7M4W7D) data set where derivatives were calculated as a pre-processing method, season of 2011/2012

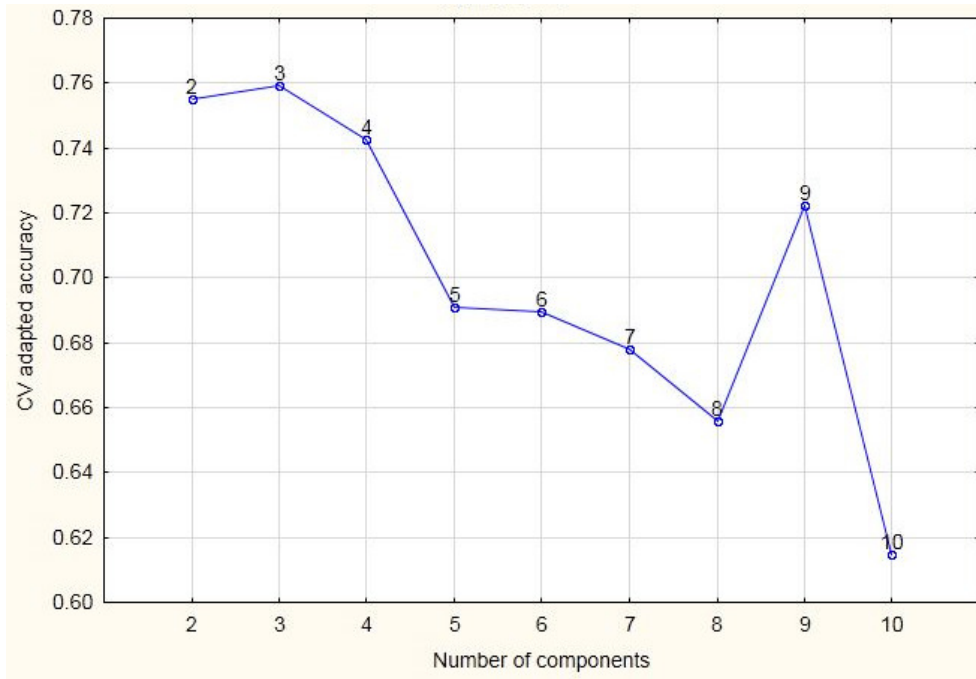


Figure 6: PCA components calculated for the after CA storage (7M) data set where multiplicative scatter correction was used as the pre-processing method, season of 2012/2013

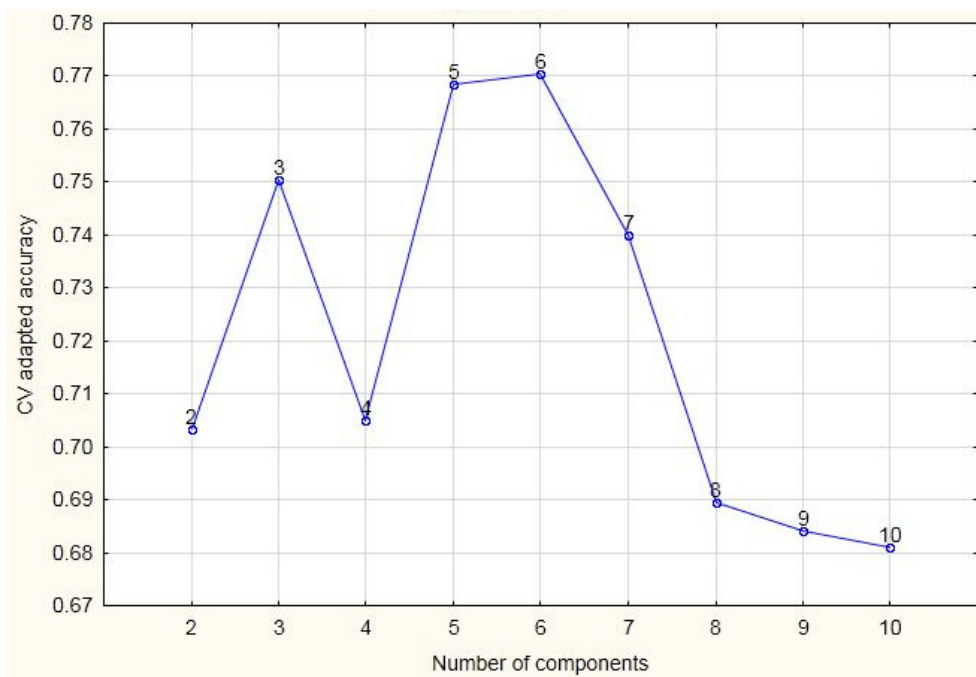


Figure 7: PCA components calculated for the after shipment and shelf-life (7M4W7D) data set where multiplicative scatter correction was used as a pre-processing method, season of 2012/2013

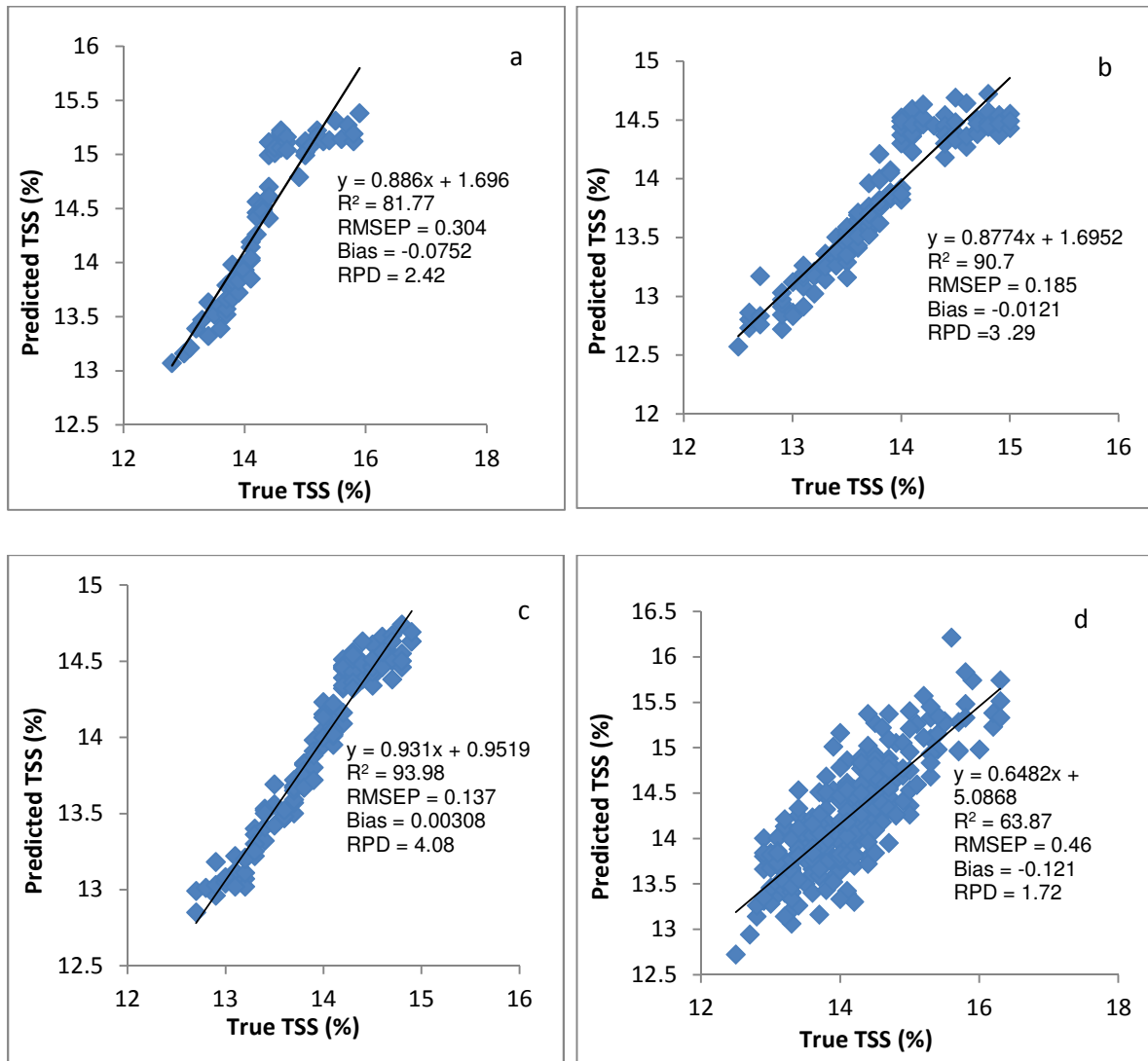


Figure 8: The prediction model of NIRs on 'Cripps' Pink' apples for total soluble solids (TSS) scanned on the (a) blush side, (b) green side, (c) cheeks and (d) all sides of the fruit after storage (7 months CA storage at -0.5 °C).

PAPER 4

DETERMINING THE CELLULAR STRUCTURE OF “COMBINATION” BROWNING, A TYPE OF FLESH BROWNING FOUND IN ‘CRIPPS’ PINK’ APPLES, USING SCANNING ELECTRON MICROSCOPY

ABSTRACT

A new type of browning was identified developing browning patterns of diffuse and radial browning in one fruit and called “combination” browning. The aim of this study was to establish if “combination” browning is truly affected by both browning types on a cellular level. Affected and non-affected samples were examined under a Scanning Electron Microscope (SEM). This browning type was named “combination” browning, referring to the browning pattern below the fruit peel as well as browning of tissue around vascular bundles. Five fruit from each browning type; radial-, diffuse- and “combination” browning; and five non-affected fruit were selected from existing trials. Six triangular sections from each fruit were selected and fixed in formaldehyde solution (5 % formaldehyde, 45 % ethanol, 5 % acetic acid and 45 % water) at room temperature. Samples were dissected from these sections for each of the browning types and non-affected fruit. Samples were dehydrated in an ethanol series, dried in a critical point drying (CPD) apparatus and gold coated before examination under the SEM. Tissue of non-affected fruit did not show any signs of damage. Fruit samples affected by diffuse browning had collapsed cells in the cortical tissue below the peel as well as large intercellular spaces. Tissue surrounding vascular bundles of fruit affected by radial browning showed cell collapse, cell disintegration and fractured cells while cortical tissue below the peel remained unaffected. Fruit samples affected by “combination” browning showed cell collapse and disintegration of cortical tissue below the peel of the fruit with fractured cells surrounding the vascular bundles. These results confirm that “combination” browning is truly a product of radial and diffuse browning in the same fruit and implies that fruit are susceptible to the same causal factors of radial and diffuse browning

Keywords: *Malus domestica*, Pink Lady™, radial browning, diffuse browning, SEM

INTRODUCTION

Different types of internal browning have been identified in 'Cripps' Pink' apples (James et al., 2005; Lau, 1998; Wilkinson and Fidler, 1973). Browning occurs when disruption of cell membranes leads to the oxidation of phenolic compounds by the enzyme, polyphenol oxidase (PPO), to quinones (Mathew and Parpia, 1971; Mayer, 1987). When cells rupture, quinones come into contact with oxygen (O_2) and are oxidized to the characteristic brown coloured polymers (Macheix et al., 1990). An anatomical study of the browning types revealed that they are structurally unique (James, 2007). They are recognized by the difference in browning pattern when the fruit are sectioned across the equatorial area. Examples of diffuse and radial browning found from the 2011/2012 season are illustrated in Fig. 1. Determining the structural differences between browning types may lead to the identification of different events related to the cause of the specific browning types, and make management of the problem possible.

The incidence and severity of internal browning in 'Cripps Pink' apples is unpredictable which makes it difficult for growers to manage. Diffuse flesh browning is a disorder mainly induced by chilling injuries (James et al., 2005) and is associated with over-maturity of fruit and long term CA storage at low temperature ($-0.5\text{ }^\circ\text{C}$) (Majoni, 2012). Incidence increases following storage in regular atmosphere (shipment) and room temperature (shelf-life) (Paper 3) (Jackman et al., 1988; Parkin et al., 1989). Radial browning development and appearance is unpredictable in South African production regions and appears to be related more to seasonal variables than growing regions (Paper 1). Both types of browning lead to high financial losses as symptoms are unpleasant and affected fruit cannot be marketed as they are not accepted by consumers.

Diffuse browning is associated with browning of the cortical tissue under the peel while radial browning is associated with browning of the cells surrounding the vascular tissue of the fruit (James, 2007). A third type of browning, caused by carbon dioxide (CO_2), is characterised by disintegration of tissue in the fruit flesh resulting in pits and cavities (de Castro et al., 2008). Active oxygen species (AOS), which form when fruits are under stress due to extended storage periods under CA conditions, disrupt normal cell function and lead to browning development (Zhuang et al., 1994). Temperature during the growing season of the fruit (James et al., 2005), or parts of the growing season (Paper 1), may leave fruit susceptible to AOS attack and browning development during long term CA storage. Australian researchers have correlated incidence of browning types to different production regions based on the accumulated growing day degrees above $10\text{ }^\circ\text{C}$ ($GDD_{>10^\circ\text{C}}$) for the specific region (James, 2007; Jobling et al., 2004). 'Cripps' Pink' apples harvested from "warm" production regions developed radial browning and fruit harvested from "cold" production regions developed diffuse browning (James et al., 2005). Although South African

production regions have been classified as “warm”, investigation of the browning types that develop in ‘Cripps’ Pink’ apples harvested from these regions showed diffuse browning to be the more dominant type of browning occurring (Paper 1).

During trials to investigate the effect of temperature on browning incidence under South African growing conditions radial browning, diffuse browning and fruit with a combination of both diffuse and radial browning, were found (Paper 1). Both tissue below the fruit peel and tissue surrounding the vascular bundles were affected in these fruit. This display of diffuse and radial browning symptoms in the same fruit led to the naming and identification of this anomaly as “combination” browning.

Susceptibility of fruit to the different types of browning is influenced by different causal factors (Paper 1 and 2). To manage the incidence of browning the causal factors should be minimized. The aim of this study was to investigate the affected tissue of “combination” browning with the use of scanning electron microscopy (SEM). A comparison between diffuse and radial browning with “combination” browning would indicate if the latter is truly radial and diffuse browning combined in the same fruit. By identifying the type, or types, of browning which are associated with “combination” browning, the causal factors could be identified and a management strategy for this browning type could be developed.

MATERIALS AND METHODS

Experimental sites

Two production regions of ‘Cripps’ Pink’ apples in South Africa were identified for the experimental sites, namely: the Koue Bokkeveld (lat. 33°05′00″S long. 19°25′00″E) and Elgin (lat. 34°8′55″S, long. 19°2′34″E) in the Western Cape (Table 1). These sites where chosen as radial-, diffuse- and “combination” browning were successfully induced in fruit harvested from these regions in the 2011/2012 season. It is general practice for growers to harvest ‘Cripps’ Pink’ apples more than once during the harvest season. The first ‘through-pick’ removes well-coloured apples on the outside of the tree. The second and third harvests (depending on crop load) remove well-coloured fruit as blush develops. For this study, ‘Cripps’ Pink’ apples from the 2012/2013 season were harvested at the last possible commercial harvest to increase the chances of browning being induced after extended storage (Majoni, 2012). The first harvest date was on 29 April 2013 (Esperanto, Koue Bokkeveld) and the last was on 7 May 2013 (Beaulieu, Fine Farms, Dennegeur and Elgin Orchards, in Elgin) (Table 1). Fruit were harvested once from every farm and harvest dates differed between farms as fruit matured or coloured at different times. Fruit were accumulated for 1 week in RA at -0.5 °C during the harvesting period and CA storage commenced in two main batches according to harvest dates. Fruit were stored for an

extended period (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) in an attempt to induce internal browning. Management practices followed at the different orchards were according to commercial practices in the different regions.

Sampling procedure

Apples available from previous trials (2012/2013 season) were combined and classified according to their symptoms based on the type of browning. Five samples were analysed for each type of browning (Table 1). 'Cripps' Pink' apples affected by each browning type; radial, diffuse and "combination"; as well as non-affected fruit were selected during quality indexing of fruit after storage. Five fruit were sectioned across their equatorial area horizontally, and six triangular sections were taken from each fruit from the peel towards the center of the fruit so that all sections showed cortical tissue below the peel and vascular bundles nearer to the center of the fruit (Fig. 2). Samples contained both affected as well as unaffected tissue. Tissue samples were fixed with formalin acetic acid solution for one month and dehydrated in an ethanol series (50 %, 60 %, 70 %, 80 %, 90 %, 95 % and 100 %). Ten minute intervals were used for each of the first five solutions (50 %, 60 %, 70 %, 80 % and 90 %) and fifteen minute intervals for the last two solutions (95 % and 100 %) in order to dehydrate the samples.

These samples were then dissected with a razor blade diagonally across the radius of the fruit to a thin slice (1-2 mm) (Fig. 3). Dissected slices were dried in a critical point drying (CPD) apparatus (E3000 Series, Quorum Technologies Ltd., Kent, United Kingdom) by placing tissue in the transfer boat with acetone and taking care not to allow air drying of the sample. Cold water was run through the system to cool the chamber to 20 °C or below while all gas control valves were closed. The transfer boat with samples was loaded into the gas cylinder, the door of the chamber closed and the gas cylinder supply valve opened. Liquid CO₂ flooded the chamber rapidly while the vent was opened to maintain the liquid level. The drain was opened to remove substitution liquid and this "flushing" action continued for 3 to 5 minutes. After flushing of the substitution liquid, the chamber was filled with CO₂ and all gas controls were closed. The system was left for one hour to allow impregnation of the tissue with the liquid gas. After impregnation of tissue with liquid gas the inlet valve was opened and "flushing" repeated. All gas control valves were closed and hot water run through the system to slowly raise the temperature of the chamber. As soon as the CO₂ had passed through its critical point (31.5 °C, 1100 psi), the water flow to the chamber was closed off and the vent gradually opened to release pressure within the chamber. The transfer boat was then removed from the system and samples mounted onto a stub with double sided carbon tape. Samples were gold coated to make the sample surface electrically conducting

and examined with a Leo® 1430VP Scanning Electron Microscope (Carl Zeiss (Pty) Limited, Cape Town, South Africa) at the SEM Micro Beam Unit, Stellenbosch University. Surface analysis of samples were conducted with beam conditions of 7 KV and approximately 1.5 nm, with a working distance of 13 mm and a spot size of 150 nm.

RESULTS AND DISCUSSION

Unaffected cortical tissue

Tissue of unaffected fruit was compared to that of fruit with tissue affected by browning development. Figure 4 A and B illustrate cortical cells of unaffected tissues of 'Cripps' Pink' viewed under the SEM. In these images, the structure of the cortical cells below the peel and in the tissue surrounding vascular bundles of non-affected fruit, present sound cells with normal intercellular spaces (Fig. 4 A and B, respectively). These cells are undamaged, the cell structure is intact and they are comparable to unaffected cells found in studies by James (2007). In turn images of tissue affected by browning showed cell collapse or fractured cells and large intercellular spaces in the area affected by the different types of browning (Fig. 4C, D and Fig. 5A, respectively).

Diffuse browning

Fruit samples that presented diffuse browning exhibited collapsed cells of cortical tissue below the peel and large intercellular spaces (Fig. 4C and D). Diffuse browning is associated with chilling injury (CI) (James et al., 2005) and over maturity of fruit when stored for longer than 4 months under controlled atmosphere (CA) conditions at -0.5 °C (Majoni, 2012). Ion leakage caused by senescence of apple fruit is associated with increased membrane viscosity and a decrease in unsaturated fatty acids (Lurie and Ben-Arie, 1983; Lurie et al., 1987). This ion leakage leads to impaired diffusion of CO₂ and O₂ from one cell to another and causes an accumulation of CO₂ in and around the cells (Finean et al., 1978). Chilling injury causes membrane lipids to change from a liquid phase to a gel phase (Moeller et al., 1981). This phase change of membrane lipids leads to increased leakiness and the loss of permeability properties which are necessary for functioning of the cell (Haest et al., 1972). The resulting membrane dysfunction leads to cell death and the formation of large intercellular spaces where cells have lost their form and function (lysigeny) (James and Jobling, 2009). This is comparable to cell collapse found in images of fruit affected by diffuse browning (Fig 4C and D). In comparison with non-affected fruit, various biochemical factors such as increased sterols, less reduced glutathione and higher saturated to unsaturated fatty acid ratios were found in brown 'Cripps' Pink' fruit by Majoni et al. (2013).

This was linked to oxidative damage caused by extended storage duration and over maturity of fruit.

Radial browning

In fruit samples characterized by radial browning, the tissue surrounding the vascular bundles showed cell collapse and disintegration (Fig 5A). These characteristics of tissue affected by radial browning were confirmed by the findings of James (2007). James and Jobling (2009) associated fractured cell walls with radial browning while cell collapse was associated with diffuse browning. Damage to cell walls disrupts gas transport as the cytoplasm of dying cells fill up the intercellular spaces (Larrigaudière et al., 2004). The leakage from damaged cells into intercellular spaces slows down diffusion of O₂ and CO₂ which in turn increases susceptibility towards low O₂ and high CO₂ damage during long term storage (Lau, 1998; Schotsmans et al., 2004). This type of leakage is associated with membrane deterioration during senescence of fruit (Larrigaudière et al., 2004).

Cortical tissue below the peel of fruit affected by radial browning was intact with a well-organized structure (Fig. 5B). Incidence of radial browning has a strong relationship with storage temperature and storage atmosphere (Jobling and James, 2008). Jobling and James (2008) found that storage temperature below 1 °C and increased concentration of CO₂ (> 1 %) during storage increased the likelihood of 'Cripps' Pink' apples developing radial browning. A build-up of CO₂ in the vascular bundles could lead to membrane peroxidation of cortical tissue in the surrounding area. In fruit affected by flesh browning associated with a high build-up of CO₂ a decrease in ascorbate and lipid soluble antioxidants and an increase in antioxidant enzymes are found (Hodges, 2003). Exactly why the cortical tissue below the fruit peel stays unaffected is not known.

Results from Paper 1 indicated that average maximum temperatures during the early cell expansion stage (50 to 100 DAFB) correlated negatively ($R^2 = -0.66$) with incidence of radial browning. Low temperatures slow down cell growth in the cortical region (Bergh, 1990) leading to development of fruit with smaller cells and a higher density and smaller intercellular spaces (Little and Holmes, 2000). Oxidative stress caused by the accumulation of CO₂ in tissues would typically lead to the build-up of AOS followed by lipid peroxidation and browning (Larrigaudière et al., 2001). However, fruit density and gas diffusion may not be related as James and Jobling (2009) found that the area that was least affected by radial browning had the highest tissue density. It has been found that rather, the interconnectivity between intercellular spaces is linked to gas diffusion in apples (Wünsche et al., 2000). In this study, radial browning samples with fractured cells appeared to be associated with compromised cell walls. More studies are needed to determine whether gas diffusivity and an accumulation of CO₂ and AOS are related to radial browning and fractured cells.

“Combination” browning

Samples affected by “combination” browning showed cell collapse of cortical tissue near the peel of the fruit as well as degradation of cells next to the vascular bundles (Fig. 6A). The damage found in the cortex region below the fruit peel was similar to damage found for fruit affected by diffuse browning. Degradation of cells associated with vascular bundles were similar to damage caused by radial browning. Results from Paper 3 showed that radial browning develops in fruit during long term CA storage while diffuse browning develops progressively from the CA period during the RA and shelf-life period. “Combination” browning forms when diffuse browning develops in fruit which were subjected to radial browning development during the CA storage period. “Combination” browning may therefore increase during RA and shelf-life in radial-brown affected fruit. Often chilling symptoms occur in sensitive fruit after being transferred to non-chilling temperatures (Jackman et al., 1988; Parkin et al., 1989). For example in mango chilling injury presents itself as discoloured and pitted areas when fruit are left at room temperature for one or two days after cold storage (Ketsa et al. 2000). Fruit subjected to “combination” browning would be susceptible to factors which influence radial browning. Development of diffuse browning in fruit where radial browning is already present indicates that fruit affected by “combination” browning was then sensitive to chilling injury which is thought to cause diffuse browning.

Results from Paper 1 indicated that the type of browning developing in ‘Cripps’ Pink’ apples grown under South African conditions does not relate to a “warm” or “cold” region as incidence of radial and diffuse browning did not differ significantly between Elgin and the Koue Bokkeveld. Strong correlations between temperatures during specific growth periods and browning incidence indicated the important influence of temperature on fruit growth and development which in turn affected susceptibility of fruit to internal browning development.

Further investigation into the calcium, ascorbate, anthocyanin, peroxidase, glutathione reductase and dehydroascorbate reductase content of cortical cells beneath the fruit peel compared to that of cortical cells associated with vascular bundles could help explain the physiological factors causing radial and diffuse browning. Quantitative studies in which intercellular spaces of non-affected fruit, and fruit affected by radial browning and diffuse browning are measured and compared to each other, could indicate how physiological differences in fruit growth may influence the susceptibility of fruit to the different types of browning. These studies should assess the growth rate of cells, climatic conditions, nutrition and water availability throughout the growth season of fruit, to relate orchard conditions with browning incidence and morphological characteristics of fruit as affected by different types of browning.

CONCLUSION

Radial and diffuse browning has been found to be structurally different (James and Jobling, 2009). In this study samples with diffuse browning exhibited collapsed cortical cells below the peel and the presence of large intercellular spaces. Samples with radial browning showed cell collapse near the vascular bundles. However, cortical cells near the fruit peel were unaffected. In this study samples presenting “combination” browning showed cell collapse associated with vascular bundles as well as disintegration of cortical cells below the fruit peel. These results, in addition to a reduced incidence of radial browning from post-CA to shelf-life combined with an increase in diffuse- and “combination” browning incidence, indicate that “combination” browning forms in fruit which are susceptible to both radial and diffuse browning development. Cells of non-brown fruit were well arranged and did not show signs of disruption or collapse.

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Tables:

Table 1: Summary of types of browning incidence found for each paper and the number of ‘Cripps’ Pink’ apples sourced from each paper for this scanning electron microscopy (SEM) study.

Paper	Production region used for trial	Total number of fruit harvested	Number of orchards fruit were harvested from	Number of fruit affected by radial browning	Number of fruit affected by diffuse browning	Number of fruit affected by “combination” browning	Number of fruit used for SEM
1	Elgin Koue Bokkeveld	1200	20	0	118	0	2 Diffuse 1 Non-brown
2	Koue Bokkeveld	1800	6	11	216	39	2 Radial 2 Diffuse 3 Combination 3 Non-brown
3	Koue Bokkeveld	400	1	31	74	21	3 Radial 1 Diffuse 2 Combination 1 Non-brown

Figures:



Figure 1: Browning patterns of A: diffuse browning and B: radial browning found after storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7 days shelf-life at $20\text{ }^{\circ}\text{C}$) in a 'Cripps' Pink' apple harvested in 2011/2012 in the Western Cape, South Africa.



Figure 2: Sectioned apple pieces with cortex and vascular tissue selected from a 'Cripps' Pink' apple grown in the Western Cape, South Africa, after long term storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks at RA storage at $-0.5\text{ }^{\circ}\text{C}$ and 7 days of shelf-life $20\text{ }^{\circ}\text{C}$) used for dissection of smaller samples.



Figure 3: Dissected apple samples after drying by critical point drying, used for SEM imaging of browning of a 'Cripps' Pink' apple grown in the Western Cape, South Africa, after long term storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks at RA storage at $-0.5\text{ }^{\circ}\text{C}$ and 7 days of shelf-life at $20\text{ }^{\circ}\text{C}$).

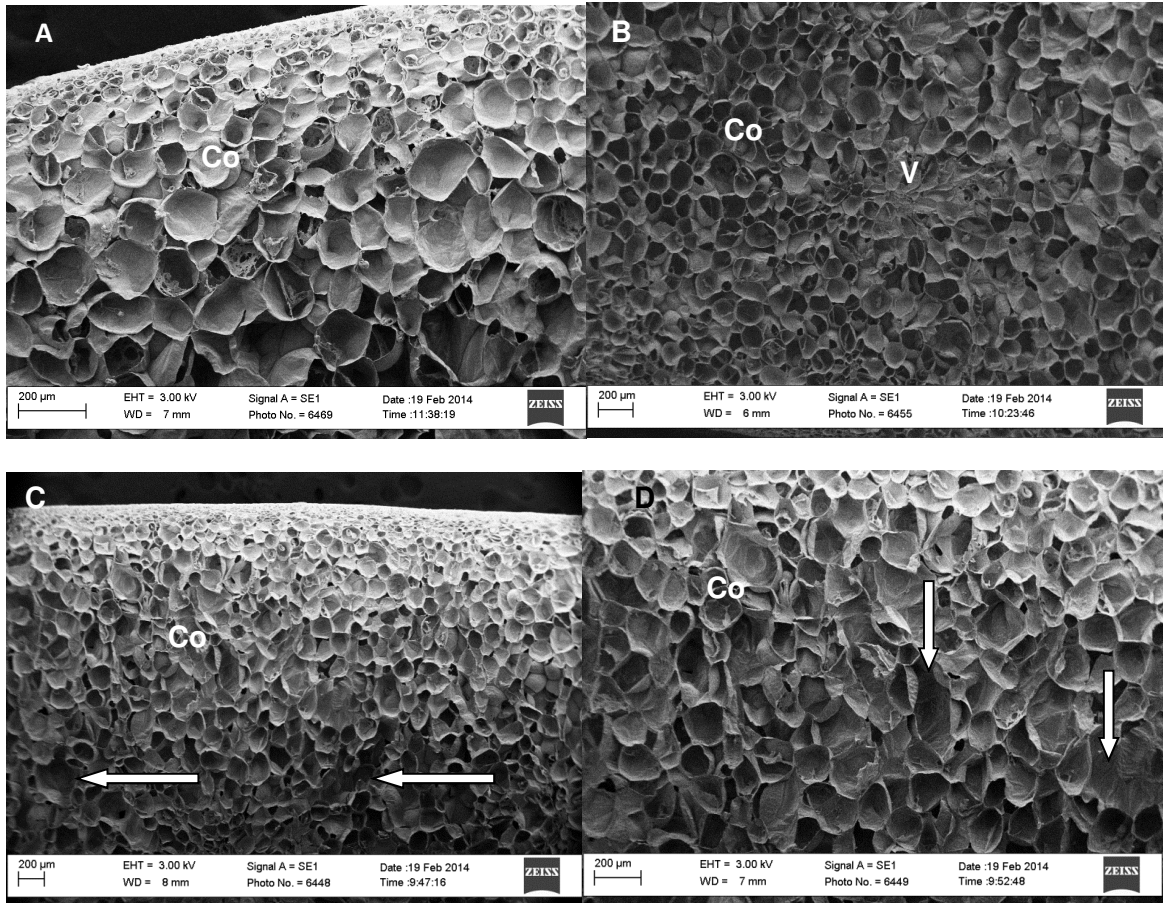


Figure 4: Scanning electron microscope (SEM) micrographs showing normal cortex tissue (Co) below the peel of an unaffected fruit (A) and normal cortical tissue surrounding vascular bundles of an unaffected fruit (B) damaged cortical tissue affected by diffuse browning (C), cortex tissue showing cell collapse and large intercellular spaces (D) of 'Cripps' Pink' apple grown in the Western Cape, South Africa, after long term storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks at RA storage at $-0.5\text{ }^{\circ}\text{C}$ and 7 days of shelf-life).

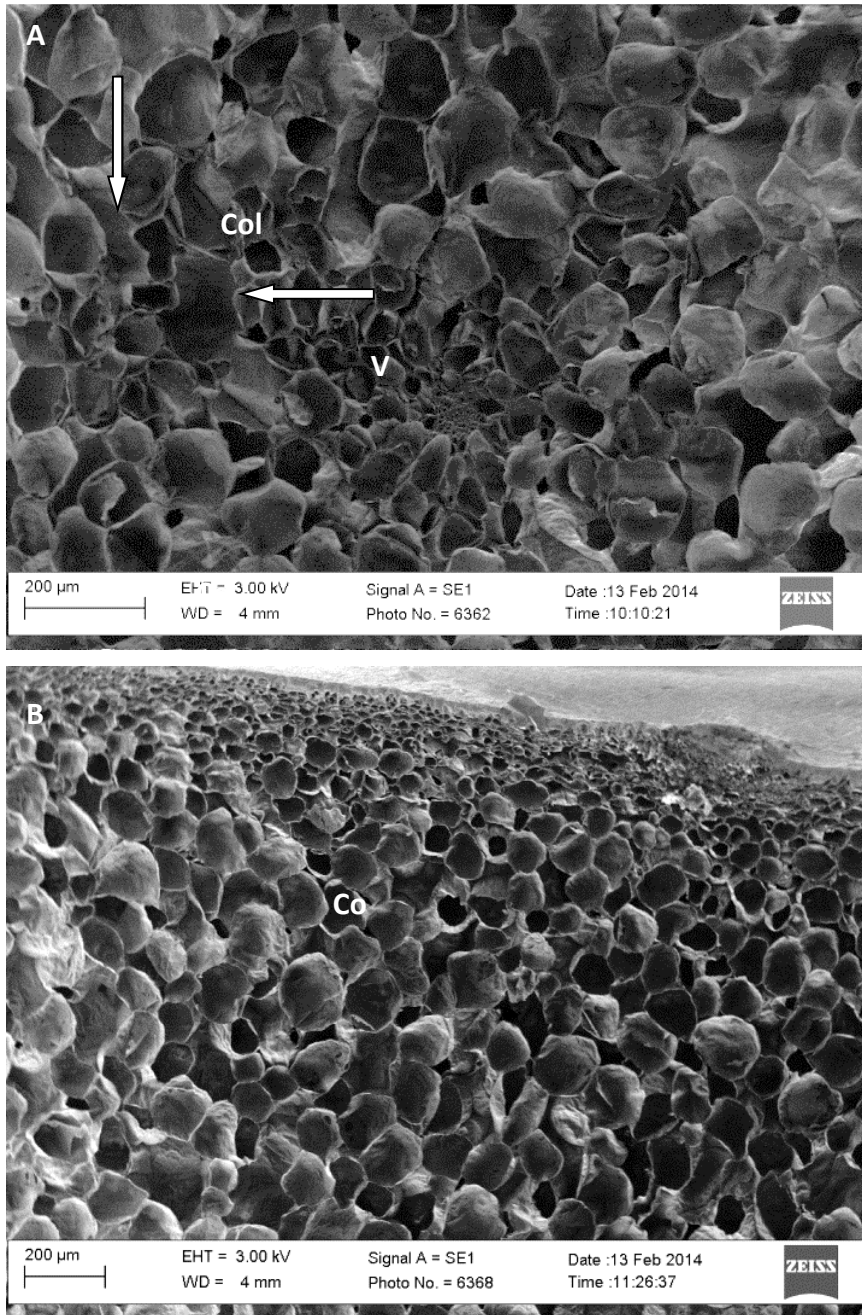


Figure 5: Scanning electron microscope (SEM) micrographs showing fruit tissue affected by radial browning. Collapse of cells surrounding (Col) vascular bundles (V) (A) and unaffected cortex tissue (Co) below the peel (B) of a 'Cripps' Pink' apple grown in the Western Cape, South Africa, after long term storage (7 months of CA storage at -0.5 °C + 4 weeks at RA storage at -0.5 °C and 7 days of shelf-life) is visible.

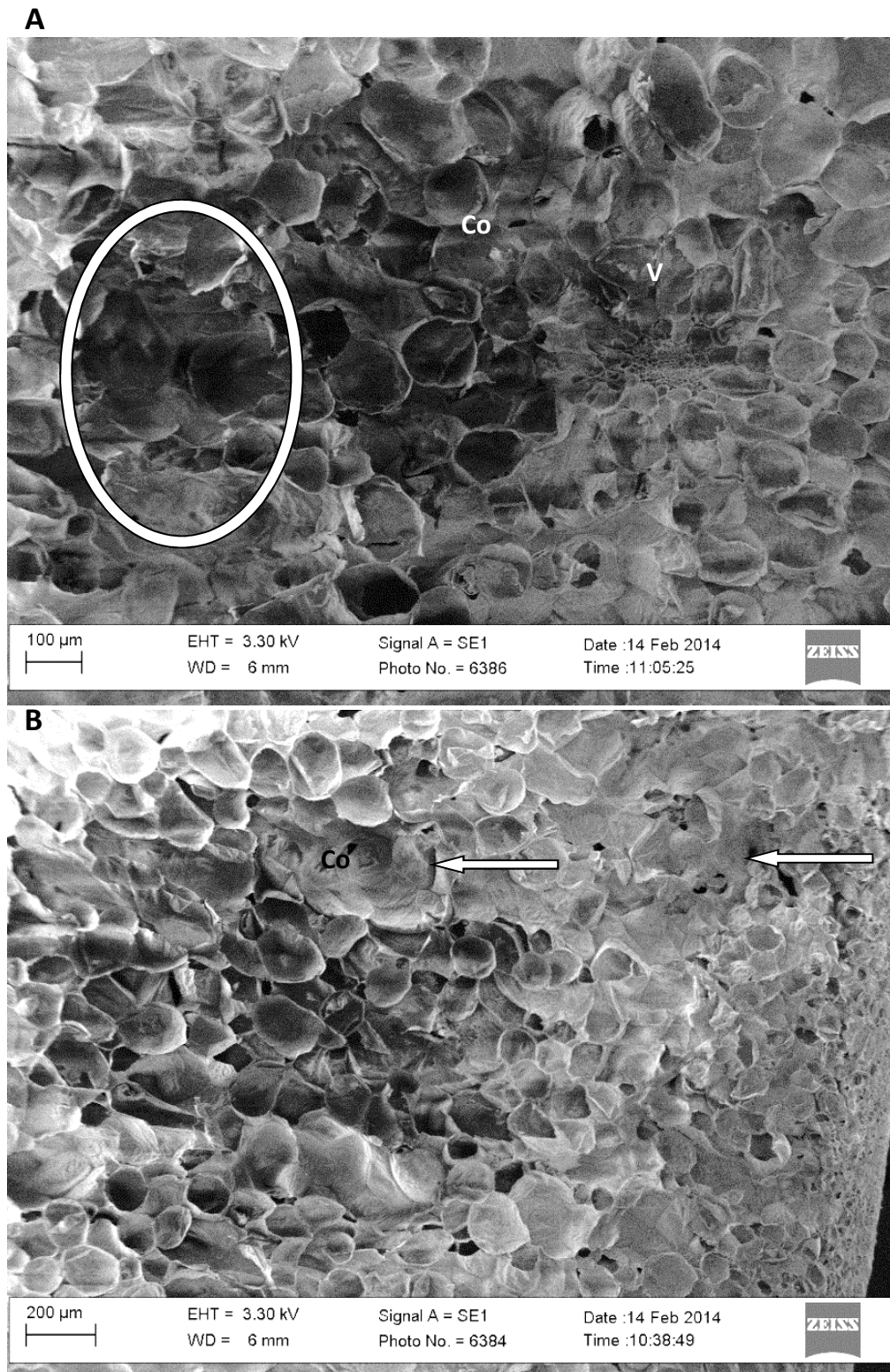


Figure 6: Scanning electron microscope (SEM) micrographs showing visual symptoms of “combination” browning. Cell disruption and large intercellular spaces (arrows) are visible in the cortex tissue (Co) surrounding vascular bundles (V) (A) and cortex tissue below the fruit peel (B) of ‘Cripps’ Pink’ apple grown in the Western Cape, South Africa, after long term storage (7 months of CA storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 weeks at RA storage at $-0.5\text{ }^{\circ}\text{C}$ and 7 days of shelf-life at $20\text{ }^{\circ}\text{C}$).

GENERAL DISCUSSION AND CONCLUSION

The aim of our first trial was to investigate a possible relationship between pre-harvest temperatures in two climatically different apple production regions on the occurrence of flesh browning in fruit tissues of 'Cripps' Pink' apples. Elgin and the Koue Bokkeveld differed according to average maximum temperatures, minimum, difference between minimum and maximum and accumulated growing day degrees above 10 °C ($GDD_{>10^{\circ}C}$) for different stages of fruit development. However, no differences in incidence of any type of browning occurred between the production regions. Seasonal temperatures differed according to maximum, accumulated $GDD_{>10^{\circ}C}$ and the difference between maximum and minimum temperatures for various growth stages, with the 2011/2012 season in general being milder compared to the 2012/2013 season. These differences in temperature affected incidence of radial browning as it was only found in fruit harvested in the 2011/2012 season and indicates that susceptibility of fruit towards radial browning could be affected by lower temperatures particularly during the early cell expansion stage. Overcast and cooler days during December in the Western Cape can occur in some seasons and may contribute to milder temperatures and therefore possibly to the occurrence of radial browning. Fruit were susceptible to diffuse browning in both seasons as the incidence did not differ significantly between seasons, even though 2012/2013 was a warmer season. This further supports the idea that diffuse browning is mainly influenced by harvest maturity and storage temperature and time in controlled atmosphere. Literature also supports that diffuse flesh browning is a disorder mainly induced by chilling injuries (James et al., 2005), extended periods in CA storage at low temperature (-0.5 °C) and overmaturity of fruit (Majoni, 2012). However, incidence of diffuse browning correlated with different temperature conditions for each season but, always with temperatures during the maturation stage. This indicates that factors associated with maturity of fruit or factors which influence susceptibility towards chilling injury during this maturation phase is affected by pre-harvest temperature and in turn affects the susceptibility toward diffuse browning development. Low temperatures during this period may have also predisposed fruit to chilling injury by altering the lipid ratio of the fruit (DeEll et al., 2005). Majoni et al. (2013) found that brown fruit had significantly higher ratios of saturated to unsaturated fatty acids compared to that of non-brown fruit which indicates susceptibility to cold storage caused by a change in the lipid ratio of fruit. Majoni et al. (2013) also found that non-brown fruit harvested at optimum maturity had the highest ascorbic acid and glutathione content compared to brown fruit and fruit harvested at post-optimal maturity, which may in turn influence susceptibility during storage. Possible exacerbating effects of diffuse browning could be small maximum – minimum (max-min) temperature differences during cell division (0-50 DAFB) which may have led to the development of fruit with a higher

density and increased susceptibility towards diffuse browning. In contrast to research findings by Jobling and James (2008), diffuse flesh browning was the most dominant type of browning to develop after long term storage even though both production regions in this study accumulated between 1636 and 1979 GDD_{>10°C} for both seasons which according to the findings of Jobling and James (2008) should be associated with incidence of radial browning or no browning at all.

Low maximum temperatures (20 °C) during the early cell expansion stage (50-100 DAFB) could have influenced the accumulation of photosynthates in fruit at a crucial time of development and may have increased susceptibility towards radial browning development and cell expansion around the affected tissue surrounding the vascular bundles in turn possibly affecting photosynthate accumulation and gas exchange in these tissues. Low temperatures during the cell expansion phase (50-100 DAFB) could possibly be associated with overcast weather and low rates of photosynthesis especially for the months of November, December and early January under South African conditions. The cell expansion phase of 2011/2012 was preceded by high temperatures during the cell division stage which could have led to a heavy fruit set (Kondo et al., 1987). Competition between fruit, when photosynthates are low (overcast weather during cell expansion), could lead to the development of fruit with carbohydrate and mineral deficits depending on the position of the fruit within the canopy and fruit cluster (Hayashi and Tanabe, 1991).

A third type of browning which exhibited browning patterns of both radial and diffuse browning was found and named “combination” browning. “Combination” browning was only found in fruit harvested in 2011/2012 indicating a possible relationship with radial browning. Incidence of “combination” browning correlated positively with fruit size for the 2011/2012 season when it occurred but did not correlate with any of the temperature parameters for any of the growth periods, possibly due to being a combination of both radial and diffuse browning.

Temperatures during different growth periods correlated to incidence of different browning types. This indicates that susceptibility of radial browning which is seasonal may be more likely to be influenced by pre-harvest temperature which was different for the seasons examined compared to diffuse browning which may be mainly affected by harvest maturity, storage temperature and duration under CA (Majoni, 2012). Data over more seasons are crucial to make any further conclusions.

The aim of our second trial, carried out over the seasons of 2011/2012 and 2012/2013, was to investigate the influence of tree age, soil type and mineral composition of fruit on internal flesh browning of ‘Cripps’ Pink’ apples. Trials were conducted on six farms in the Koue Bokkeveld production region. Browning incidence and mineral composition of fruit were compared between old and young orchards, and between sandy soil and clay soil orchards.

Mineral composition of brown fruit was compared to non-brown fruit and correlated to the incidence of browning. Browning of fruit was associated with low potassium (K) concentrations and K:magnesium (Mg) ratios for both seasons and higher calcium (Ca):phosphorous (P) and Ca:Mg ratios for the 2012/2013 season. K and Mg are strongly antagonistic in the nutrient uptake process and can result in a deficiency of the depressed nutrient (Voogt, 1998). Diffuse- and “combination” browning susceptibility of fruit in this study seems more related to the effect of high Mg in combination with low K rather than a specific mineral on its own. Correlations between Mg concentration of fruit and incidence of the total amount of browning indicated that fruit with a high Mg concentration were prone to browning development. Incidence of radial- and “combination” browning differed significantly between seasons and a very low incidence of these browning types were found for 2012/2013. Incidence of diffuse browning did not differ significantly between seasons. Fruit harvested from young trees (both seasons) and sandy soil orchards (2011/2012) were riper and prone to development of diffuse and “combination” browning. Incidence of radial browning was not affected by tree age or soil type. Sandy soils are prone to leaching of minerals (Su et al., 2004). Susceptibility of fruit harvested from orchards with sandy soil could be caused by increased leaching of minerals from sandy soils compared to clay soils. Fruit from orchards with sandy soils had a lower K concentration and K:Mg ratios for both seasons. The vigorous growth of young trees and its association with poor quality retention after harvest may have predisposed fruit to development of diffuse and “combination” browning (de Villiers, 1961; Sharples, 1973). Mineral analysis of fruit affected by radial browning and fruit affected by diffuse browning separately could indicate which of the browning types is affected by mineral concentration. The effect of soil type on browning incidence might have been contorted by the strong influence of tree age. More orchards with different soil types but with a similar tree age should be used in future studies and over more seasons.

The aim of our third trial was to assess near infrared reflectance spectroscopy (NIRs) as a non-destructive tool for detecting internal browning of ‘Cripps’ Pink’ apples. It was important for this study to establish if internal browning could be detected after CA storage and before shipment. Fruit were harvested from Esperanto, a farm in the Koue Bokkeveld for the seasons of 2011/2012, 2012/2013 and 2013/2014. Fruit were divided into two storage groups (2011/2012 and 2012/2013) and near infrared reflectance (NIR) spectra was collected from the first group after 7 months CA storage (7M) and after 7 months CA + 4 weeks RA + one week shelf-life for the second group (7M4W7D). Analytical tools such as partial least squares (PLS) regression (2013/2014 season) and PLS- discriminant analysis (DA) (2011/2012 and 2012/2013) were used to correlate NIR spectra to TSS of fruit, or to distinguish between brown and non-brown fruit, by means of NIR spectra. Browning was

assessed destructively after NIR spectra were collected. Diffuse and total browning incidence increased dramatically from the 7M storage to the 7M4W7D storage period for both seasons which is similar to characteristics of chilling injury as found by Jackman et al. (1988) and Parkin et al. (1989). As incidence of diffuse browning was not measured after RA and again after shelf-life it cannot be confirmed whether the increase in incidence of diffuse browning was caused by the extended storage period at RA in combination with shelf-life conditions, or only the ambient temperature of shelf-life conditions in this study. However, Majoni (2012) noted that diffuse browning generally increased further during the shelf-life period after the RA storage period at $-0.5\text{ }^{\circ}\text{C}$. Radial browning found in fruit after the 7M CA storage evaluation did not develop further after 4 weeks at RA at $-0.5\text{ }^{\circ}\text{C}$ and 7 days at $20\text{ }^{\circ}\text{C}$ (7M4W7D). When diffuse browning developed in fruit already affected by radial browning, “combination” browning was formed. Discriminant analysis using PLS, successfully identified browning in fruit. The accuracy of identification of browning increased as the percentage of diffuse and “combination” browning increased. Radial browning may be detected with more difficulty compared to diffuse- or “combination” browning due to the shallow penetration depth of the NIRs’ light source and the affected cortical regions associated with vascular bundles deeper in the fruit (James, 2007). The accuracy of prediction using PLS-DA is determined by sample size of affected and unaffected fruit, and should be tested on a larger sample size at a semi-commercial level. TSS was measured at the 7M stage as reference data to correlate with NIR spectra as it was found to correlate with the incidence of diffuse browning after storage and ripening (7 months CA + 4 weeks RA + one week shelf-life). The high predictive R^2 generated by PLS validation models found for this trial, confirms that NIRs can accurately predict TSS of ‘Cripps’ Pink’ apples. Significant positive correlations between TSS (measured at 7M) and incidence of diffuse browning (measured at 7M4W7D) in combination with the strong correlation of TSS with spectra generated at the 7M stage indicates that TSS can possibly be used to predict incidence of diffuse browning after shipment and ripening and to sort fruit based on their spectra at the 7M stage. The higher TSS in diffuse brown fruit may be linked to fruit maturity which is known to influence diffuse browning incidence, but may also be influenced by other factors and be coincidental. The relationship between diffuse browning and TSS should be investigated in detail to understand how and why TSS is able to predict browning, before sorting of fruit based on spectra and its correlating TSS is performed.

The discovery of “combination” browning as a combination of radial and diffuse browning in the same fruit led to the investigation of this anomaly on a cellular level with the help of scanning electron microscopy (SEM). James and Jobling (2009) indicated that cortical tissue below the fruit peel is affected in fruit with diffuse browning while cortical tissue surrounding

the vascular bundles remained unaffected. Radial browning affects cortical tissue associated with the vascular bundles while cortical tissue below the fruit peel remained unaffected (Jobling and James, 2008). James (2007) observed that brown tissue of fruit affected by diffuse browning shows cell collapse whereas brown tissue of fruit affected by radial browning shows fractured cell walls. SEM of fruit affected by radial browning in this study showed collapsed and fractured cortical cells associated with vascular bundles while the cortical tissue below the fruit peel remained unaffected. Tissue of samples taken from fruit affected by diffuse browning showed collapsed cortical tissue and large intercellular spaces below the fruit peel while cortical cells near vascular bundles remained unaffected. Investigation of samples from fruit affected by “combination” browning showed collapsed cortical cells near the vascular bundles as well as cortical cells below the peel, thus, confirming that “combination” browning truly is affected by both radial and diffuse browning. Tissue from non-brown fruit contained sound cells and had a well-organized structure. The cell collapse and large intercellular spaces found for fruit affected by diffuse browning could have been due to increase ion leakage and membrane viscosity caused by overmaturity of fruit and chilling injury (Finean et al., 1978; Majoni, 2012; Moeller et al., 1981; Lurie et al., 1987). Cell collapse and cell fracture associated with radial browning could possibly have been caused by the build-up of active oxygen species (AOS) associated with the accumulation of CO₂ in the dense tissues of ‘Cripps’ Pink’ fruit (Larrigaudière et al., 2001). The reason for browning of tissue around vascular bundles and not below the peel of fruit affected by radial browning could not be determined and the relationship between tissue density and CO₂ build up during long term CA storage should be further researched.

The different browning types are affected by different causal factors (James, 2007). The trials conducted in this study showed that radial and diffuse browning are influenced in different ways by pre-harvest temperature, soil type, tree age and mineral composition of fruit. The correlations found between temperature for specific growth periods and incidence of browning indicates that specific physiological characteristics affects susceptibility of fruit towards browning. This was supported by the difference in affected cells for different browning types. These physiological weak links are affected by temperature and possibly by competition between fruit for minerals and photosynthates, especially K concentrations and ratios of K:Mg. The different effects of pre-harvest temperature, soil type, tree age and mineral concentration of fruit on the susceptibility of fruit towards radial and diffuse browning have confirmed that these browning types are physiologically different and management of these browning types should be approached differently. To avoid development of diffuse flesh fruit should be harvested at optimal maturity (<40 % starch breakdown) as was previously suggested by Majoni, 2012 and stored at a higher temperature than -0.5 °C

(James, 2007) as these seem to be the main factors involved in diffuse browning as they did not differ between regions and seasons.

Future studies should include the documentation of temperatures according to growth stages for different farms in different production regions and relate this to incidence of browning types over a longer period of time. The development of “combination” browning in fruit subjected to radial browning masks the incidence of radial browning at shelf-life. The incidence of radial browning should therefore be determined after CA storage and then related to quality and maturity parameters. This would give a better perspective of the true incidence of radial browning and the relationship of radial browning with possible causal factors. The possible relationship between fractured and collapsed cells for radial and diffuse browning, respectively, and the compositional differences of the cell walls and cell membranes as affected by temperature (lipid composition of membranes) and mineral concentration of fruit cells (Ca and cell strength) could be investigated to better understand the causal effects. Assessment of NIRs on South African commercial packlines with a larger fruit sample should occur to test the accuracy and viability of the tool for sorting brown and non-brown fruit before and after the shipment and shelf-life periods. Using NIRs technology to sort fruit for internal browning would aid packhouses in making informed decisions before shipment and supply to distant sensitive markets.

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