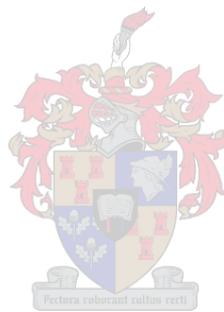


The effect of maturity and crop load on the browning and concentration of phenolic compounds of Thompson Seedless and Regal Seedless

by

WD Kamfer



Thesis presented in partial fulfillment of the requirements for the degree of

Master of Agricultural Science

at

Stellenbosch University

Department of Viticulture and Oenology, Faculty of AgriSciences

Supervisor: Dr PJ Raath

Desember 2014

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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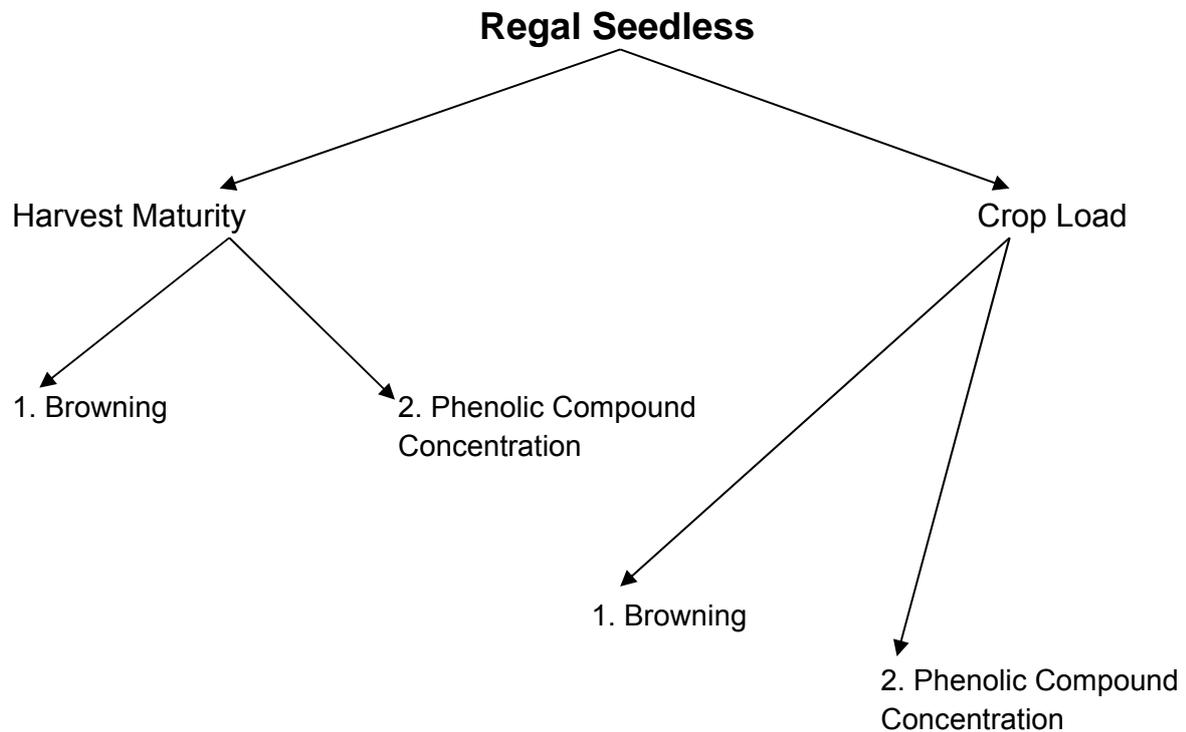
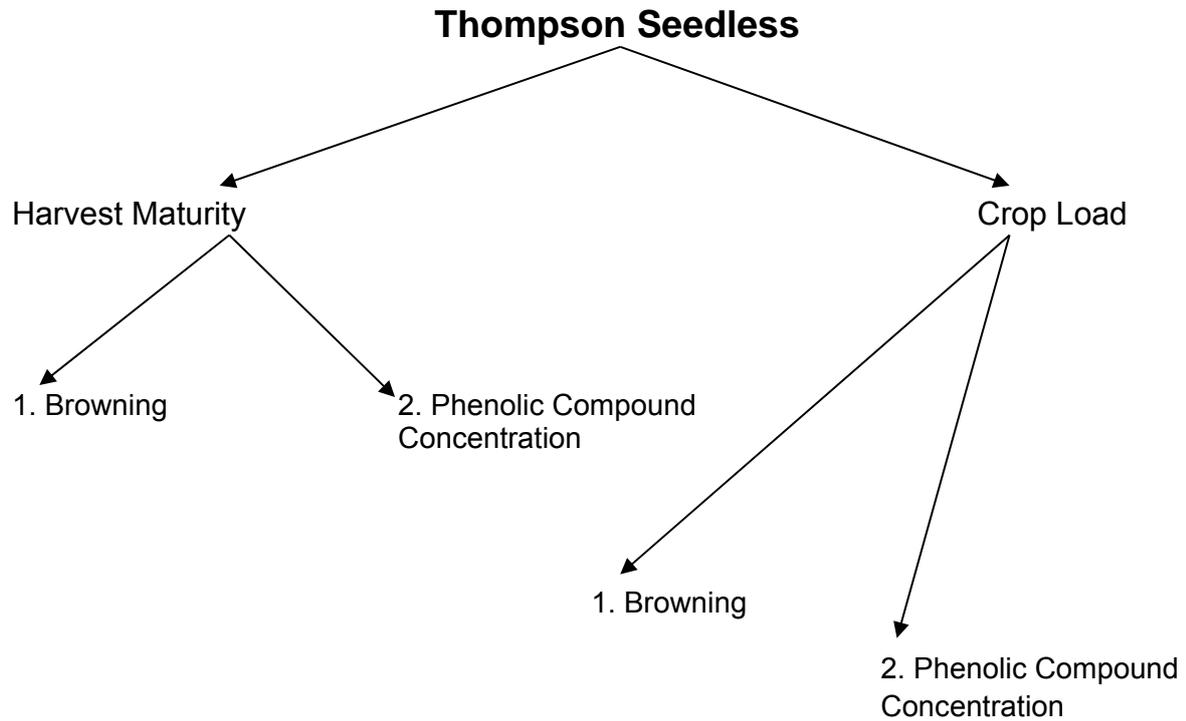
SUMMARY

Thompson Seedless and Regal Seedless are two white seedless table grape cultivars widely produced in South Africa. Both cultivars are susceptible to berry browning, especially Regal Seedless. Browning leads to annual financial losses for table grape growers. Although a correlation between harvest maturity and the occurrence of browning seems to exist, it is still unclear whether maturity levels are the actual contributing factor. The aim of the study was to establish if harvest maturity and crop load could influence the occurrence of browning of both cultivars. The impact of harvest maturity and crop load on phenolic compound concentration in the berry skin of both cultivars was also investigated. Total external browning of Regal Seedless and Thompson Seedless occurred in much higher percentages than internal browning. Regal Seedless showed a tendency to decreased total external browning with harvest maturity. The main reason for this is that net-like browning, which is the greatest contributor to total external browning, decreased with harvest maturity, in all three seasons. External browning of Thompson Seedless increased with harvest maturity in both seasons. Contact browning was the greatest contributor to total external browning of Thompson Seedless. Crop load did not significantly influence berry browning of Regal Seedless or Thompson Seedless grapes. The flavan-3-ol concentration (catechin, epicatechin, procyanidin B1 and procyanidin B2) in Regal Seedless generally increased with harvest maturity, whereas in Thompson Seedless the general tendency was a decrease in the flavan-3-ol concentration with harvest maturity. The development of phenolic compound concentration with maturity could not be correlated with the occurrence of berry browning. Crop load did not affect flavan-3-ol concentration. When the flavan-3-ol concentration of Regal Seedless and Thompson Seedless were compared at different harvest maturities the concentrations of flavan-3-ols were clearly much higher in the skin of Regal Seedless than in the skin of Thompson Seedless (for both the 2008 & 2009 seasons). Comparison of the browning incidence with harvest maturity for these two cultivars (see above) clearly reveals that external browning of Regal Seedless occurred in much higher percentages than on Thompson Seedless. Regal Seedless had much higher levels of external browning than Thompson Seedless. The concentration of flavan-3-ols in the skin of white seedless cultivars may be an indication of the cultivar's susceptibility to external browning.

OPSOMMING

Thompson Seedless en Regal Seedless is twee wit pitlose tafeldruif kultivars wat ekstensief in Suid-Afrika verbou word. Verbruining kan 'n probleem wees by beide kultivars, spesifiek Regal Seedless. Die faktore wat aanleiding gee tot verbruining is nog nie duidelik bepaal nie. Alhoewel dit lyk of daar 'n korrelasie tussen rypheidsgraad van die oes en verbruining kan wees is dit steeds onduidelik of oesrypheidsvlakke die werklike oorsaak van verbruining is. Die doel van die studie was om vas te stel of die rypheidsgraad van die oes en oeslading verbruining van beide kultivars kan beïnvloed. Die effek van oes rypheidsgraad en oeslading op konsentrasie van fenoliese verbindings in die korrelskil van beide kultivars is ook ondersoek. Totale eksterne verbruining van Regal Seedless en Thompson Seedless het in baie hoër persentasies voorgekom as interne verbruining. Daar was 'n tendens by Regal Seedless dat totale eksterne verbruining verminder het soos die oes ryper geraak het as gevolg van netagtige verbruining, wat die grootste bydrae tot totale eksterne verbruining veroorsaak het. Netagtige verbruining se voorkoms het verminder oor al drie seisoene. Eksterne verbruining van Thompson Seedless het toegeneem met oes rypheid in beide seisoene. Kontak verbruining het grootste bydrae gelewer tot totale eksterne verbruining van Thompson Seedless. Oeslading het nie 'n betekenisvolle invloed op verbruining van Regal Seedless en Thompson Seedless gehad nie. Die flavan-3-ol (katesjien, epikatesjien, prosianidien B1 en prosianidien B2) konsentrasie van Regal Seedless het met oes rypheid toegeneem. By Thompson Seedless was daar 'n afname in die flavan-3-ol konsentrasie met oes rypheid. Daar was geen korrelasie tussen die konsentrasie van fenoliese verbinding en die voorkoms van verbruining vir beide kultivars. Oeslading het nie 'n betekenisvolle effek op die konsentrasie van fenoliese verbindings gehad nie. Vergelyking van die flavan-3-ol konsentrasie van Regal Seedless en Thompson Seedless by verskillende rypheidsgrade wys dat die konsentrasie baie hoër in die korrel skil van Regal Seedless as in die van Thompson Seedless (vir beide 2008 & 2009 seisoene). Die vergelyking van die voorkoms van verbruining met oesrypheid van beide kultivars wys duidelik dat eksterne verbruining van Regal Seedless in baie hoër persentasies voorkom as in Thompson Seedless. Flavan-3-ol konsentrasie in die skil van wit pitlose kultivars kan 'n aanduiding wees van die kultivar se moontlike risiko vir die voorkoms van eksterne verbruining.

SCHMATIC OUTLINE OF STUDY



ACKNOWLEDGEMENTS

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My family, for their support and encouragement.

Mari Kamfer, for her understanding, support and encouragement. She is a true companion.

God, for granting me the patience and dedication to continue when I wanted to give up.

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This thesis is presented as a compilation of five chapters.

The research results are contained in two separate chapters: one with the focus on investigations into browning (Chapter 3) and the other with the focus on investigations into phenol concentration (Chapter 4).

The layout of the document is as follows:

- Chapter 1 Introduction and project aims**
- Chapter 2 Literature review: Browning in table grapes**
- Chapter 3 The effect of harvest maturity and crop load on browning of Thompson Seedless and Regal Seedless**
- Chapter 4 The effect of harvest maturity and crop load on phenol concentration of both Thompson Seedless and Regal Seedless**
- Chapter 5 General discussion and conclusions**

LIST OF ABBREVIATIONS

Abbreviations used in text

DAD	Diode Array
HM	Harvest Maturity
HPLC	High-performance Liquid Chromatography
LSD	Least significant differences
mDP	mean Degree of Polymerisation
PPO	Polyphenol Oxidase
TA	Titrateable Acidity
TSS	Total Soluble Solids
TMT	Treatment

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CHAPTER 1

INTRODUCTION AND AIMS

1.1 General introduction

The table grape industry is a very important sector in South African agriculture. In the 2008/09 season 48.4 million cartons (4.5 kg equivalent) were exported, in the 2009/10 season 51.5 million cartons and in the 2010/11 season 44.7 million cartons. The table grape industry in 2011 employed more than 10 000 permanent workers and more than 40 000 seasonal workers. A vine census carried out in 2011 showed that there were 412 ha of Regal Seedless and 1432 ha of Thompson Seedless planted in South Africa. Production of Regal Seedless and Thompson Seedless is responsible for almost 20% of export grape volumes. Increasing costs of electricity, fuel and labour are putting increasing pressure on table grape growers, and profit margins have also decreased. The number of table grape producers in South Africa has recently been decreasing, from 466 in 2009 to 382 in 2011 (SATI, 2011).

Table grapes are an aesthetic product and the impact of browning can have a severe influence on the commercial value of the grapes. The browning of white seedless table grapes can result in financial losses to growers. The factors contributing to the development of browning of white table grapes have not yet been adequately established. A correlation seems to exist between the sugar levels of harvested grapes and the occurrence of browning (Vial *et al.*, 2005), but it is still unclear whether maturity levels is the actual contributing factor. Crop load, especially over-cropping, has also been implicated as negatively impacting grape quality. Over-cropped Flame Seedless vines have shown inadequate development of fruit soluble solids, reduced packable yields and variable effects on fruit composition (Dokoozlian & Hirschfelt, 1995).

The browning potential in grape juice is calculated by taking into account the browning index of each phenolic compound and its concentration in the grape juice. It seems that the concentration of phenolic compounds is more closely related to the browning of juice and wines than the enzymatic activities of a given grape cultivar (Macheix *et al.*, 1991). The content of the phenolic substrate is of great importance in the enzymatic activity of browning (Sapis *et al.*, 1983).

The Hex River Valley is the largest table grape producing area in South Africa. Approximately 4500 ha of table grapes are planted and the area produces about 19

million cartons (4.5 kg equivalent) of grapes annually (SATI 2011). The trials reported in this study were carried out in the Hex River Valley in the Western Cape Province of South Africa.

1.2 Problem statement and research questions

The financial impact of the browning of grapes on the South African table grape industry is very unfavourable. Some studies indicate that, in certain varieties, maturity can play a role in browning incidence (Wolf 1996; Vial *et al.*, 2005). Overcropping can also negatively impact grape quality (Dokoozlian & Hirschfelt, 1995). Sapis *et al.*, (1983) state that the phenolic substrate is of high importance in browning.

The hypothesis is that there is a breakdown of cells in grape berries after the onset of ripening (Lang & Doring, 1991) and this breakdown could lead to a mixing of phenolic compounds and polyphenol oxidase (PPO). Mixing of phenolic compounds and PPO will trigger oxidation reactions, which could lead to browning. The degradation of membrane integrity is at the centre of this hypothesis.

1. The first research question is: Does (1) harvest maturity and (2) crop load have an effect on the occurrence of browning of Regal Seedless and Thompson Seedless grapes?
2. The second question: How do (1) harvest maturity and (2) crop load influence phenolic concentration in the berry skins of Regal Seedless and Thompson Seedless?
3. The third question: Is there a correlation between phenolic concentration development in the berry skin and berry browning?
4. The fourth question: How do the phenolic concentration in the skin of Regal Seedless and Thompson Seedless compare with each other?

1.3 Project aims

1. Establish whether (1) harvest maturity and (2) crop load influence berry browning of Regal Seedless and Thompson Seedless.
2. Determine the influence (1) harvest maturity and (2) crop load would have on concentration of phenolic compounds in the berry skin of Thompson Seedless and Regal Seedless.
3. Establish whether there is any correlation between phenolic concentration development and berry browning.
4. Compare the phenolic concentration in the skin of Regal Seedless and Thompson Seedless.

In order to achieve the above aims, the following tasks were to be carried out:

1. Select suitable Regal Seedless and Thompson Seedless vineyards.
2. Determine different crop loads in accordance with established treatments just after set for both cultivars.
3. Harvest both cultivars at different harvest maturities, from 16°Brix until 20°Brix.
4. Transport grapes to Experico and store at -0.5°C within 2 h after packing.
5. Determine the TSS (Total Soluble Solids) and TA (Titratable Acidity) content, which serves as indicators of maturity levels of the grapes.
6. Determine the occurrence of browning, as established by standardised Experico protocols.
7. Determining the phenolic compound concentration in the skin of both Regal Seedless and Thompson Seedless grape (a) different crop load levels and at (b) different harvest maturities.

1.4 Layout of document

This thesis is presented as a compilation of five chapters. The research results are contained in two separate chapters: one with the focus on investigations into browning (Chapter 3) and the other with the focus on investigations into phenol concentration (Chapter 4).

1.5 References

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CHAPTER 2

LITERATURE REVIEW: BROWNING IN TABLE GRAPES

2.1 Introduction

The two cultivars considered in this study, Regal Seedless and Thompson Seedless are extensively cultivated in South Africa. In the 2010/11 season, the combination of Thompson Seedless and Regal Seedless accounted for more than 15% of the total grape export volume. For the 2011 season, these two white seedless varieties comprised 46% of the total white seedless volume exported (SATI, 2011).

Regal Seedless and, to a lesser extent, Thompson Seedless, like many other white seedless cultivars, are susceptible to browning. Regal Seedless, particularly, has been under huge commercial pressure and the cultivar has been omitted from most retailers' preferred lists of choice in the United Kingdom and the EU. Therefore, table grape growers in South Africa have begun replacing this variety with more commercially acceptable varieties like Prime and Sugraone. Regal Seedless plantings have decreased from 647 ha in 2008 to 412 ha in 2011 (SATI, 2008 and 2011), which is a reduction of 34% in four years. Browning of Thompson Seedless is less common than browning of Regal Seedless. It is restricted to seasonal variation.

2.2 Browning of table grapes

2.2.1 Possible causes of browning of table grapes

Browning of fruit, including table grapes is a very complex problem. A disruption of cell membranes, which allows mixing of the enzyme polyphenol oxidase (PPO) with phenolic substrates occurring naturally in fruit, is the first step in browning (Ferreira, 1997; Golding *et al.*, 1998). The process involves two phases: an enzymatic phase and a spontaneous polymerisation phase. The first phase is characterised by conversion of monophenols to diphenols (Kruger *et al.*, 1999), whereafter, diphenols are then oxidised by means of hydroxylation enzymes and *o*-quinone through PPO located in the cytoplasm (Macheix *et al.*, 1991; Liyanage *et al.*, 1993). The second phase is characterised by spontaneous polymerisation during which quinones are polymerised, which leads to the formation of melanin (brown pigments), which are responsible for the brown colour or browning phenomenon (Sapis *et al.*, 1983).

The three factors that could possibly influence the occurrence of browning and the rate at which it appears in grapes and grape juice are the following: (i) the cell wall and cell membrane integrity, (ii) the phenolic substrates in the vacuoles of cells that can be oxidised, and (iii) the PPO activity and oxygen availability (Macheix *et al.*, 1991).

2.2.2 Types of browning

The table grape industry of South Africa has identified six main groups of browning: external, internal, low-temperature, chemical, physical, and pathogenic browning (Fourie, 2009). The two most common types of browning that occur on white seedless table grapes are internal and external browning, in their various forms.

External browning can be subdivided into different types of which net-like, mottled, friction, and contact browning are the most common symptom on grapes. Internal browning is expressed as chocolate-, water-, and glassy berry (Fourie, 2009).

2.2.2.1 External browning

External browning can manifest in many different phenotypes (Fourie, 2009):

- Net-like browning: Necrotic streaks (dashed-like), progressing from the stylar-end towards the pedicel-end of the berry (Fig. 1a)
- Mottled browning: Brown blotches and/or or spots on the berry surface (Fig. 1b)
- Friction browning: Circle-like browning close to the pedicel area, associated with rolling of berries against each other (Fig. 2a)
- Contact browning: Brown marks on the berry surface, where berries touch, often associated with square-like flattened areas at the pedicel-end of the berry (Fig. 2b)
- Peacock spot: Brown circles, or half-circles, with a clear centre, on the surface of berries where adjacent berries touch, with symptoms already present in the vineyard (Fig. 3a)

- Styler-end russet spots browning: Brown russet-like damage at the styler-end of the berry, characterised by irregular shaped spots, exhibiting a circular damaged area (Fig. 3b)
- Styler-end necrotic spots browning: Brown spots at the styler-end of the berry, characterised by slightly sunken necrotic tissue, often associated with secondary pathogenic infection (Fig. 4a)
- Sunburn: Brownish colouration of the berry surface, as a result of direct exposure to damage by the sun, often characterised by a leathery, rough touch (Fig. 4b).

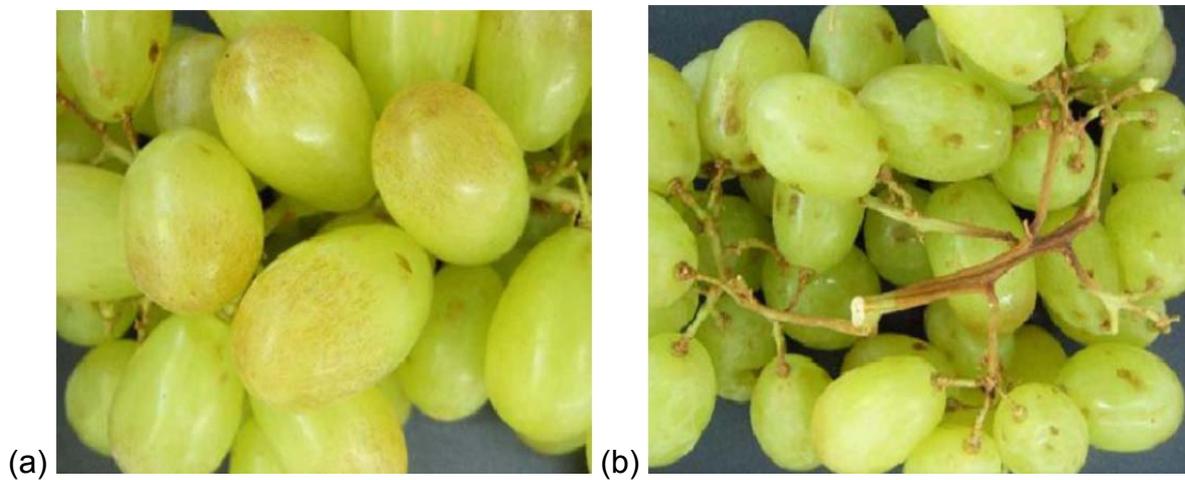


Figure 2.1 (a) Net-like browning and (b) mottled browning on Regal Seedless.

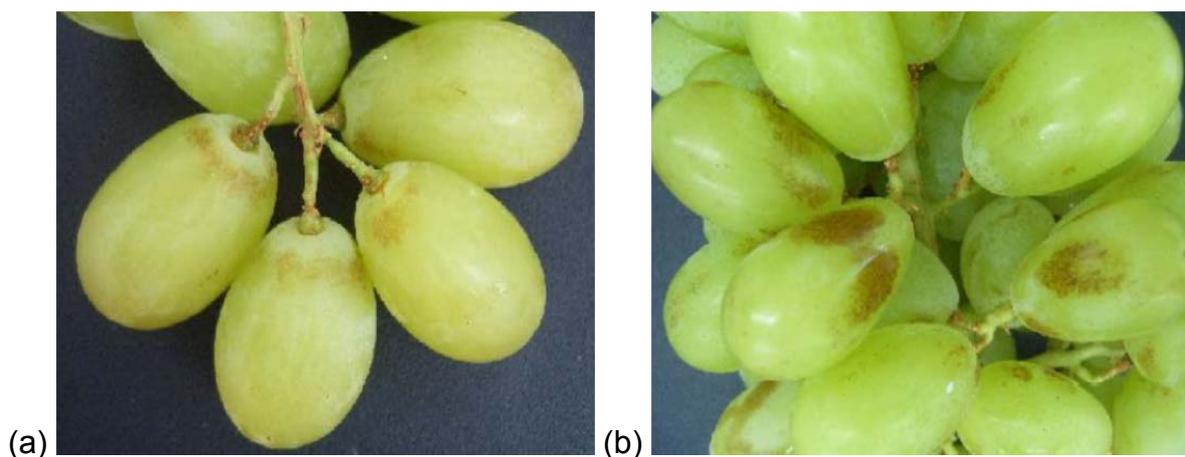


Figure 2.2 (a) Friction browning and (b) contact browning on Regal Seedless.

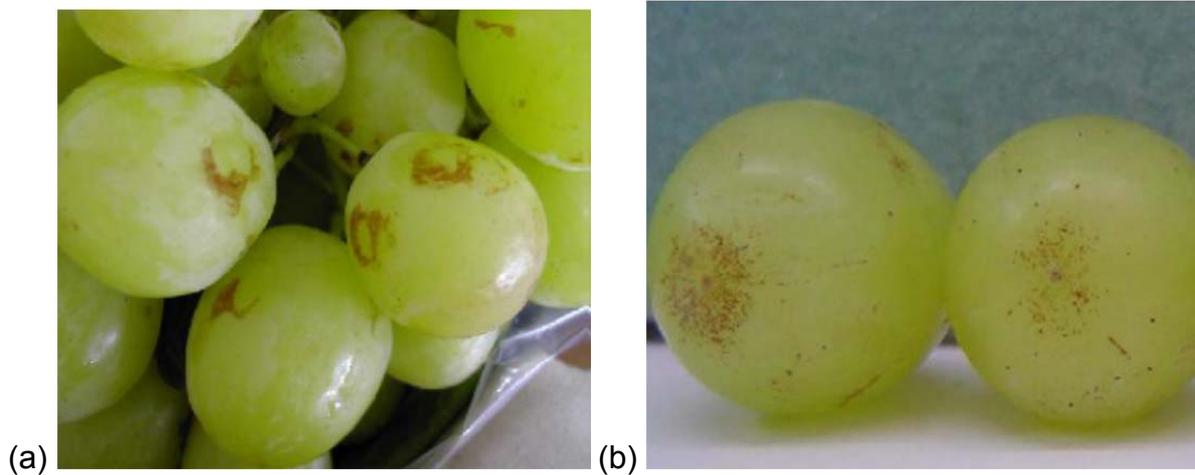


Figure 2.3 (a) Peacock spot and (b) stylar-end russet spots browning.

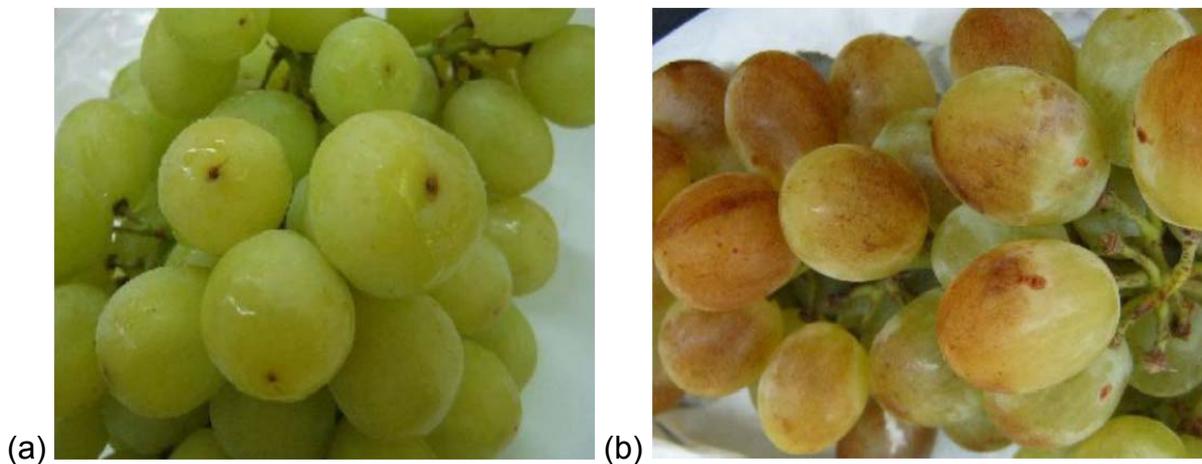


Figure 2.4 (a) Stylar-end necrotic spots browning and (b) Sunburn.

2.2.2.2 Internal browning

Chocolate berry internal symptoms show a brown discoloration, which originates mostly from the stylar end of the berry. In severe cases, the whole berry may appear brown, as in Fig. 5(a). Chocolate berry external symptoms originate from the stylar end, progressing upwards towards the pedicel end of the berry, with a clearly visible distinct line between the affected and sound tissue, as in Fig. 5(b).

Water berry symptoms, as in Fig. 6(a), refer to the browning of berries, associated with desiccation, often related to damage to pedicels, starting at the pedicel end and extending towards the stylar end of the berry as the disorder progresses. Symptoms

include berries exhibiting a dull, translucent, brown appearance, with browning progressing from the inside, outwards.

Glassy berry symptoms, as in Fig. 6(b), exhibit a dull, translucent, brown appearance, with browning progressing from the inside, outwards (Fourie, 2009).

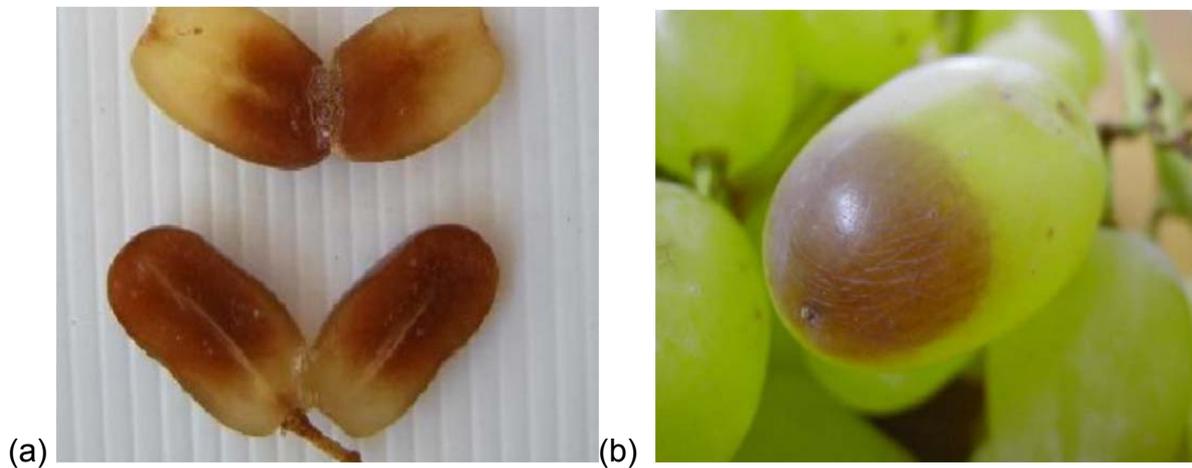


Figure 2.5 (a) Chocolate berry (external symptoms) and (b) chocolate berry (internal symptoms).

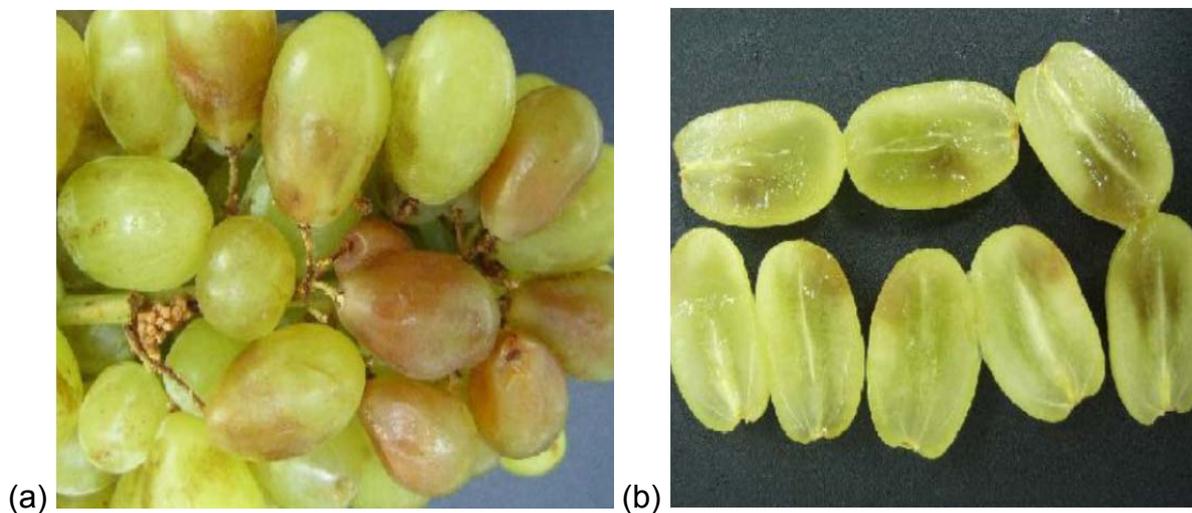


Figure 2.6 (a) Water berry and (b) glassy berry symptoms.

Table 2.1 The six main types of browning found in table grapes and the different formats in which they are expressed in berries, adapted from Fourie (2009).

External browning	Internal browning	Physical browning	Chemical browning	Low-temp. browning	Pathogenic browning
• Net-like browning	• Chocolate browning	• Bruising	• Methyl bromide damage	• Freezing damage	• Fungal infection
• Friction browning	• Water berry	• Abrasions	• CO ₂ damage	• Cold damage	
• Contact browning	• Glassy berry				
• Mottled browning					
• Styler-end russet spots					
• Styler-end necrotic spots					
• Sunburn					
• Peacock spot					

2.2.3 Harvest maturity and browning

Although a correlation between sugar levels of harvested grapes and the occurrence of browning seems to exist, it is still not clear whether the maturity levels are the actual contributing factor. Singleton (1966) observed that, for white grapes, there generally appears to be greater browning tendency in the juice of the riper harvests. Wolf (1996) observed that, for the cultivar Waltham Cross (white seeded), skin browning is directly related to fruit maturity. Princess (white seedless) bunches in California harvested at higher TSS levels showed increased browning (Vial *et al.*, 2005). Harvest time had a significant effect on browning in both these cultivars.

Increasing values of both solubilised PPO activities and total crude PPO activities from the beginning of véraison of different cultivars (Sapis *et al.*, 1983) and the fact that there is a breakdown of cells in grape berries after the onset of ripening (Lang & During, 1991) are all possible contributing factors to increased browning with higher maturity levels in table grapes.

2.2.4 Crop load and browning

A grapevine has the capacity to produce a given weight of fruit and to bring that fruit to normal maturity within a given number of degree-days of heating, characteristic for the cultivar and the climatic region (Winkler, 1958). Over-cropped vines are generally characterised by delayed fruit maturation, small berries, reduced vine growth, higher sugar/acid ratio at a given fruit maturity, poor fruit colouration, and

softness of berry texture (Dokoozlian & Hirschfeld, 1995). Therefore, crop load has also been implicated to have an impact on the quality of grapes. Some of the obvious effects of over-cropping are lower colour (in the case of red varieties), lower pH, and a delay of fruit maturation (Weaver, 1961). The capacity of a vine to ripen grapes is largely determined by its total leaf area and the percentage of the total leaf surface area that is at light saturation or above, provided other factors are not limiting growth, and the initiation of fruit primordia (Kliewer & Weaver *et al.*, 1971). Over-cropping of the cultivar Tokay was found to have a negative impact on fruit coloration and concentrations of proline and arginine in berry juice compared to the control (Kliewer & Weaver *et al.*, 1971). Bravdo *et al.* (1985) found that over-cropping impacted the quality of the must and the wine content, specifically malic acid, wine colour, ash, and tartaric acid content of Cabernet Sauvignon. Dokoozlian & Hirschfeld (1995) found a delay in colour development as well as inadequate development of fruit soluble solids and a reduction in packable yields in over-cropped Flame Seedless vines.

It has been hypothesised that a reduction in crop level could benefit the grape quality by accelerating maturity. Grapes will reach optimum maturity earlier with cells more intact. Production practices used to maximise grape quality parameters or yield can have a significant effect on the source–sink relationship of the grapevine (Williams, 1996). TSS for cv. Tas-A-Ganesh (*Vitis vinifera L.*) decreased with an increase in yield per vine and there was a reduction in berry diameter (Somkuwar & Ramteke, 2006). This data may be explained by source–sink relationships. A greater photoassimilate source due to higher leaf area and bigger root surface area will result in a higher concentration and total amount of photoassimilate in the fruits (Williams, 1996). Theoretically, as the crop size decreases, there is less competition for photosynthate and therefore a greater supply of photoassimilate available for the remaining fruits.

2.2.5 Customer perception of table grape quality

Rolle *et al.* (2012) suggested that for table grapes to be accepted by customers as a good quality product is reliant on some measurable qualitative properties such as firmness and taste, as well as the quantitative properties such as sugar and acid content. It is very important to constantly ensure customer satisfaction. Cliff *et al.*

(1996) and Mencarelli *et al.* (2005) have shown that customers prefer table grapes with good taste and flavour. Deng *et al.* (2005) have shown that the visual appearance of the fruit, the stems and the skin as well as flesh firmness are all critically important. Jayasena & Cameron (2008) reported that table grape quality is highly dependent on the maturity level at which the grapes are harvested.

The main parameters to determine table grape maturity in South Africa are TSS and TA. In some other countries, TSS is referred to as soluble solids concentration. TSS is measured in °Brix and refers to the amount of sugars (glucose and fructose) present (Baiano *et al.*, 2012). The organic acid composition is measured as TA and expressed as g/L tartaric acid or percentage titratable acidity (Shiraishi *et al.*, 2010).

2.2.6 Optimum maturity of Regal Seedless

Gütschow (2000), Avenant (2007) and Fraser (2007) studied the optimum eating quality for Regal Seedless. The main aim of their research was to determine optimum eating quality for this variety with lowest possible astringency.

Gütschow (2000) established 'picking windows' (harvest dates) for various newly released cultivars to establish industry maturity standards by which seasonal and area ripening could be identified. This was done by determining the effect of different harvest maturities on long-term storage which would result in the optimum eating quality of Regal Seedless. The recommendation on Regal Seedless was that the TSS (°Brix) should be increased to 18°Brix. At 17°Brix, the skin components were still very astringent (Gütschow, 2000).

Avenant (2007) suggested that Regal Seedless should not be harvested before the grapes reached a sugar concentration of 17°Brix because an increase in sugar content disguises the astringent taste of Regal Seedless.

Fraser (2007) evaluated the sensory profiling of Regal Seedless at different maturity levels. Organoleptic parameters such as astringency, skin tenacity, and eating quality were evaluated. The phenolic content of Regal Seedless at different harvest maturities and, more specifically, the flavanols, which are responsible for the astringency perception, was also evaluated (Fraser, 2007). The recommendation

was that the eating quality of Regal Seedless improved from 17°Brix and upwards. The total flavanols, which are mainly responsible for astringency, were the lowest between 18 and 19°Brix. The recommended maturity level for Regal Seedless was between 17 and 19°Brix (Fraser, 2007).

2.3 The role of phenolic compounds

Phenolic compounds play an important role in the quality of grapes and wines. They are classified in two major groups: flavonoids and non-flavonoids.

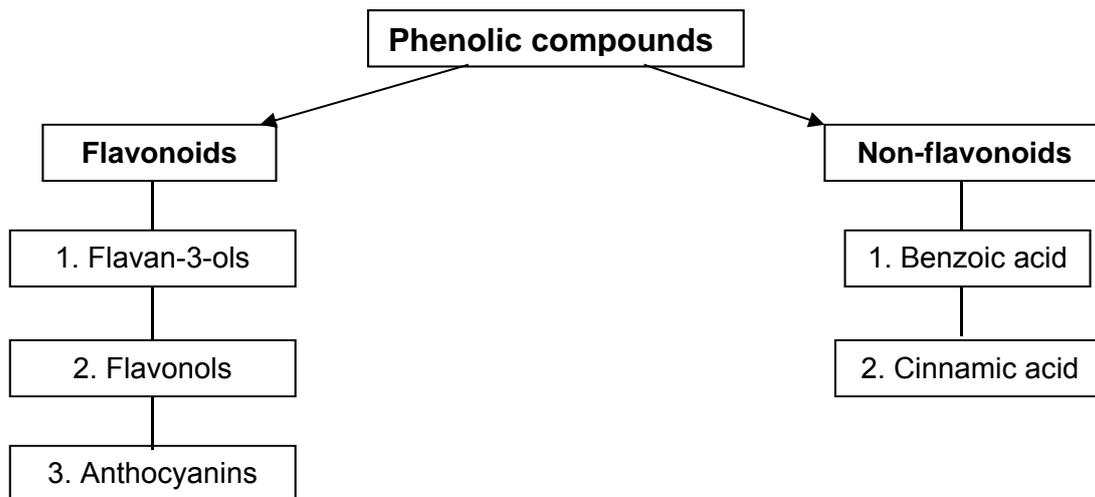


Figure 2.7 Phenolic compounds

2.3.1 Flavonoids

Flavonoids can be divided into three main groups: (1) flavan-3-ols, (2) flavonols, and (3) anthocyanins. All flavonoids are based on a common C₆-C₃-C₆ skeleton which consists in two phenolic rings (A and B) linked via a heterocyclic pyran ring (C ring). This large group is subdivided in several families based on the oxidation state of their C-ring. In flavan-3-ols, which bear one hydroxyl group in the 3 position, the C-ring is saturated and thus shows two asymmetric carbons (in C₂ and C₃). This opens the possibility for different stereoisomers. The A-ring of flavan-3-ols is generally hydroxylated in C₅ and C₇ and the B-ring in C₄ (Fig. 1: R, R′=H, afzelechin series), but further diversity arises from the substitution pattern of the B-ring (Terrier *et al.*, 2009).

Flavan-3-ols are the most abundant of the flavonoids, followed by anthocyanins and flavonols which are prevalent in the grape skins (Adams 2006). Flavan-3-ols are the

basic building blocks (monomers). The flavan-3-ols occur free or polymerise to form dimers, trimers, or higher oligomers (polymers) through (C4–C6/C4–C8) interflavan linkages. These polymeric flavan-3-ols are called proanthocyanidins or condensed tannins. (Boulton *et al.*, 1996; Cheynier & Rigaud 1986).

Anthocyanins are responsible for the red colour in grapes. They are the red pigments that are present in grape skins (Boulton *et al.*, 1996). They are mainly located in the vacuoles of the skin cells. Malvidin-3-glucoside is the most abundant in red cultivars, representing about 40% of the total anthocyanins (Boulton *et al.*, 1996). Anthocyanin development is very important to the production of Flame Seedless and Crimson Seedless. In warmer production areas, like the Orange River in South Africa, colour development on these varieties is very challenging. Very few studies have examined the impact of ripening stage of the grapes on the extractability of phenolic compounds. Fournand *et al.* 2006 investigated anthocyanin and proanthocyanidin quantitative and compositional modifications in grape skins during sugar accumulation in the pulp. The aim was to determine whether date of harvest may have influence on the skin phenolic extraction. The proportion of methoxylated anthocyanins continued to increase in the skin as sugar accumulated while the proportion of coumaroylated anthocyanins initially increased and then rapidly decreased. No major quantitative nor qualitative change was observed for tannins except for a slight increase of the mean degree of polymerization. Regal Seedless and Thompson Seedless are both white seedless cultivars and therefore contain no anthocyanins. For this reason, anthocyanins will not be discussed further.

The flavonols kaempferol, quercetin, myricetin, and isorhamnetin are found in wines, but in the berry they are present as the corresponding glucosides, galactosides, and glucuronides (Adams 2006). In Pinot noir, Shiraz, and Merlot fruit, the amount of these compounds has been shown to be highly dependent on light exposure of the tissues in which they accumulate (Price *et al.*, 1995, Spayd *et al.*, 2002, Downey *et al.*, 2004).

2.3.1.1 Flavan-3-ols

The flavan-3-ols, a large family of polyphenolic compounds, are mainly responsible for the astringency, bitterness, and structure of wines (Singleton & Esau, 1969).

The primary flavan-3-ols are (+)-catechin and (-)-epicatechin and (-)-epicatechin-3-gallate (Ribéreau-Gayon *et al.*, 2000). They differ around the two stereo centres of the flavan-3-ols: (+)-catechin has the 2,3-*trans* configuration and (-)-epicatechin the 2,3-*cis* configuration. When the hydrogen at R₁ is replaced by a hydroxyl group, it is known as (+)-gallocatechin and (-)-epigallocatechin. (+)-Catechin and (-)-epicatechin can be esterified to gallic acid (Ribéreau-Gayon *et al.*, 2000).

Flavan-3-ols are located in the solid parts of the berry of both red and white grape cultivars (Lea *et al.*, 1979). The highest concentrations of flavan-3-ols are present in the seeds and lower concentrations are present in the skins (Boulton *et al.*, 1996; Ricardo-da-Silva *et al.*, 1992).

Kennedy *et al.* (2001) reported that berry development is correlated with an increase in proanthocyanidin mDP (mean Degree of Polymerisation), an increase in proportion of (-)-epigallocatechin extension subunits, and increases in the level of anthocyanins associated with the proanthocyanidin fraction.

Dimeric proanthocyanidins can be divided into two groups, identified by a letter and a number (Weinges *et al.*, 1968; Thompson *et al.*, 1972): types A and B. Trimeric proanthocyanidins are divided in two categories: types C and D. The proanthocyanidin dimers and some of the trimers have been fully identified. Isolation and separation of (+)-catechin, (-)-epicatechin, dimeric, trimeric, oligomeric, and condensed procyanidins is possible (Ribéreau-Gayon *et al.*, 2000).

The procyanidins have been intensively studied by groups led by Weinges *et al.* (1968) and by Haslam *et al.* (1975; 1977). It is clear that a widely distributed family of procyanidins is the B series; they may be regarded as dimers of (+)-catechin and (-)-epicatechin units, whose major members are B1–B4.

Cantos *et al.* (2002) identified the following flavan-3-ols in red and white table grape cultivars by liquid chromatography-mass spectroscopy (LC-MS): (+)-catechin, (+)-gallocatechin, (-)-epigallocatechin, procyanidin B1, procyanidin B2, procyanidin B4, and procyanidin C1. The total amount of flavan-3-ols ranged from 18 (in Napoleon) to 109 (in Flame) mg/kg fresh weight in red cultivars, while in the white cultivars it was in the order of 57 (in Dominga) to 81 (in Moscatel Italica) mg/kg fresh weight.

The contribution of flavan-3-ols to the total phenolics is greater in the white cultivars than the red (Cantos *et al.*, 2002).

In a study by Souquet *et al.* (1996), the degradation products released by thioacidolysis of Merlot skin extract showed that (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, and (-)-epigallocatechin are the major constitutive units of grape skin tannins. (+)-Gallocatechin and (-)-epigallocatechin gallate were also detected.

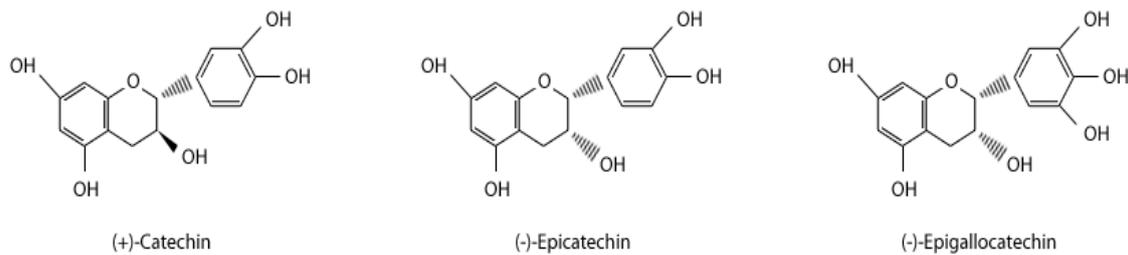


Figure 2.8 (+)-Catechin, (-)-epicatechin and (-)-epigallocatechin (Shoji *et al.*, 2008).

(+)-Catechin is the major flavan-3-ol in skins and grape seeds of both Semillon and Ugni Blanc in the early period (stage 1) and its concentration decreases during ripening. Dimer B1 is always the primary dimer in the skin (De Freitas & Glories, 1999). Bourzeix *et al.* (1986) found that dimer B2 is generally the predominant dimer (38%) in grape seeds, followed by dimer B1 (29%) and dimer B4 (21%). Dimer B1 is the predominant dimer in grape skin (64%). The level of (+)-catechin was found to be about four times superior to that of (-)-epicatechin in the skins.

2.3.1.2 Flavonols

Flavonols are yellow pigments that occur mainly in the skins of both red and white grapes (Ribéreau-Gayon *et al.*, 2000). Flavonols, although colourless, contribute to wine colour as anthocyanin copigments (Asen *et al.*, 1972; Boulton, 2001). The flavonols are found in both red and white grapes in the glycoside form in the vacuoles of epidermal tissue (Ribéreau-Gayon *et al.*, 2000).

Flavonols are products of the flavonoid biosynthetic pathway, which also yields anthocyanins and condensed tannins in grapes (Mattivi *et al.*, 2006). Flavonols are very important for their antioxidant properties and other biological activities (Makris *et*

al., 2006). They are generally considered to act as UV protectants and free-radical scavengers (Flint *et al.*, 1985; Smith & Markham, 1998).

In grapes, the most common flavonols are kaempferol, quercetin and myricetin (Ribéreau-Gayon *et al.*, 2000). According to Cantos *et al.* (2002), the flavonol content makes a greater contribution to the total phenolic content in white cultivars than in red cultivars.

2.3.2 The role of flavonoids in browning

According to Sapis *et al.* (1983), the content of phenolic substrates is of prime importance in the enzyme activity of browning. As mentioned earlier, it seems that the concentration of phenolic compounds is more closely related to the browning of juice and wines than the enzymatic activities of a given grape cultivar (Macheix *et al.*, 1991).

Lee & Jaworski (1986) reported that the combined content of (+)-catechin and (-)-epicatechin in some white grape cultivars are closely correlated with the rate of browning of the grapes. Lee & Jaworski (1988) found that (+)-catechin and (-)-epicatechin had the fastest rate of browning in white grapes, reaching a maximum within 6 h. Procyanidin B2 and B3 were initially slow, but increased with time, reaching a maximum at 48 h.

Browning potential was calculated for 15 white grape varieties grown in New York. A high correlation between browning potential and actual browning was observed (Lee & Jaworski, 1988).

Simpson (1982) reported that monomeric (+)-catechins and dimeric procyanidins, despite their relatively low concentrations, are important indicators of the browning susceptibility of white wines. Browning susceptibility of wines appears to be mostly related to their flavan-3-ol content (Cheynier *et al.*, 1988). Oxidised (-)-epicatechin solutions were found to be highly coloured compared to those derived from phenolic acids (Lee & Jaworski, 1988; Oszmianski & Lee, 1990). Grape must oxidative browning is greatly enhanced by the addition of flavan-3-ols (Ricardo-da-Silva *et al.*, 1991). During fermentation, it was observed that increased flavonoid content from an

extraction of flavan-3-ol, (+)-catechins and derivatives increased the browning capacity in wines (Singleton & Cilliers, 1995).

It is clear that flavonoids and more specifically, the flavan-3-ol content may contribute to the browning susceptibility of a grape cultivar. Determining the individual phenolic compounds in the berry skin at harvest could possibly be used as an indicator, to predict the browning potential of a grape cultivar.

2.3.3 Non-flavonoids

Non-flavonoids are represented in grapes and wine by the following phenolic acids and their derivatives: benzoic acid (C₆–C₁) and cinnamic acid (C₆–C₃) (Boulton *et al.*, 1996). The hydroxybenzoic and hydroxycinnamic acids are predominant in the pulp of white wine grape cultivars although the total phenolic content in the pulp is usually low Fernández de Simón *et al.* (1992). Ribéreau-Gayon *et al.* (2000) found that the non-flavonoids are the main phenolic components in the flesh, where the concentrations of the other phenolic compounds are very low.

The most important benzoic acid in wine grapes is gallic acid (Boulton *et al.*, 1996). The other benzoic acids most commonly found in grapes are protocatechic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid (Boulton *et al.*, 1996). The major source of gallic acid is the hydrolysis of (-)-epicatechin gallate (Boulton *et al.*, 1996). The hydroxycinnamic acids caffeic, *p*-coumaric, and ferulic acids are mainly esterified with tartaric acid to form caftaric, coutaric, and fertaric acids (Boulton *et al.*, 1996). Cantos *et al.* (2002) identified cafteric acid and *p*-coumaric acid in three white table grape cultivars (Superior Seedless, Dominga, and Moscatel Italica) and in four red cultivars (Flame Seedless, Red Globe, Crimson Seedless and Napoleon). There were no significant differences in the amounts of hydroxycinnamic acids between red and white cultivars.

2.3.4 Relationship between phenolic concentration and maturity

The possible relationship between grape maturity and phenolic compound concentration has been studied by a few researchers. Singleton & Esau (1969) could not find a correlation between berry °Brix and polyphenol content. No relationship

between the TSS in the berry and the total phenolic concentration in the skins of ripening Shiraz and Cabernet Sauvignon grapes could be found (Pirie & Mullins, 1977). The total phenolic and anthocyanin levels of Shiraz increased rapidly from one week after véraison and continued with maturity before reaching stability at a very mature stage (Pirie & Mullins, 1980).

Cultivar differences also seem to play a big part in the accumulation of phenolic compound concentration with maturity. The total phenolic concentration per gram of berry weight varies with cultivar (Singleton, 1966). Patterns of phenolic substances are considerably influenced by the genetics of the grapevine (Singleton & Trousdale, 1983). Seasonal, regional, and environmental factors influence the quantity and rate of accumulation as well as the maximum amount of phenolic concentration (Lee & Jaworski, 1989; Ribéreau-Gayon *et al.*, 2000).

Singleton (1966) found that there was a general trend downward in total phenolic compound concentration per unit weight of berry as the berry developed toward maturation. The total phenolic content per berry, however, actually increased rather rapidly over a considerable portion of the development and ripening period. The decrease can be due to an increase in berry weight. Although the concentrations of different phenolic compounds decreased, the total phenolic content per berry increased. During the last month of ripening the total phenolic content per berry remained quite constant, but it could decrease at high maturity levels (Boulton *et al.*, 1996).

Comparisons between different studies are very difficult because different measurements and different techniques are used. The main difference between red and white varieties, in terms of their total phenolic compound composition is that red grapes contain anthocyanins and white grapes do not.

Ribéreau-Gayon *et al.* (2000) showed that the phenolic compounds of white and red grapes followed the same trend of accumulation/breakdown; phenolic compounds increased in the skin but decreased in the seed. Czochanska *et al.* (1979) found that the highest concentration of flavan-3-ols were present at around véraison. The level then decreased to a more or less steady level. The flavanols (+)-catechin and (-)-epicatechin remained stable right through the ripening process. Lee & Jaworski

(1989) also found that the flavan-3-ols and proanthocyanidins increased sharply at véraison and then decreased to their lowest concentration at harvest. Kennedy *et al.* (2002) and Downey *et al.* (2003) observed that (+)-catechin in the skin decreased rapidly from véraison, while there was an increase in level of (-)-epicatechin (Downey *et al.*, 2003).

In the cultivars Tinto Fino and Cabernet Sauvignon, the level of (+)-catechin monomers in the wines decreased with increasing grape maturity. While (+)-catechin decreased with maturity, other flavan-3-ol components such as (-)-epicatechin and proanthocyanidin dimers and trimers increased in concentration in more mature fruit. Of the many factors that influence flavonoid content and composition of a grape cultivar, the site and season are the most important (Pérez-Magariño & González-San José, 2004).

2.3.5 Relationship between the phenolic concentration and crop load

From an oenological point of view, cluster thinning may result in an increased grape quality, especially in the compounds related to wine colour (Peña-Neira *et al.* 2007).

Berry size of table grape varieties is much larger than that of wine grape varieties. Du *et al.* (2012) compared four wine grape cultivars: Cabernet Sauvignon, Cabernet Franc, Merlot, and Cabernet Gernischt, and four table grapes cultivars: Muscat, Red Globe, *Vitis labruscana* (Kyoho), and Milk grape with each other. Eight grape varieties were studied; the concentrations of the phenolic, flavonoid, anthocyanin, and resveratrol content were compared. The table grapes had lower total phenolic content, flavonoids and total anthocyanins and less antioxidant capacity. The larger berries and in most cases larger crop of the table grape varieties are the main reasons for this diluted effect of phenolic content in table grapes.

The effect of pruning severity on quercetin and (+)-catechin content in berry skin of cv. Blaufrankisch (*Vitis vinifera* L.) was studied over 3 years. The quercetin content has been shown to be highly dependent on the light exposure of the berries in which it accumulates. An increase in node number linearly decreased skin (+)-catechin, and it is suggested that the decrease was caused by increased yield per vine (Beslic *et al.*, 2010).

2.3.6 The post-véraison berry

According to Lang & Thorpe (1989), referring to the post-véraison berry, when the cell membranes are in good order the tissues will be extremely turgid and the berries hard, as indeed is the case just prior to the onset of ripening. The author suggests that after the onset of ripening a grape berry may probably be more accurately thought of as a small bag of sugary water rather than as a heterogeneous and complex plant tissue. Lang & During (1991) proposed that the decline in firmness with maturity is due to a decline in turgor caused by a substantial loss of compartmentation of the berry mesocarp cells. The general belief in the table grape industry regarding the influence of maturity on browning and work done by Wolf (1996) and Vial *et al.* (2005) has strengthened this hypothesis. On the other hand, Krasnow *et al.* (2008) reported that membrane integrity and cell viability, assessed by fluorescein diacetate fluorescent staining of the berry pulp and confocal microscopy imaging, clearly demonstrated that mesocarp cells stay viable throughout development and ripening of grape berries. This study was further supported the research of Fontes *et al.* (2011), in which individual cells were isolated from pulp tissue of fully ripened grape berries through enzymatic digestion. Flow cytometry and bright-field, epifluorescence and confocal microscopy confirmed that cells were viable, complex, structurally intact and physiologically active, and able to incorporate fluorescent sugars. The intactness of the plasma membrane and the intricate acidic vacuolar apparatus confirmed that berry softening during ripening is not strictly associated with loss in membrane integrity. Lastly, Vicens *et al.* (2009) reported on changes in cell walls of Shiraz during ripening and over-ripening; moderate changes were observed in skin cell walls during ripening. Modifications in skin cell walls could be considered restrained compared to what is generally described in other fruits or other tissues in grape.

2.4 Regal Seedless and Thompson Seedless

The two cultivars researched in this study were Regal Seedless and Thompson Seedless.

Regal Seedless

Regal Seedless was originally known as Regent Seedless (1991–1984). It was developed and patented by the ARC Nietvoorbij Research Institute (South Africa). The variety ripens in the early to mid-season window. Regal Seedless has large berries for a seedless cultivar; its natural size is ± 7 g/berry. Bunches are well-filled, needing little or no thinning. The berries have a strong skin. Occasionally complaints are received about an astringent taste in the skin. This might be a problem when grapes are not fully matured. There are no problems with uneven berry size. Regal Seedless is a highly fertile variety and is capable of very good production with very little labour inputs by the grower (Van der Merwe, 2012).

Thompson Seedless

Thompson Seedless or Sultana is an old cultivar that has been used as breeding parent for many seedless cultivars. Originally, Sultana was used for wine and raisins, but it has been cultivated as a table grape in South Africa since 1982/3. The berry weight of Thompson Seedless is 5.2 g/berry (after treatment with gibberellic acid). It is a mid-season variety, and one of the most important table grape cultivars in many countries, e.g., California, Chile, and Australia (Van der Merwe, 2012).

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CHAPTER 3

The effect of harvest/berry maturity and crop load on browning of Thompson Seedless and Regal Seedless

3.1 Introduction

Table grapes are an aesthetic product and the financial impact of browning can therefore be detrimental. The factors contributing to the development of browning of white table grapes have not yet been adequately established. Thompson Seedless and Regal Seedless are white seedless grape cultivars which are sensitive to browning.

Although a correlation between the maturity levels of harvested grapes and the occurrence of browning seems to exist, it is still not clear whether maturity levels are the actual contributing factor (Wolf, 1996; Vial *et al.*, 2005). Vial *et al.* (2005) found that on the variety Princess (California), skin browning is directly related to fruit maturity. Wolf (1996) found that Waltham Cross bunches harvested at higher TSS levels show increased browning. Singleton (1966) observed that there generally appears to be a greater browning tendency in the juice in the riper harvests of white grapes.

A hypothesis concerning the cause of browning is that there is a disruption of cellular membranes which allows mixing of polyphenol oxidase (PPO) and the phenolic substrates (Sapis *et al.*, 1983). In the development of browning, the fundamental step is the enzymatic oxidation of phenolic compounds to *o*-quinones, catalysed by PPO (polyphenol oxidase) (Singleton, 1987). These products then undergo further reactions, leading to the formation of brown pigments (Macheix *et al.*, 1991; Liyanage *et al.*, 1993).

Increasing solubilised PPO activity and total crude PPO activity, in several white grape varieties, from the beginning of véraison (Sapis *et al.*, 1983), and the fact that there is a breakdown of cells in grape berries after the onset of ripening (Lang & Daring, 1991) provided motivation for this part of the trial, namely, to determine the effect of maturity on browning of the two cultivars Thompson Seedless and Regal Seedless.

Crop load has also been implicated as having an effect on browning; it has been claimed to affect the keeping quality of grapes (Dokoozlian & Hirschfeldt, 1995). A grapevine has the capacity to produce a given weight of fruit and to bring that fruit to

normal maturity within a given number of degree–days of heat, which is characteristic for the cultivar and the climatic region (Winkler, 1958). The capacity of a vine to ripen a grape harvest is largely determined by the total leaf area. The percentage of the total leaf surface also influences the grape-ripening capacity and the initiation of the fruit primordium (Kliewer & Weaver, 1971).

Over-cropped vines are generally characterised by delayed fruit maturation (Weaver *et al.*, 1961), small berries, reduced vine growth, higher sugar/acid ratio at a given fruit maturity, poor fruit colouration and softness of berry texture (Somkuwar & Ramteke, 2006). Cultivation practices used to maximise grape quality parameters or yield can have a significant effect on the source–sink relationships of grapevines (Williams, 1996). Some of the obvious effects of over-cropping are less colour (in the case of red varieties), lower pH and poorer quality (Weaver *et al.*, 1957). Over-cropping negatively influences must and wine composition, specifically must malic acid, wine colour, ash and tartaric acid content (Bravdo *et al.*, 1985). Over-cropping on the cultivar had a negative impact on fruit coloration and concentrations of proline and arginine in berry juice compared to the controls (Kliewer & Weaver, 1971). This data may be explained by source–sink relationships. As the crop size decreases, there is a greater supply of photosynthate available for the remaining fruits.

The aims of this study was to establish the effect of (1) harvest maturity and (2) crop load on the berry browning of Regal Seedless and Thompson Seedless.

3.2 Materials and methods

3.2.1 Experimental vineyards

3.2.1.1 Regal Seedless

The trial was conducted in a commercial vineyard of Regal Seedless, grafted on Ramsey, on the farm Carpe Diem in the Hex River Valley during the 2008 and 2009 seasons. This block was planted in 1999 on a double gable trellising system with a 3 × 1.2 m, with vines developed in alternative directions, and was subjected to micro-irrigation. In 2009, a difference in crop load levels could not be achieved due to vigorous growth that led to extreme over-thinning of bunches. Only five maturity

levels, determined based on different picking dates, were therefore considered as treatments in the 2009 season (n=30). In 2010, the trial was shifted to an alternative block on the farm Moselle in the Hex River Valley. This Regal Seedless/Ramsey block was planted in 1999 on a double gable trellising system with a vine spacing of 3 × 1.5 m, and was subjected to micro-sprinkler irrigation.

3.2.1.2 Thompson Seedless

A commercial vineyard of Thompson Seedless grafted on Ramsey, on the farm Mountain Lodge in the Hex River Valley, was used during the 2008 and 2009 season. The block was planted in 2001 on a flat roof trellising system with vine spacing of 3 × 2 m vine, and was subjected to drip irrigation. Crop load and maturity levels were considered for both the 2008 and 2009 seasons in the Thompson Seedless vineyard (2 x 6, n=12).

3.2.2 Experimental design and treatments

Both the Regal Seedless and Thompson Seedless trial were laid out in a complete random block design. Three predetermined crop load levels were applied to Regal Seedless as treatments (Table 3.1). Thompson Seedless is a less fertile variety than Regal Seedless. The higher crop load, i.e. the third treatment of 7.5 bunches/m² could therefore not be achieved and only two crop load treatments were applied (Tables 3.2). The crop load treatments were replicated six times (3 x 6, n = 18), except for Regal Seedless in the 2009 season. Crop load was expressed as bunches per square metre (bunches/m²), with an industry norm of 4 to 5.5 bunches/m², which gives 40 000 to 55 000 bunches/ha. This calculates to between 24 and 33 tons per hectare (t/ha), which serves as an industry average. Just after crop loads were applied, bunches were shortened as follows: Regal Seedless (14 cm) and Thompson Seedless (10 cm).

In 2008, the two cultivars were each harvested three times during the season, as follows:

- (1) early: ±16°Brix;
- (2) optimum maturity: ±18°Brix, and
- (3) late maturity: ±20°Brix.

For each of the harvesting stages the following codes were assigned HM1; HM2 and HM3).

In 2009 an additional harvest date, advanced maturity, were added for Thompson Seedless and grapes were harvested four times during the season:

- (1) early: $\pm 16^{\circ}$ Brix,
- (2) at the optimum maturity: at $\pm 18^{\circ}$ Brix,
- (3) late maturity: $\pm 19^{\circ}$ Brix and
- (4) advanced maturity: $\pm 20^{\circ}$ Brix

Four harvest maturities stages were assigned by codes HM1, HM2, HM3 and HM4.

Regal Seedless were harvested at five different harvest maturities with a very late harvest maturity $> \pm 20$ Brix also evaluated: HM1-HM5. In 2010 only Regal Seedless were harvested at only three harvest maturities: HM1-HM3 (Table 3.5 & 3.6).

In the Regal Seedless block each experimental unit consisted of 6 vines. In 2008, three different crop load treatments (TMT1–TMT3) were applied. In the 2009 season, no difference in crop load could be achieved; hence only maturity levels were taken into account.

In the Thompson Seedless block crop load and maturity levels were considered for both the 2008 and 2009 seasons in the Thompson Seedless vineyard (n=12). Only two different crop load treatments (TMT1–TMT2) could be achieved for both seasons. An experimental unit consisted of 4 vines.

Since each experimental unit represented a repetition, for each harvest date (or maturity level) 18 samples were obtained for Regal Seedless and 12 samples for Thompson Seedless.

A quantity of 4.5 kg of grapes was harvested randomly from all the vines in each experimental unit and packed in a box. An Uvasys SO₂ sheet (Grapetek; Cape Town, South Africa) was immediately placed on top of the grapes, as per commercial farming procedures. A paper moisture absorbing material (MAM) sheet (Superior Packaging; Cape Town, South Africa) was placed on top of the SO₂ sheet. A 52 x 2

mm box liner (Astrapak; Johannesburg, South Africa) was used. The boxes were taken to Experico (storage facilities) within 2 h after packing the grapes. There, the grapes were placed in a cold room at -0.5°C , to be stored for 5 weeks. Thereafter, the grapes were kept for another week at 7°C .

3.2.2.1 Regal Seedless

The aim of shortening the Regal Seedless bunches to 14 cm (mentioned above) was to achieve ± 600 g bunches. The different treatments (TMTs) aimed to give more or less the following crop loads (Table 3.1):

TMT1: 24 t/ha

TMT2: 36 t/ha

TMT3: 45 t/ha

Each of the three crop load treatments was repeated six times (3×6 , $n = 18$).

On the farm Carpe Diem, an experimental unit consisted of 6 vines (2008 & 2009).

On the farm Moselle, an experimental unit consisted of 5 vines (2010).

Table 3.1 Crop load treatments applied to a Regal Seedless trial on the farms Carpe Diem and Moselle in the Hex River Valley: 2008 & 2010 seasons.

Regal Seedless crop load (bunches/m ²)		
Treatment 1	Treatment 2	Treatment 3
4.5	6.0	7.5

3.2.2.2 Thompson Seedless

Thompson Seedless bunches were shortened to an estimated 600 g per bunch just after crop load was applied. The different treatments (TMTs) aimed to give more or less the following crop loads (Table 3.2):

TMT1: 24 t/ha

TMT2: 36 t/ha

Each crop load treatment was repeated 6 times (2×6 , $n = 12$).

Each experimental unit consisted of 4 vines.

Table 3.2 Crop load treatments applied to a Thompson Seedless trial on the farm Mountain Lodge in the Hex River Valley: 2008 & 2009 seasons.

Thompson Seedless crop load (bunches/m ²)	
Treatment 1	Treatment 2
4.5	6.0

3.2.3 Bunch sampling for browning analysis

Sampling of grape browning was carried out by Experico (Stellenbosch). Bunches were randomly picked at different maturity levels to obtain homogenous TSS (Table 3.6 & 3.9). The boxes were taken to Experico (storage facilities) within 2 h after packing the grapes. At Experico the grapes were placed in a cold room at -0.5°C, to be stored for 5 weeks. Thereafter, the grapes were kept for another week at 7°C. TSS values was determined after six weeks of storage using a hand-held Westover Brix refractometer (RHB-32ATC; Westover, Owatonna, MN, USA). The TA content of the grapes was determined by titration with NaOH to pH (phenolphthalein indicator) (Tables 3.6 & 3.10). The occurrence of browning, as established by Experico standardised protocols (Fourie, 2009), was quantified and expressed as a percentage on a weight/weight basis, weight of brown berries to the total weight of berries (abbreviated hereafter as wt/wt %).

3.2.4 Statistical analysis

One carton of grapes of approximately 4.5 kg served as an experimental unit. The statistical software programme SAS version 8.2 (SAS, 1999) was used for all statistical analyses. The data were subjected to an analysis of variance (ANOVA) using the general linear means procedure. The Shapiro–Wilk test (Shapiro & Wilk, 1965) was performed to test for non-normality. Student's t-test least significant differences (LSD) were calculated at a 5% significance level ($P \leq 0.05$) to compare the means of the treatments.

3.3 Results and discussion

3.3.1 Regal Seedless

3.3.1.1 The effect of crop load on browning of Regal Seedless

In 2008, no significant effect of crop load on internal or external browning was found. In 2010, there was an increase in internal browning at the higher crop load (Table 3.3). The level of internal browning was very low level, compared to external browning, and would not have had a commercial impact on the value of the grapes. In 2008, with higher crop load, there was a slight, but insignificant, decrease in external browning. In 2010, there was an increase in external browning with higher, but insignificant, increase in crop load (Table 3.3).

Table 3.3 Impact of crop load on the occurrence of browning of Regal Seedless: 2008 & 2010 seasons.

Treatment*	Internal browning (wt/wt %)		External browning (wt/wt %)	
	2008	2010	2008	2010
TMT1	0.64a	0.00b	26.34a	47.50a
TMT2	0.94a	0.12b	23.44a	53.25a
TMT3	0.78a	0.58a	22.90a	58.46a
LSD**	0.58	0.26	8.36	15.72

*TMT1 = 4.5 bunches per m²; TMT2 = 6 bunches per m²; TMT3 = 7.5 bunches per m².

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at ($P \leq 0.05$).

Similar results of increased external browning with increased crop load were obtained for Regal Seedless in Riebeek-Kasteel in the 2002 season (J. Avenant, personal communication, 19-06-2012). It seems that higher crop load therefore can contribute to increased levels of internal and external browning, but the site and season also has an influence. The effect does not seem to be consistent, however, and does not seem to be the contributing factor to both internal and external browning.

Crop load did not consistently influence browning. The reason for this could be that the crop load did not consistently affect berry ripening. For example, in 2008, the different crop loads did not have a significant effect on both the TSS (°Brix) and TA

of Regal Seedless (Table 3.4). Winkler and Williams (1939) defined a grapevine as being well balanced and not over-cropped when the vine brings its fruit from flowering to a given °Brix, with a given summation of degree days of heat, which is constant for a given variety. To be defined as 'over-cropped', there must be a delay in sugar accumulation (Dokoozlian & Hirschfeld, 1995). Although the heavier crop load in the 2008 season showed a slight decrease in sugar accumulation, it was not significant (Table 3.4).

The high density spacing of vines (2777 vines/ha) combined with vigorous vegetative growth of the block of Regal Seedless resulted in straggly bunches. In 2009, the thinning effect of bunches was even higher, which resulted in more straggly bunches; hence the decision was made not to use the crop load treatment for that year. These bunches have fewer berries per bunch, probably resulting in lower bunch weights (not measured). Although bunches per vine were sufficiently altered, the total crop weight per vine was probably not affected, to the extent that a clear distinction between different crop loads and accumulation of sugar could be obtained. In the 2010 season, a different trial block (on the farm Moselle) with a less dense vine spacing of 2222 vines/ha was used in the trial. In this block, bunches set with more berries per bunch than in previous seasons at the farm Carpe Diem. The total bunch weight per vine was higher, resulting in crop loads that significantly affected the accumulation in TSS (°B). It was expected that the highest crop load (TMT 3) (would show a significant decrease in TA (%), but this not the case (Table 3.4).

Table 3.4 Effect of crop load treatment on TSS (°B) and TA (%) for Regal Seedless: 2008 & 2010 seasons.

Treatment*	TSS (°B)		TA (%)	
	2008	2010	2008	2010
TMT1	17.31a	20.47a	0.49a	0.48a
TMT2	16.83a	19.63a	0.50a	0.53b
TMT3	16.67a	18.08b	0.51a	0.55b
LSD*	1.06	0.90	0.04	0.04

*TMT1 = 4.5 bunches per m²; TMT2 = 6 bunches per m²; TMT3 = 7.5 bunches per m²

**LSD Least significant difference (P ≤ 0.05)

Values followed by different letters indicate significant difference at (P ≤ 0.05)

3.3.1.2 The effect of maturity level on browning of Regal Seedless

The different harvest maturities had a significant impact on the TSS and TA of Regal Seedless (Table 3.5 – 3.6). In 2009 it was decided to measure five harvest maturities, because crop load treatments could not be achieved in the vineyard.

Table 3.5 Average TSS levels at different harvest maturities of Regal Seedless.

TSS (°Brix)			
HM*	2008	2009	2010
HM1	15.48a	14.96a	16.73a
HM2	16.76b	17.66b	20.03b
HM3	18.57c	18.72c	21.49c
HM4		20.24d	
HM5		20.67d	
LSD**	0.60	0.81	1.03

*HM Harvest Maturity

**LSD Lowest significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 3.6 Average TA levels at different harvest maturities of Regal Seedless.

TA (%)			
HM*	2008	2009	2010
HM1	0.59a	0.90a	0.58a
HM2	0.45b	0.67b	0.49b
HM3	0.45b	0.55c	0.48b
HM4		0.53c	
HM5		0.47d	
LSD**	0.03	0.045	0.038

*HM Harvest Maturity

**LSD Lowest significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

External browning

External browning occurred in all three seasons on Regal Seedless at a level that would have had a negative impact on the commercial value of the grapes (Table 3.7). Net-like browning was the main contributor to total external browning.

In 2008, external browning did not change significantly with harvest maturity, although net-like browning significantly decreased from HM 2 (16.76°Brix and 0.45 TA %) to HM 3 (18.57°Brix and 0.45 TA %).

Furthermore, in 2009, total external browning decreased significantly HM 3 (18.72°Brix and 0.55 TA %) to HM 4 (20.24°Brix and 0.53 TA %).

In 2010, a significant decrease was observed between HM 1 (16.73°Brix and 0.59 TA) and HM 2 (20.03°Brix and 0.50 TA). The decreasing trend in total external browning was mainly due to net-like browning, which showed a significant decrease with increased berry maturity. Other contributors to external browning, i.e., contact, mottled, physical and friction browning, did not show a constant significant trend. In 2010, only friction browning had a commercial impact as part of the total external browning with a significant increase with harvest maturity (Table 3.7).

During the 2009 season, Witbooi & Fourie (2009) also found that Regal Seedless produced in the Paarl region showed a significant decrease in net-like browning from Harvest 1 (15.7°Brix and 0.6 TA %) to Harvest 2 (19.2°Brix and 0.6 TA %). However, the same trend was not observed in 2009 in the Hex River Valley. It was therefore, concluded that the development of total external browning of the Regal Seedless populations did not show a specific trend.

Sebola & Fourie (2010), however, found that in Regal Seedless from the Paarl area higher levels of contact browning (the main contributor to total external browning), occurred on grapes harvested more mature. Friction browning, on the other hand, contributed most towards total external browning in the Hex River Valley and total external browning significantly decreased with harvest maturity, mainly because of a decrease in friction browning. Avenant (2010) found that a delay in harvest time resulted in decreased net-like browning of Regal Seedless. This is in accordance with the results obtained in my study.

Although it seems that there is a tendency for total external browning to decrease with harvest maturity, differences in the pattern and extent of its occurrence do occur between regions and seasons. These differences exceed the impact of harvest maturity. The decrease in total external browning is mainly due to a decrease of net-

like browning with harvest maturity. Other forms of external browning (mottled, contact, friction and physical) showed contradictory trends between seasons and did not seem to be related to harvest maturity at all.

The results of Vial *et al.* (2005), who found that cv. Princess showed increased levels of external browning at higher maturity, and of Wolf (1996), who found that Waltham Cross bunches harvested at higher TSS levels showed increased browning, convinced growers to commence harvesting at minimum TSS values in order to reduce the risk of browning. Results of the research carried out in the present study, however, indicate that early harvesting could actually increase the browning problem of Regal Seedless and that varietal differences in terms of browning can occur. Research on a single white seedless variety should therefore not be used as a guideline for other varieties.

Internal browning

In all three seasons, the level of internal browning was very low, with low potential commercial impact (Table 3.7). Low levels of internal browning compared to external browning have also been observed on Regal Seedless by both Witbooi & Fourie (2009) and Sebola & Fourie (2010). It is also reported that, in one trial, carried out in the 2009 season, internal browning of Regal Seedless increased with harvest maturity in Paarl area but it was not observed in another trial carried out in the Hex River Valley (Witbooi, 2009).

In 2010, internal browning first increased and then decreased with harvest maturity in the Paarl trial, while in the Hex River Valley no significant trend was observed. It seems that an increase in harvest maturity does not have a consistent effect on internal browning of Regal Seedless. Vial *et al.* (2005) ascribed a high level of internal browning found in a Princess block, compared to another block in a consecutive year, to the poor vigour of first mentioned block. All Regal Seedless blocks used in this trial were growing optimally, with no lack of vigour. It is possible that optimum growth of Regal Seedless block was part of the reason for low internal browning.

Table 3.7 Impact of harvest maturity on browning of cv. Regal Seedless in the Hex River Valley: 2008, 2009 & 2010 seasons.

	Sugar (°B)			External browning			Internal browning			Net-like browning			Friction browning			
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	
HM*																
HM1	15.48a	14.96a	16.73a	24.61a	26.33a	62.85a	0.68ab	0.17a	0.36a	9.27a	20.84a	41.43a				13.95b
HM2	16.76b	17.66b	20.03b	26.44a	28.51a	47.58b	0.54b	0.07a	0.20a	8.23a	26.56a	1.89b				39.80a
HM3	18.56	18.71c	21.49c	21.63a	25.76a	48.77b	1.13a	0.22a	0.14a	0.11b	22.80a	0.53b				36.20a
HM4		20.23d			15.17b			0.33a			9.84b					
HM5		20.67d			13.77b			0.21a			6.65b					
LSD**	0.60	0.81	1.04	7.62	7.38	11.35	0.58	0.26	0.23	4.59	7.17	6.31				9.143

*HM Harvest maturity

**LSD Lowest significant difference at ($P \leq 0.05$)Values followed by different letters indicate significant difference at ($P \leq 0.05$).

From the results obtained it is concluded that internal browning levels of Regal Seedless are generally very low and are not impacted by harvest maturity. In future research, Regal Seedless blocks with poor vigour can be researched, to see if excessive sunlight will influence internal browning.

3.3.1.3 Optimum maturity for harvesting Regal Seedless

The minimum export standard for Regal Seedless is TSS 16°Brix and a sugar–acid ratio of 30:1 (DAFF, 1999). The cultivar must comply with both mentioned indices or with a minimum maturity of 17°Brix (DAFF, 1998–1999). Fraser (2007) studied the phenolic content of Regal Seedless at different maturities and found that the eating quality of Regal Seedless improved from 17°Brix upwards. The recommended maturity level for Regal Seedless that emerged from that study was between 17 and 19°Brix (Fraser, 2007). Avenant (2007) also recommended that Regal Seedless should not be harvested before the grapes reached a sugar concentration of 17°Brix, because an increase in sugar content disguises the astringent taste of Regal Seedless. Gütschow (2000) determined the effect of different harvest maturities on the long term storage of Regal Seedless and recommended that harvest should be at 18°Brix.

3.3.2 Thompson Seedless

3.3.2.1 The effect of crop load on browning of Thompson Seedless

In 2008, the heavier crop load of 6 bunches/m² significantly increased external browning. Internal browning, however, did not differ significantly. The greatest contributor to external browning in the 2008 season was contact browning and friction browning, both of which significantly increased with heavier crop load (Table 3.8).

In 2009, neither internal nor external browning was significantly affected by crop load. External browning showed only a slight decrease with the heavier crop load. The greatest contributor to external browning was mottled browning, which also showed a slight decrease with heavier crop load (Table 3.8).

Table 3.8 Impact of crop load on browning of Thompson Seedless in the Hex River Valley: 2008 & 2009 seasons.

Treatment*	Internal browning (wt/wt %)		External browning (wt/wt %)		Contact browning (wt/wt %)
	2008	2009	2008	2009	2008
TMT1	1.22a	1.34a	7.33a	4.67a	3.29a
TMT2	1.18a	1.03a	14.32b	3.67a	6.63b
LSD**	0.42	1.35	4.74	3.02	2.38

*TMT1: 4.5 bunches/m²; TMT2: 6 bunches/m²

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at ($P \leq 0.05$)

Results of this study lead to the conclusion that crop load does not significantly influence browning of Thompson Seedless. The Thompson Seedless vineyard was growing optimally and one can argue that the crop load of 6 bunches/m² was not high enough to have a significant effect on decreasing the ripening process and possibly affect browning and overall quality.

3.3.2.2 The effect of maturity level on browning of Thompson Seedless

The different harvest maturities had a significant impact on the TSS and TA of Thompson Seedless (Table 3.9 – 3.10). In 2009 it was decided to measure four harvest maturities, because crop load treatments could not be achieved in the vineyard.

Table 3.9 Average TSS levels at different harvest maturities of Thompson Seedless.

HM*	TSS (°Brix)	
	2008	2009
HM1	16.38a	15.02a
HM2	18.24b	16.42b
HM3	20.45c	17.84bc
HM4		18.59c
LSD**	0.91	1.17

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at ($P \leq 0.05$)

Table 3.10 Average TA levels at different harvest maturities of Thompson Seedless.

TA (%)		
HM*	2008	2009
HM1	0.57a	0.83a
HM2	0.52b	0.68b
HM3	0.49b	0.61c
HM4		0.53d
LSD**	0.04	0.04

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at ($P \leq 0.05$)

External browning

External browning of Thompson Seedless increased with harvest maturity in both seasons. Total external browning values were high enough to have a significant commercial impact on the value of the grapes. In 2008 the biggest contributor to external browning was contact browning and friction browning. Both showed a significant increase from the second harvest. The greatest contributors to external browning in 2009 were mottled, contact and friction browning (Table 3.11).

Witbooi & Fourie (2009) found that total external browning for Thompson Seedless from Saron area slightly decreased with harvest maturity although not significantly. The reason for this decrease was the significant decrease of contact browning. Thompson Seedless from Paarl in the same season increased in total external browning from HM1 to HM2 and then decreased again from HM2 to HM 4. Again, the greatest contributor was contact browning, which also first increased and then decreased.

Sebola & Fourie (2010) found a significant decrease in total external browning with harvest maturity for Thompson Seedless in Saron area. The greatest contributor was contact browning, which significantly decreased with harvest maturity. In the same season (2010), Thompson Seedless from Paarl area did not show a significant trend with harvest maturity.

In the present study external browning of Thompson Seedless significantly increased with harvest maturity and this result is in accordance with Vial *et al.* (2005) which

showed that external browning on Princess increased with harvest maturity and Wolf (1996) who found the same for Waltham Cross. However, research on Thompson Seedless by Witbooi & Fourie (2009) and Sebola & Fourie (2010) did not confirm the results of the present study. There seem to be differences in browning accumulation between varieties, production areas and different seasons. External browning is not consistently affected by harvest maturity.

Although in the present study an increase in external browning with harvest maturity was consistent, it was not confirmed by other studies on Thompson Seedless. In the majority of other relevant studies, contact browning seemed to be the greatest contributor to total external browning on Thompson Seedless. Contact browning did not show a significant trend with harvest maturity. External browning was a greater contributor to browning of Thompson Seedless than internal browning. Internal browning occurrence was relatively low and would have had minimum commercial impact. Internal browning however did show a tendency to increase with harvest maturity, but it was not consistent.

Internal browning

In the 2008 season, internal browning was not affected by harvest maturity, but in the 2009 season internal browning increased from HM1 to HM2, although it was < 2.5% (Table 3.11). Witbooi & Fourie (2009) found that internal browning, for Thompson Seedless from Saron (2009 season), significantly increased from 21.5°Brix to 22.6°Brix. Thompson Seedless from the Paarl (2009 season) showed no significant increase with harvest maturity. Sebola & Fourie (2010) found that internal browning for Thompson Seedless from Saron (2010 season) significantly increased from 19.2°Brix to 20.3°Brix and again to 22.2°Brix. Thompson Seedless from Paarl (2010) did not increase significantly with harvest maturity. Occurrence of internal browning did not consistently increase with harvest maturity. Its extent was relatively low and would not have had significant commercial impact.

Table 3.11 Impact of harvest maturity on browning of Thompson Seedless in the Hex River Valley: 2009 & 2010 seasons.

	Total internal browning (wt/wt %)		External browning (wt/wt %)							
	2008	2009	2008			2009				
			Contact	Friction	Total	Mottled	Contact	Friction	Total	
HM*										
HM1	1.06a	0.2b	0.11b	0.16b	1.47b	0.00b	0.11b	0.03b	0.66b	
HM2	1.38a	2.1a	7.01a	7.84a	17.13a	0.00b	0.00b	0.62b	1.71b	
HM3	1.16a	2.43a	7.22a	2.09b	12.81a	0.07b	1.97a	2.62a	6.10a	
HM4		0				7.08a	0.09b	0.00b	8.23a	
LSD**	0.71	1.10	2.90	1.93	4.78	1.37	0.87	1.05	2.52	

*HM Harvest Maturity

**LSD Lowest significant difference at ($P \leq 0.05$)

Values followed by different letters indicate significant difference at ($P \leq 0.05$)

3.4 Conclusions

It can be concluded that the optimum harvest maturity of Regal Seedless is between 17 and 19°Brix and that growers should be very cautious to harvest Regal Seedless too early because of the high risk of net-like browning at early maturity.

3.4.1 External browning

Total external browning for Regal Seedless grapes was very high and would definitely have had a negative impact on the commercial value of the grapes. Crop load did not have any significant effect on external browning. Regal Seedless showed a tendency to decrease in total external browning with harvest maturity. The main reason for this was that net-like browning, which made the biggest contribution to total external browning, decreased with harvest maturity for all three seasons.

The recommendation to the table grape industry is that external browning on Regal Seedless remains the biggest contributor to berry browning. Net-like browning seemed to be the biggest contributor to total external browning. Net-like browning decreased with harvest maturity. The industry practice to harvest Regal Seedless at very early maturity levels to try and decrease browning can actually increase

external browning. The recommendation is to harvest between the optimum maturity levels of between 17 and 19°Brix. Further research need to be done on the possible causes of net-like browning and possible controls of this phenomenon. Further research should be done to study the effect of gibberellic acid (GA3) applications on net-like browning on Regal Seedless.

Total external browning of Thompson Seedless occurred in higher percentages than internal browning. Crop load did not consistently influence external browning of Thompson Seedless. External browning however increased with harvest maturity in both seasons. Contact browning was the biggest contributor to total external browning on Thompson Seedless.

3.4.2 Internal browning

Total internal browning in Regal Seedless was very low. Indications were that a higher crop load led to increased internal browning. Furthermore, in two out of three seasons, internal browning increased with harvest maturity, however only significantly in 2008 season. The occurrence of internal browning was, however, not consistent, and also not the most important contributor to browning.

Total internal browning in Thompson Seedless was also very low, for all crop load treatments. Crop load did not consistently influence internal browning, internal browning was not affected by harvest maturity during the 2008 season, but it did increase with harvest maturity in the 2009 season.

Internal browning in both Regal Seedless and Thompson Seedless was much less than anticipated. It is therefore recommended that future research should focus more on external browning than on internal browning.

The hypothesis of this study was that over cropping and higher maturity could lead to a disruption of cellular membranes, which allows mixing of PPO and phenolic substrates and would then be the possible cause of browning. From the research results it became evident that browning was a far more complex and enigmatic problem. Seasonal and varietal differences as well as differences between different production areas contributed to the complexity of the problem. Crop loads as applied

in the vineyard did not create the dramatic delayed maturation we have hoped for in this study. For future research these treatments of 4.5, 6 and 7.5 bunches/m² should be increased. The risk of increasing these treatments and possibly also bunch lengths to induce a heavier crop load can be that research is not parallel with the norms of the industry anymore.

The complexity of browning was confirmed in this study. Season, variety and location all influence the accumulation of browning. This study addressed the table grape industry perception that maturity is related to browning. Interestingly, it was found here that early harvesting of Regal Seedless could actually increase browning and that over-cropping did not significantly influence browning. It is important to bear in mind here that over-cropping can lead to other quality problems.

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CHAPTER 4

**The effect of harvest/berry maturity
and crop load on phenol concentration
of Thompson and Regal Seedless**

4.1 Introduction

There has been considerable interest in determining the composition and contents of phenolic compounds in wine grape cultivars (Sapis *et al.*, 1983; Lee & Jaworski, 1988; Macheix *et al.*, 1991; Souquet *et al.*, 1996). The aim of most of these studies was to understand the physiological role of phenolic compounds in wine grape cultivars. Very few similar studies have been undertaken on table grape cultivars. Recently, some research was carried out to determine the anti-oxidant activity and health promoting properties of some table grape varieties (Lago-Vanzela *et al.*, 2011; Lutz *et al.*, 2011).

Enzyme activity with phenols as substrate and the possible influence on wine quality is considered important. Among the chemical reactions that occur during the different phases from grapes to wine, the oxidation of phenolic compounds is responsible for profound modifications of the initial plant polyphenols. This includes the appearance of more or less condensed brown substances characterising the browning process. Browning in the early stages of juice or must preparation before any treatment is primarily the result of enzymatic oxidation of phenolic substances naturally present in the grapes. The browning of musts and wines depends on numerous parameters. The three most important are the availability of oxygen, the nature and levels of oxidisable substrates, and phenoloxidase activity during the first phases of wine making (Macheix *et al.*, 1991).

As the occurrence of browning in Regal Seedless and, to a lesser extent, Thompson Seedless is a great problem for the South African table grape industry, it has been speculated that this chemical reaction can contribute to berry browning.

The process consists of two phases: the first is enzymatic and the second is a spontaneous polymerisation. The first phase is characterised by a conversion of monophenols to diphenols (Kruger *et al.*, 1999), whereafter the diphenols are oxidised by means of hydroxylation enzymes and *o*-quinone through PPO located in the cytoplasm (Liyanage *et al.*, 1993; Macheix *et al.*, 1991). The substrates most rapidly oxidised by grape PPO are catechin, epicatechin, caffeic acid and catechol or 4-methylcatechol (Cash *et al.*, 1976; Harel & Mayer 1971).

The second phase is characterised by spontaneous polymerisation during which quinones are polymerised, which leads to the formation of melanin (brown pigments) that is responsible for/characteristic of the brown colour/browning phenomenon (Sapis *et al.*, 1983).

TSS and TA are characteristic ripening parameters (Baiano *et al.*, 2012). A correlation has often been sought between these features and polyphenol oxidase activity. PPO activity is an important factor in grape and grape juice browning and, consequently, in must preparation and wine production (Traverso-Rueda & Singleton 1973). It is reported, however, that it is not frequently correlated with the browning of different grape cultivars (Romeyer *et al.*, 1985; Sapis, *et al.*, 1983). However, in experiments carried out, it was found that when excess phenolic substrate was supplied, there was an increase in browning in experimental tests and in grape juice (Lee & Jaworski, 1987 ;Sapis *et al.*, 1983).

It seems that the concentration of phenolic compounds is more closely related to the browning of juice and wines than the enzymatic activities of a given grape cultivar (Macheix *et al.*, 1991). It is possible that a high flavan-3-ol content of grapes can be a very important indicator of susceptibility to browning.

Lee & Jaworski (1988) found that catechin and epicatechin had the fastest rate of browning in white grapes. Browning potential has been calculated taking into account the browning index of each phenolic compound (phenolic acids, catechin, epicatechin and procyanidins and its concentration in the grape juice (Lee & Jaworski, 1988). The browning potential of 12 selected white grape cultivars showed a high correlation with the degree of browning as measured. These results showed that it is possible to predict the browning potential of the white grape juice or wine, if you determine the individual phenols in the grapes after harvest (Lee & Jaworski, 1988). The amount and types of phenols present within a cultivar is genetically controlled causing grape cultivars to differ over a considerable range (Boulton *et al.*, 1996).

Considerable variations are generally observed in the levels of phenolic compounds in grape berries during growth and maturation (Lee & Jaworski, 1989). The total

phenolic content decreases steadily during the last stages of growth and maturation of white grape cultivars.

Studies by Wolf (1996) and Vial et al., (2005) showed that cultivars Princess (white seedless) and Waltham Cross (white seeded) both increased in browning / external browning as fruit reached higher maturity.

The aims of this study was

To establish the impact of (1) harvest maturity and (2) crop load on the phenolic concentration in the berry skin of Regal Seedless and Thompson Seedless.

Furthermore, to establish if there is a correlation between phenolic concentration development and berry browning of Regal Seedless and Thompson Seedless and to make a comparison between phenolic concentration in the berry skins of Regal Seedless and Thompson Seedless.

The hypothesis was that, with over-cropping and harvest maturity, membrane integrity would degenerate, leading to polyphenols and PPO increasingly coming in contact resulting in increased browning.

4.2. Material and methods

4.2.1 Experimental vineyards

Refer to 3.2.1 for experimental vineyard information.

4.2.2 Experimental design and treatments

Refer to 3.2.2 for experimental design and treatments.

4.2.3 Phenol analysis

A random sample of 20 berries per box was taken from each carton. Berries were stored at -20°C until further analysis. Berries were removed without damaging the skin. The pedicel of berries remained intact. Berries were removed from the -20°C freezer and left overnight in a refrigerator. The next morning berries were peeled and then homogenized. Phenol analysis was determined by high-performance liquid chromatography (HPLC). Analyses were conducted for grapes from the 2008 and

2009 seasons on 20 randomly selected berries at each harvest maturity level from each replication. An Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA) HPLC instrument equipped with diode array (DAD) and refractive index (RID) detectors with same manufacturing specifications was used. A (polystyrene/divinylbenzene) reversed phase column (PLRP-S, 100Å, 150 x 4.6 mm, 3 µm) from Polymer Laboratories (Ltd) (Shropshire, UK) protected with a guard cartridge (PLRP-S, 10 X 4.6 mm) (Polymer Laboratories (Ltd), Shropshire, UK) with the same packaging material was used. The mobile phase consisted of Solvent A: 1.5% aqueous o-phosphoric acid (Sigma-Aldrich, Kempton Park, South Africa) and Solvent B: 80% HPLC grade acetonitrile (Sigma-Aldrich)/20% Solvent A, establishing the following gradient: 0 min: A 94%, B 6%; 73 min: A 69%, B 31%; 78 to 86 min: A 38%, B 62%; and 90 min: A 94%, B 6%. The flow rate was 1 mL/min. An injection volume of 100 µL was used. After every ten samples, a blank and a known standard mix were run. The column temperature was held at 35°C and the system was equilibrated for 15 min at the starting solvent conditions between samples. This was done to ensure a stable baseline, consistent retention times and validity of the standard curves. The spectra were recorded from 250 nm to 400 nm. Data processing was done with Chemstation software (Hewlett Packard, Waldbronn, Germany). Table 4.1 contains a list of the compounds analysed with this method with their retention times in minutes.

Table 4.1 Phenols analysed by HPLC-DAD: retention times on a reversed phase column (PLRP-S; Polymer Laboratories, UK).

Compound	Retention time (min)
Gallic acid	6.60
Gallocatechin	16.40
Epigallocatechin	22.00
Catechin	27.10
B1	27.90
Epicatechin	31.30
B2	39.70
Epicatechingallate	54.12
Polyphenols	78.00

4.2.4 Statistical analysis

The statistical software programme SAS version 8.2 (SAS, 1999) was used for all the statistical analysis. The data were subjected to an analysis of variance (ANOVA) using the general linear means procedure. The Shapiro–Wilk test (Shapiro & Wilk, 1965) was performed to test for non-normality. Student's t-Least Significant Differences (LSD) were calculated at a 5% significance level ($p \leq 0.05$) to compare the means of the treatments.

4.3 Results and discussion

4.3.1 Concentration of phenolic compounds in grape skins

4.3.1.1 Regal Seedless

In the 2008 season, quantities of epicatechin in the skin of Regal Seedless were higher than catechin (Table 4.3). Singleton & Trousdale (1983), Lee & Jaworski (1986, 1987 and 1989) have also found that in some white cultivars the epicatechin content is higher than catechin. Others studies have found that the catechin content is higher than epicatechin (Montealegre *et al.*, 2006; Lago-Vanzela *et al.*, 2011). In the 2009 season, the situation was the opposite: catechin content was slightly higher than epicatechin (Table 4.3). Lee & Jaworski (1989); Singleton & Trousdale (1983) and De Freitas & Glories (1999) all confirmed that phenolic levels can differ greatly due to seasonal variability. The influence of weather conditions during the two seasons on the grape ripening process could be one of the main reasons for these differences in seasons (Jackson & Lombard, 1993; Kliewer, 1970 and Singleton & Esau, 1969).

In both seasons in the skin of Regal Seedless, procyanidin B1 was present in the highest concentration of all the flavan-3-ols (Table 4.3). The content of procyanidin B2 was only tested in the 2009 season, when it had a lower concentration than B1, but it was more abundant than epicatechin and almost equal to catechin (Table 4.3). This was in accordance with the findings of Bourzeix *et al.* (1986), De Freitas & Glories (1999) and Lago-Vanzela *et al.* (2011), who suggested that dimer B1 is the major procyanidin found in grape skins.

In 2008, gallic acid and epigallocatechin made a small contribution to the total flavan-3-ol content in the skin of Regal Seedless (Table 4.4). The concentration of gallic acid was also very low for both the 2008 and 2009 seasons (Table 4.4). Low gallic acid content has also been confirmed in an extensive study carried out by Liang *et al.* (2011) on 344 European grape (*Vitis vinifera*) cultivars; they found that hydroxybenzoic acids, including both gallic and vanillic acids, were present in relatively low concentrations. The mean content of gallic acid was 0.006 mg/g.

4.3.1.2 Thompson Seedless

In the 2008 season, similar to in the case of Regal Seedless, the quantity of epicatechin was higher than catechin in the skin of Thompson Seedless. The opposite occurred in 2009, when catechin was higher than epicatechin. In 2009, procyanidin B1 had the highest concentration of all flavan-3-ols (Table 4.5). Epigallocatechin and gallic acid also constituted a small contribution to the total flavan-3-ol content in the skin of Thompson Seedless. Again, in both seasons, gallic acid was present in very low concentrations (Table 4.6)

Comparative weather data from the weather station in De Doorns (Hex River Valley) shows that there were big differences in rain fall between the two seasons. The 2009 season had a total 264,8mm of rain compared to 145.2 in the 2008 season. Abnormal high rain in November 2009 of 121.4mm was very high for the Hex River Valley which is a winter rainfall area (Table 4.2). This abnormal high rainfall could have influenced the difference between the phenolic content between the two seasons. As previously mentioned seasonal, regional, and environmental factors influence the quantity and rate of accumulation as well as the maximum amount of phenolic concentration (Lee & Jaworski, 1989; Ribéreau-Gayon *et al.*, 2000).

Table 4.2 Comparative weather data for the 2008 & 2009 seasons (Source: ARC-ISCW).

Season	Values	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Total
2008	Sum of Rain mm	1,6	11,4	67,4	44,4	0,4	20	145,2
	Ave Temp	14,1	17,0	18,1	21,9	23,0	23,0	19,6
	Max Temp	22,5	25,3	25,8	29,6	31,1	31,2	27,7
	Min Temp	5,6	8,6	10,5	14,4	15,4	15,1	11,7
2009	Sum of Rain mm	117,6	11,0	121,4	10,4	0,0	4,4	264,8
	Ave Temp	12,2	17,1	19,2	22,0	22,1	23,3	19,3
	Max Temp	19,3	25,3	27,0	30,1	30,3	32,5	27,4
	Min Temp	5,0	9,2	11,8	14,1	14,1	15,1	11,5

4.3.2 Phenolic concentration and maturity

4.3.2.1 Regal Seedless

In Regal Seedless, in both seasons, the catechin concentration increased, except for in the case of the last harvest date (H4) in 2009 (Table 4.3). The epicatechin concentration significantly increased with maturity in both seasons (Table 4.3). Downey *et al.* (2003) also reported that epicatechin increased in more mature wine grapes.

In 2008, procyanidin B1 did not show a significant trend. In 2009, however, it increased significantly with maturity (Table 4.3). This is in agreement with results of a study of Romeyer *et al.* (1985), which showed that procyanidins B1, B2 and B4 increased continuously during maturation in four *Vitis vinifera* varieties. The concentration of procyanidin B2 was only measured in 2009 for Regal Seedless (Table 4.3) and Thompson Seedless (Table 4.5). Its concentration in Regal Seedless showed no significant trend while Thompson Seedless a significant decrease with maturity was observed. In the 2008 season, polyphenols and total polyphenols for Regal Seedless both increased with harvest maturity, but not in the 2009 season (Table 4.3).

Great seasonal differences in the concentrations of catechin, procyanidin B1, polyphenols and total polyphenols occurred between the 2008 and 2009 seasons. In

all instances, 2009 had higher values than 2008. A comparison of the browning incidence for the two seasons showed that internal browning and total external browning occurred in very similar percentages between the two seasons, but there was a great difference in the occurrence of net-like browning between the 2008 and 2009 seasons. Net-like browning of Regal Seedless was much higher in 2009 than in 2008.

Table 4.3 Concentration (mg/kg skin) of phenols of Regal Seedless at different harvest maturities in the Hex River Valley: 2008 & 2009 seasons.

	Epicatechin		Catechin		B1		B2	Polyphenols		Total polyphenols	
	2008	2009	2008	2009	2008	2009	2009	2008	2009	2008	2009
HM*	2008	2009	2008	2009	2008	2009	2009	2008	2009	2008	2009
HM1	6.51b	6.42c	4.02b	35.52b	15.26a	66.19b	40.54a	970.10b	2401.60a	996.50b	2551.00a
HM2	8.44b	6.95c	5.57a	70.19a	17.83a	78.54a	40.99a	1047.40b	2252.50a	1079.60b	2450.00a
HM3	18.44a	9.51b	6.39a	62.57a	19.25a	82.95a	44.72a	1490.20a	2384.30a	1539.00a	2651.00a
HM4		12.69a		36.57b		80.23a	44.87a		2480.06a		2680.00a
LSD**	2.40	1.59	1.29	14.14	5.42	11.59	6.21	240.65	251.00	244.79	595.21

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 4.4 Concentration (mg/g skin) of more phenols of Regal Seedless at different harvest maturities in the Hex River Valley: 2008 & 2009 seasons.

	Gallic acid		Gallocatechin	Epigallocatechin
	2008	2009	2008	2008
HM*	2008	2009	2008	2008
HM1	0.12b	0.52b	1.23b	1.96ab
HM2	0.18b	0.57ab	1.10b	1.69b
HM3	0.27a	0.66a	2.03a	2.38a
HM4		0.62ab		
LSD**	0.08	0.11	0.47	0.46

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

4.3.2.2 Thompson Seedless

In 2008, the catechin concentration did not show any increasing or decreasing pattern (Table 4.5). In 2009, the catechin levels significantly decreased from the first harvest time (H1) (Table 4.5). This finding is in agreement with other studies, where it was found that the concentration of catechin in the berry skin decreased during grape maturation (De Freitas & Glories, 1999; De Freitas *et al.*, 2000). In 2008, the epicatechin concentration of Thompson Seedless significantly decreased but in the 2009 season it significantly increased with maturity (Table 4.5). Lee & Jaworski (1989) have found that epicatechin increased until véraison and then decreased to the lowest concentration at harvest.

In the 2008 season, both the polyphenols and total polyphenols of Thompson Seedless decreased significantly with harvest maturity. In the 2009 season, the polyphenols first increased and then decreased significantly, while total polyphenols did not show a significant trend (Table 4.5). Singleton (1966) also reported on a general downward trend in total phenols per unit weight of berry, this was mainly due to growth of the berry. However the total phenol content per berry actually increased. Giovanelli & Brenna (2007) showed total phenolics in three Italian grape varieties showed a progressive increase up to veraison, then a decrease occurred until harvest.

In 2008, Thompson Seedless showed no significant trend for procyanidin B1 concentration, however, in 2009 its concentration decreased significantly (Table 4.5). This is in agreement with the findings of Czochanska *et al.* (1979) and Jordão *et al.* (2001) who suggested that procyanidin B1 exhibited highest concentration in the early stages of development followed by a decrease in the last stages of maturation.

The values of catechin and procyanidin B1 were higher in 2009 than in 2008. However, total external browning was higher in 2008 than in 2009. Pirie & Mullins (1977) and Singleton & Esau (1969) found no relationship between TSS in berry and total polyphenols in the berry skin. Fraser (2007) determined the total phenolics of whole berries as well as per berry weight of Regal Seedless. Total phenols per berry weight first increased and then decreased. The total phenols per berry weight

showed no specific trend. When seeking correlations between TSS and total phenols per berry and per berry weight each season differed from another.

Table 4.5 Concentration (mg/g skin) of phenols of Thompson Seedless at different maturity levels in the Hex River Valley: 2008 & 2009 seasons.

	Epicatechin		Catechin		B1		B2	Polyphenols		Total polyphenols	
	2008	2009	2008	2009	2008	2009	2009	2008	2009	2008	2009
HM*											
HM1	11.01a	3.32b	2.75a	18.42a	6.49a	19.86a	10.28a	499.02a	341.45ab	513.85a	532.00a
HM2	2.60c	3.69b	2.47a	4.87b	5.44a	11.23b	7.25b	186.93b	414.56a	202.90b	438.00a
HM3	3.46b	3.77b	2.86a	6.50b	5.57a	13.78b	6.93b	276.77b	259.26b	294.15b	297.00a
HM4		4.61a		6.24b		15.46b	8.27b		284.44b		310.00a
LSD**	0.77	0.69	0.64	3.54	1.54	4.36	1.984	190.04	88.60	194.43	196.32

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 4.6 Concentration (mg/g skin) of more phenols of Thompson Seedless at different maturity levels in the Hex River Valley: 2008 & 2009 seasons.

	Gallic acid		Gallocatechin	Epigallocatechin
	2008	2009	2008	2008
HM*				
HM1	0.30a	0.082a	0.98a	3.28b
HM2	0.03a	0.167a	0.87a	5.44a
HM3	0.19a	0.021a	1.08a	5.61a
HM4		0.101a		
LSD**	0.30	0.16	0.32	0.88

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

4.3.3 Phenolic concentration and crop load

Peña-Neira *et al.* (2007) found that the flavan-3-ol, catechin, and certain flavanols resulted in higher concentration in berry skins from low yield plants. A study by Fanzone *et al.* (2011) on Malbec grape skins and seeds reported that cluster thinning significantly affected catechin, catechin-3-gallate, procyanidin and anthocyanin content of grape skins.

The impact of crop load on phenol concentration was much less than the impact of harvest maturity. For Regal Seedless, in the 2009 season, no definite difference in crop load could be (practically) achieved in the vineyard, hence that year is not reported on.

In the 2008 season, for Regal Seedless, epigallocatechin, gallic acid and gallo catechin concentrations decreased significantly with heavier crop load. For the major phenols, however no significant trend was observed (Table 4.7). The rest of the phenols as well as total polyphenols increased with crop load, but it was not significant, except procyanidin B1 which decreased with crop load, but not significantly.

For both 2008 and 2009 seasons, for Thompson Seedless, the effect of crop load on phenol concentration was not significant (Table 4.8). The reason why no significant differences in phenol concentration could be observed might be because the crop load effects on Regal Seedless and specifically Thompson Seedless was not sufficiently extreme enough to enforce adequate differences between phenolic compounds development. For future studies, greater crop loads and longer bunch lengths should be applied to achieve higher crop loads.

Table 4.7 Concentration of phenols of Regal Seedless at different crop load levels in the Hex River Valley: 2008 season.

	B1	Epigallo- catechin	Gallic acid	Gallo- catechin	Catechin	Epicatechin	Epicatechin- gallate	Polyphenols	Total polyphenols
T1	21.23a	2.23a	0.26a	1.99a	5.90a	12.63a	0.78a	1298.90a	1341.00a
T2	16.36a	1.89b	0.18b	1.17b	4.87a	11.23a	0.45a	1057.10a	1091.20a
T3	14.81a	1.85b	0.16b	1.34b	5.11a	11.78a	4.69a	1151.60a	1182.90a
LSD*	10.09	0.34	0.08	0.56	1.95	10.09	4.27	269.49	282.63

*LSD = Least significant difference ($P \leq 0.05$).

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 4.8 Concentration of phenols of Thompson Seedless at different crop load levels in the Hex River Valley: 2008 & 2009 seasons.

2008	Epigallo-catechin	Gallic acid	Gallo-catechin	Catechin	Epi-catechin	B1	Polyphenols	Total polyphenols
T1	4.75a	0.16a	0.91a	2.50a	3.25a	5.05a	261.60a	276.30a
T2	5.00a	0.15a	0.99a	2.90a	4.26a	6.72a	365.36a	383.10a
LSD*	1.28	1.28	0.23	0.80	3.68	1.95	250.25	257.30
2009		Gallic acid	B2	Catechin	Epi-catechin	B1	Polyphenols	Total polyphenols
T1		0.08a	8.35a	9.15a	3.95a	15.24a	344.34a	381.11a
T2		0.12a	7.93a	8.45a	3.81a	14.71a	303.96a	360.00a
LSD*		0.11	0.86	2.41	0.67	4.35	95.61	110.31

*LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

4.3.4 Comparison between Thompson Seedless and Regal Seedless: phenol concentration and browning

A comparison of the concentrations of catechin, epicatechin, procyanidin B1 and procyanidin B2 of Regal Seedless and Thompson Seedless revealed that the concentrations of catechin and epicatechin in Regal Seedless skin were much higher than in Thompson Seedless (Table 4.9). A similar observation was made for procyanidin B1, for both the 2008 and 2009 seasons, and procyanidin B2, in the 2009 season (Table 4.10). A comparison of browning incidence with harvest maturity between these two varieties revealed that external browning of Regal Seedless occurred in much higher percentages than that of Thompson Seedless (Table 4.11). In this study, the much higher flavan-3-ol and procyanidin content in the skin of Regal Seedless could be the reason for the higher external browning, compared to Thompson Seedless. According to Sapis *et al.* (1983), the concentration of phenolic compounds is more closely related to the browning of juice and wines than the enzymatic activities of a given grape cultivar.

Lee & Jaworski (1988) defined maximum browning (the highest absorbance) as the browning index (BI). The highest values in their study were identified for procyanidin B3, procyanidin B2, catechin and epicatechin. Simpson (1982) reported that monomeric catechins and dimeric procyanidins, despite their relatively low concentrations, are important indicators of browning susceptibility of white wines.

Upon comparing the phenol concentration of Regal Seedless and Thompson Seedless, it is clear that the different flavan-3-ols tested were all present in higher concentration in the skin of Regal Seedless than in Thompson Seedless (Tables 4.9 & 4.10).

It can be concluded that the concentration of flavan-3-ols in the skins of white seedless cultivars may therefore be an indication of the risk of the cultivar's susceptibility to external browning.

Table 4.9 Comparison of the concentration of catechin and epicatechin of Regal Seedless and Thompson Seedless with harvest maturity in the Hex River Valley: 2008 and 2009.

	Catechin				Epicatechin			
	Thompson Seedless	Regal Seedless						
HM*	2008	2008	2009	2009	2008	2008	2009	2009
HM1	2.75a	4.02b	18.42a	35.52b	11.01a	6.51b	3.32b	6.42c
HM2	2.47a	5.57a	4.87b	70.19a	2.60c	8.44b	3.69b	6.95c
HM3	2.86a	6.39a	6.50b	62.57a	3.46b	18.44a	3.77b	9.51b
HM4			6.24b	36.57b			4.61a	12.69a
LSD**	0.64	1.29	3.54	11.59	0.77	2.40	0.69	1.59

*HM Harvest Maturity

**LSD = Least significant difference ($P \leq 0.05$).

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 4.10 Comparison of the concentration of procyanidin B1 and B2 of Regal Seedless and Thompson Seedless with harvest maturity in the Hex River Valley: 2008 & 2009 seasons.

	Procyanidin B1				Procyanidin B2	
	Thompson Seedless	Regal Seedless	Thompson Seedless	Regal Seedless	Thompson Seedless	Regal Seedless
HM*	2008	2008	2009	2009	2009	2009
HM1	6.49a	15.26a	19.86a	66.19b	10.28a	40.54a
HM2	5.44a	17.83a	11.23b	78.54a	7.25b	40.99a
HM3	5.57a	19.25a	13.78b	82.95a	6.93b	44.72a
HM4			15.46b	80.23a	8.27b	44.87a
LSD**	1.54	5.42	4.36	11.59	1.984	6.21

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)

Values followed by different letters indicate significant difference at $P \leq 0.05$

Table 4.11 Comparison of the browning incidence of Regal Seedless and Thompson Seedless with harvest maturity in the Hex River Valley: 2008 & 2009 seasons.

	Internal browning				External browning			
	2008		2009		2008		2009	
	Thompson Seedless	Regal Seedless						
HM1	1.06a	0.68ab	0.2b	0.17a	1.47b	24.61a	0.66b	20.84a
HM2	1.38a	0.54b	2.1a	0.07a	17.13a	26.44a	1.71b	26.56a
HM3	1.16a	1.13a	2.43a	0.22a	12.81a	21.63a	6.10a	22.80a
HM4			0.0	0.33a			8.23a	9.84b
HM5				0.21a				6.65b
LSD**	0.71	0.58	1.10	0.26	4.78	7.62	2.52	7.17

*HM Harvest Maturity

**LSD Least significant difference ($P \leq 0.05$)Values followed by different letters indicate significant difference at $P \leq 0.05$

4.4 Conclusions

Regal Seedless data showed that there is a general tendency for the flavan-3-ols to increase with harvest maturity. In Thompson Seedless there was a tendency for the flavan-3-ols to decrease with harvest maturity. For both 2008 and 2009 season procyanidin B1 was the most abundant of all phenols that was tested in the skin of Regal Seedless. In 2009, procyanidin B1 was also the most abundant in the skin of Thompson Seedless. Gallic acid content was very low both seasons and both cultivars.

The impact of crop load on phenolic compound concentration was less than expected. In the 2008 season, for Regal Seedless, epigallocatechin, gallic acid and gallic acid concentrations decreased significantly with heavier crop load. For the major phenols, however no significant trend was observed. For both 2008 and 2009 seasons, for Thompson Seedless, the effect of crop load on phenol concentration was not significant. Phenolic compound concentration could not be correlated with browning incidence for both cultivars in both seasons.

Big seasonal differences for the concentration of catechin, procyanidin B1, polyphenols and total polyphenols occurred between the 2008 and 2009 season. This however could not be correlated to the occurrence of browning. Internal

browning and external browning occurred at very similar percentages in both seasons. In 2009 net-like browning was however much higher than in 2008.

In Thompson Seedless values of catechin and procyanidin B1 were higher in 2009 than 2008. Total external browning was however higher in 2008 than in 2009 and internal browning did not differ much between the two seasons. Although there were seasonal differences between concentrations of flavan-3-ols per cultivar, these differences could not be correlated with browning incidence.

Comparing phenolic compound concentration of Regal Seedless and Thompson Seedless with each other showed interesting results. In Regal catechin, epicatechin, procyanidin B1 and procyanidin B2 were the most abundant. These flavan-3-ols were also prominent in the skin of Thompson Seedless. The concentration of these flavan-3-ols were however much higher in Regal Seedless than in Thompson Seedless. If we compare the browning occurrence of the two cultivars with each other it is clear that external browning occurred in much higher percentages in Regal Seedless than in Thompson Seedless for both seasons. We can conclude that the concentration of flavan-3-ols in the berry skin of a white seedless grape cultivar may be a possible indicator of the browning susceptibility of this variety.

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CHAPTER 5

Summary and conclusions

5.1 Introduction

Out of this study it is clear that total external browning for Regal Seedless and Thompson Seedless occurred in much higher percentages than internal browning. External browning occurred in higher percentages in Regal Seedless than in Thompson Seedless. Regal Seedless showed a tendency to decrease in total external browning with harvest maturity. The main reason for this was that net-like browning, which made the biggest contribution to total external browning, decreased with harvest maturity for all three seasons. Internal browning occurrence was too low to have any commercial impact.

The industry practice to harvest Regal Seedless at very early maturity levels to try and decrease browning can actually increase external browning. The recommendation is to harvest Regal Seedless between the optimum maturity levels of 17–19°Brix and to be careful that Regal Seedless does not over-mature, because of contact and friction browning which can occur at later harvests.

External browning for Thompson Seedless increased with harvest maturity in both seasons. Contact browning was the biggest contributor to total external browning for both seasons. The fact that external browning increased with harvest maturity for both seasons were, however contradictory to other relevant studies.

The analysis of the concentration of phenols in the berry skin of Regal Seedless showed that there is a general tendency for the flavan-3-ols to increase with harvest maturity. In the skin of Thompson Seedless there was a general tendency for the flavan-3-ols to decrease with harvest maturity. For both varieties the development of phenol concentration with maturity could not be correlated with berry browning. Crop load did not have a significant effect on phenol concentration for both cultivars.

The comparison of the flavan-3-ol concentration (catechin, epicatechin, procyanidin B1 and procyanidin B2) of Regal Seedless and Thompson Seedless was very interesting. The comparison was done at different harvest maturities only, because crop load did not have a significant effect. It is clear that the flavan-3-ol concentration were much higher in the skin of Regal Seedless compared to the skin of Thompson Seedless for both the 2008 and 2009 season. If we look at the comparison of

browning incidence with harvest maturity for these two varieties it is clear that external browning for Regal Seedless occurred in much higher percentages than for Thompson Seedless. The conclusion can be made that the concentration flavan-3-ols in the skin of white seedless cultivars may be an indication of the risk of the cultivar's susceptibility to external browning.

The hypothesis of this study was that over cropping and higher maturity could lead to a disruption of cellular membranes and degeneration of membrane integrity, this would allow mixing of polyphenol oxidase and phenolic substrates which would lead to berry browning. It however became evident that berry browning was a far more complex and enigmatic problem. Seasonal and varietal differences contributed to the complexity of the problem. Crop loads as applied in the vineyard did not create the dramatic delayed maturation we have hoped for in this study. For future research these treatments of 4.5; 6 and 7.5 bunches per m² should be increased. The risk of increasing these treatments and possibly also bunch lengths to induce a heavier crop load can however be that research is not parallel with the norms of the industry anymore.

5.2 Conclusions

The complexity of berry browning was confirmed in this study. The season and cultivar influenced accumulation of browning.

1. Total external browning for Regal Seedless showed a tendency to decrease with harvest maturity. The main reason for this was that net-like browning, which made the biggest contribution to total external browning, decreased with harvest maturity for all three seasons. External browning for Thompson Seedless increased with harvest maturity in both seasons. Contact browning was the biggest contributor to total external browning on Thompson Seedless for both seasons.

Total external browning for Regal Seedless and Thompson Seedless occurred in much higher percentages than internal browning.

2. Concentration of phenolic compounds in the berry skin of Regal Seedless showed that there is a general tendency for the flavan-3-ols to increase with harvest maturity. In the skin of Thompson Seedless there was a general tendency for the flavan-3-ols to decrease with harvest maturity.

Crop load did not have a significant effect on phenol concentration for both cultivars.

3. For both cultivars the development of phenol concentration with maturity could not be correlated with berry browning.
4. The comparison of the flavan-3-ol concentration (catechin, epicatechin, procyanidin B1 and procyanidin B2) of Regal Seedless and Thompson Seedless showed that the flavan-3-ol concentration was much higher in the skin of Regal Seedless compared to the skin of Thompson Seedless for both the 2008 and 2009 season. External browning for Regal Seedless occurred in much higher percentages than for Thompson Seedless. The conclusion can be made that the concentration of flavan-3-ols in the skin of white seedless cultivars may be an indication of the cultivar's susceptibility to external browning.

5.3 Recommendations for future research

Future research should focus on external browning. Internal browning was very low in all seasons for both cultivars.

External browning, specifically netlike browning was the biggest contributor on Regal Seedless. Future research into netlike browning and its possible cause could be of great value.

For future studies bigger crop loads and longer bunch lengths should be applied to achieve higher crop loads.