

Manipulation of the taste of Regal Seedless (*Vitis vinifera* L.) table grapes

by

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Master of AgriSciences at Stellenbosch University.*

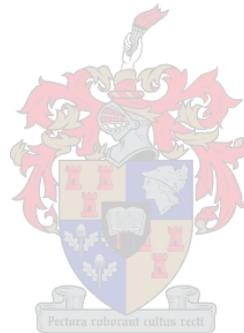
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DECLARATION

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



Name of candidate

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SUMMARY

Regal Seedless is a white, seedless grape which has the potential to become a profitable cultivar for the table grape producer since it has the advantages of early season harvesting and inherently large berries. There is, however, a downside to this cultivar, namely the seasonal occurrence of an unacceptable, astringent taste. This negative taste affects the demand by local and international markets. The astringency perception is due to the presence of phenolic compounds. It is well known that the phenolic composition and concentration change during the ripening of the grape. Different postharvest treatments are applied to fresh fruit like persimmons to remove astringency. These treatments include the use of carbon dioxide, nitrogen and ethanol.

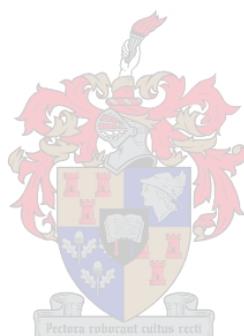
The aim of this study was to determine the optimum maturity level for Regal Seedless where the phenolic concentration is the lowest and the astringent taste acceptable. The use of postharvest treatments to manipulate the taste and the phenolic content, were also investigated. The effect on other quality parameters like total soluble solids (TSS), pH and total titratable acidity (TTA) were also evaluated.

During the maturity study grapes were randomly collected from a three-year-old Regal Seedless vineyard from véraison for a five week period. The postharvest study consisted of three trials: zero-oxygen conditions, an ethanol dip and cold storage duration. The zero-oxygen trial had a 4 x 2 factorial which included two atmospheres (air and nitrogen) in combination with four exposure times (4, 8, 16, 32 hours). During the following season an additional exposure time (64 hours) was added. The ethanol dip trial included five ethanol concentrations (0%, 10%, 20%, 40% and 80%) in combination with two SO₂ pad treatments (with and without). The treatment design was a 5 x 2 factorial. The cold storage trial consisted of three cold storage periods (0, 4, 7 weeks) in combination with a shelf life period (0 or 1 week).

The maturity study emphasized the fact that the total and individual phenolics change in concentration during ripening. The flavanols, which are mainly responsible for astringency, decreased rapidly from véraison, while the flavonols increased at an advanced maturity. With this in mind it was possible to determine the level where the phenolic content was low and the eating quality and astringency acceptable. The role of seasonal variability was evident in this study.

The application of nitrogen and ethanol can be used as alternative methods in seasons where there is a problem with the taste of Regal Seedless. Both of these methods were successful in decreasing the astringent taste, improving the eating quality

and decreasing the concentration of individual and total phenolics, although the decrease was not always that prominent. These treatments might be more effective if the trials are conducted at the commercial maturity (18°Brix) of Regal Seedless rather than on grapes with an advanced maturity. The use of nitrogen and ethanol had no significant influence on TSS, pH and TTA. The present combination of cold storage and shelf life period that are used to simulate the shipping period overseas and the period that the grapes will be on the shelf in the supermarket, is four weeks at -0.5°C and one week at 15°C respectively. The cold storage trial showed that this protocol resulted in the lowest astringency and lowest phenolic concentration.



OPSOMMING

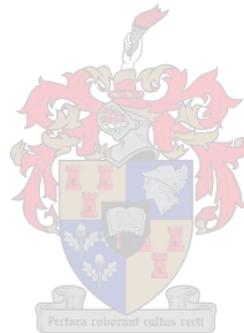
Regal Seedless is 'n wit, pitlose tafeldruif. Die kultivar het die potensiaal om 'n winsgewende kultivar vir die tafeldruifprodusent te word aangesien dit vroeg in die seisoen geoes word en natuurlike groot korrels het. Ongelukkig het die druif 'n probleem, naamlik die seisoenale voorkoms van 'n onaanvaarbare, vrank smaak. Hierdie negatiewe smaak beïnvloed die aanvraag vanaf plaaslike en internasionale markte. Die vrank smaak word veroorsaak deur die teenwoordigheid van fenoliese komponente. Dit is alombekend dat die fenoliese samestelling en konsentrasie in die druif verander tydens rypwording. Verskillende na-oesbehandelings word gebruik op vars vrugte soos kakivrugte (persimmons) om vrankheid te verwyder. Die behandelings sluit in die gebruik van koolstofdiksied, stikstof en etanol.

Die doel van hierdie studie was om die optimale oesrypheid vir Regal Seedless te bepaal, waar die konsentrasie van fenole die laagste en die vrank smaak aanvaarbaar is. Na-oes behandelings om die smaak en die fenoliese inhoud te manipuleer, is ook ondersoek. Die effek op ander kwaliteitsparameters, totale oplosbare vaste stowwe (TOVS), pH en totale titreerbare suurheid (TTS), is ook geëvalueer.

Tydens die oesrypheidstudie, is druiwe ewekansig geoes vanaf 'n drie jaar oue Regal Seedless wingerd, vanaf deurslaan vir 'n vyf week periode. Die na-oes studie het bestaan uit drie proewe: geen-suurstof kondisies, 'n etanol doop en koelopbergings tyd. Die geen-suurstof proef het bestaan uit 'n 4 x 2 faktoriaal wat twee atmosfere (lug en stikstof) ingesluit het in kombinasie met vier blootstellingstye (4, 8, 16, 32 ure). In die daaropvolgende seisoen is 'n addisionele blootstellingstyd (64 ure) bygevoeg. Die etanol doop proef het vyf etanol konsentrasies (0%, 10%, 20%, 40% en 80%) in kombinasie met twee SO₂ behandelings (met en sonder) ingesluit. Die behandelingsontwerp was 'n 5 x 2 faktoriaal. Die koelopbergingsproef het bestaan uit drie koelopbergingsperiodes (0, 4, 7 weke) in kombinasie met 'n rakkertyd (0 of 1 week).

Die oesrypheidstudie het die feit beklemtoon dat die konsentrasie van totale en individuele fenole veranderinge ondergaan gedurende rypwording. Die flavanole, wat hoofsaaklik verantwoordelik is vir vrankheid, het vinnig afgeneem in konsentrasie vanaf deurslaan, terwyl die flavonole toegeneem het by 'n hoë rypheid. Dit was dus moontlik om die oesrypheidsvlak te bepaal waar die fenool inhoud laag en die eetkwaliteit en vrankheid aanvaarbaar is. Die rol van seisonale fluktuasies was sigbaar in die studie.

In seisoene waar daar 'n probeem met Regal Seedless se smaak is, kan stikstofgas of etanol aangewend word as alternatiewe metodes. Albei die metodes was suksesvol om die vransk smaak te verminder, eetkwaliteit te verbeter en die konsentrasie individuele en totale fenole te verlaag, alhoewel die afname nie altyd prominent was nie. Die behandelings sou dalk meer effektief gewees het as die proewe by die kommersiële rypheid (18°Brix) van Regal Seedless uitgevoer was eerder as op driuwe met 'n hoër rypheid. Die gebruik van stikstofgas en etanol het geen betekenisvolle invloed op TOVS, pH en TTS gehad nie. Die huidige koelopberging en rakleef tyd wat gebruik word om die verskeppingsperiode oorsee en die tyd wat die driuwe op die rak in die supermark is te simuleer, is onderskeidelik vier weke by -0.5°C en een week by 15°C . Die koelopbergingsproef het bewys dat die laagste vranskheid en konsentrasie van fenole by dié protokol gevind is.



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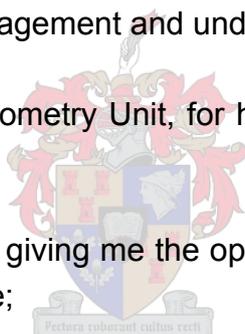
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PREFACE

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the specified journal to which the chapter is submitted for publication.

Chapter 1 **General Introduction and Project Aims**

Chapter 2 **Literature Review**

Phenol development and its relation to astringency perception

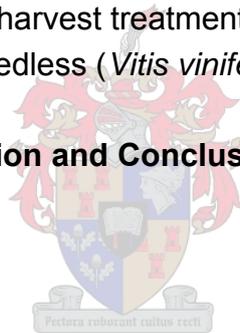
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The effect of fruit maturity on the phenolic content and taste of Regal Seedless (*Vitis vinifera* L.)

Chapter 4 **Research Results**

The effect of postharvest treatments on the phenolic content and taste of Regal Seedless (*Vitis vinifera* L.)

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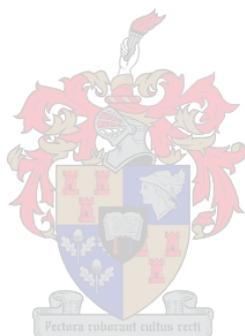
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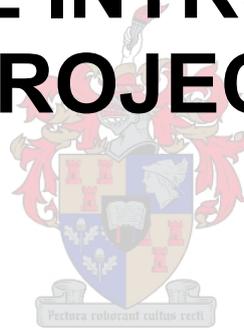
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GENERAL INTRODUCTION AND PROJECT AIMS



GENERAL INTRODUCTION AND PROJECT AIMS

The table grape industry forms a crucial part of the agricultural sector in South Africa. In 2005/06 a total of 51.3 million cartons were exported to overseas markets. This highlights that the external earnings for table grapes are very high. There is a continuous and growing international demand by table grape industries for seedless cultivars of high quality with good characteristics like inherently large berries and good storage ability. Appearance, taste and nutritional value are important aspects that convince consumers to purchase a certain table grape. Taste and nutrition are partly determined by a group of secondary metabolites called phenolic compounds.

Regal Seedless is a popular South African bred table grape cultivar which almost has all of the abovementioned positive properties. However, the cultivar is affected by a sporadic, unacceptable, astringent taste. Astringency is seen as a negative taste in fresh fruit. It is therefore important to determine the source(s) and/or reason(s) for this problem and find solutions. If this problem is not addressed the demand by overseas markets may decrease.

The astringent sensation is mainly due to the presence of phenolic compounds (Joslyn & Goldstein, 1964), in especially the group called the flavonoids (Robichaud & Noble, 1990). The concentration of phenolic compounds in the grape is influenced by environmental factors and viticultural practices. Environmental factors include: locality, climate, seasonal conditions, mineral nutrition of the soil and fruit maturity (Lee & Jaworski, 1989; Garcia et al., 1993; Jackson & Lombard, 1993; Keller & Hrazdina, 1998; Kennedy et al., 2001; Kennedy et al., 2002). Previous research has shown that phenolic content decrease with increasing fruit maturity (Lee & Jaworski, 1989). Viticultural practices include: canopy management, irrigation, growth regulators, pruning, crop load and rootstocks (Jackson & Lombard, 1993; Price et al., 1995; Dokoozlian & Kliewer, 1996; Keller & Hrazdina, 1998). Postharvest treatments with carbon dioxide (CO₂), nitrogen (N₂) and ethanol vapour are applied during storage to decrease the astringency in other fresh produce like persimmons (Matsuo & Ito, 1977; Kato, 1990; Zavrtnik et al., 1999; Arnal & Del Río, 2003).

The aim of this study was to test the effect of fruit maturity and the application of different postharvest practices on the phenolic content and taste of Regal Seedless. The effects of these treatments on other quality variables such as total soluble solids (TSS), pH and total titratable acidity (TTA) were also investigated.

In order to achieve the abovementioned goals, the following approaches were followed:

1. The choice of a suitable Regal Seedless vineyard;
2. *Maturity trial*: harvesting of grapes at different maturity levels from véraison;
Zero-oxygen trial: postharvest application of 100% nitrogen gas for different lengths of time;
Ethanol dip trial: postharvest application of different ethanol concentrations;
Cold storage trial: combination of different cold storage and shelf life periods;
3. Determine the effect of fruit maturity on TSS, TTA and pH;
4. Sensory evaluation of grapes for astringency, skin tenacity and eating quality by a tasting panel;
5. Determine the effect of different trials on the total and individual phenolic content, spectrophotometrically and via HPLC analysis.

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LITERATURE REVIEW

PHENOL DEVELOPMENT AND ITS RELATION TO ASTRINGENCY PERCEPTION



LITERATURE REVIEW

2.1 INTRODUCTION

South Africa is one of the major exporters of table grapes in the world. Thus, table grapes are of great economic importance. During the 2002/03 season 46.7 million cartons were exported overseas and during the 2003/04 season these numbers increased to 53.2 million cartons. This shows that the external earnings for table grapes are very high. There was however a decrease in 2004/05, to 46.8 million cartons (PPECB, personal communication).

The need for seedless cultivars has become an increasing world-wide demand and this is where Regal Seedless plays an important role. Regal Seedless was released in 1997 to the South African table grape industry. It is one of the most promising and newly planted white, seedless cultivars and producers hope for a continuous and growing demand from the international market.

The cultivar has many positive characteristics: seedless, inherent large berries, minimal bunch preparation (less labour) and good storage ability. However, the acceptability of the cultivar on local and international markets is affected by a sporadic, unacceptable, astringent taste. Astringency is seen as a negative taste in table grapes. This sensation is mainly due to the presence of phenolic compounds (Joslyn & Goldstein, 1964a) in especially the group called the flavonoids (Robichaud & Noble, 1990).

Phenolic compounds are secondary metabolites and are present in all plant tissues. They are widely distributed in the plant kingdom and frequently form the most abundant secondary metabolites in fruit. Here, they are often present in high concentrations. This group of substances has attracted the attention of chemists and biologists for a century or more. The development of methods used for analysing phenolic compounds has been the focus of numerous studies.

2.2 PHENOLS IN GRAPES AND WINE

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). Seasonal and regional differences can be qualitatively and quantitatively large within a specific cultivar. Climate and maturity are other parameters that affect the concentration of phenolic compounds. For example, cooler conditions result in higher phenolic

concentrations (Singleton & Trousdale, 1992). In table grapes, storage and processing can also influence the concentration of phenols present (Calabrese, 2003). These compounds play a significant role determining the characteristics and quality of grapes and wine (Boulton et al., 1996).

2.2.1 CLASSIFICATION OF PHENOLIC COMPOUNDS

The phenolics in grapes (and wine) can be divided into two major groups: non-flavonoids and flavonoids.

2.2.1.1 Non-flavonoids

The non-flavonoids are mainly phenolic acids. These phenolic acids can be divided into two subgroups namely the hydroxybenzoic acids and hydroxycinnamic acids (Boulton et al., 1996). Fernández de Simón et al. (1992b) reported that 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. Ribéreau-Gayon et al. (2000) confirmed that the non-flavonoids are the main phenol component in the flesh where the concentration of the other phenolic compounds is very low. The non-flavonoid concentration varies between 100 – 200 mg/L in red wine and 10 – 20 mg/L in white wine (Ribéreau-Gayon et al., 2000). The hydroxycinnamic acids are present in much higher amounts in the berry than the hydroxybenzoic acids (Singleton & Esau, 1969).

2.2.1.1.1 Hydroxybenzoic acids

The most important benzoic acid (C_6-C_1) (Figure 2.1) in wine grapes is gallic acid (Boulton et al., 1996). The benzoic acid derivatives differ from each other with regard to the substitution on the benzene ring (Table 2.1) (Ribéreau-Gayon et al., 2000). The rest of the benzoic acids most commonly found in grapes are protocatechuic acid, p-hydroxybenzoic acid, vanillic acid and syringic acid. Two other compounds are only present in trace amounts: salicylic acid and gentisic acid.

Hydroxybenzoic acids are primarily degradation products and mostly appear with mould action (or wine aging). The major source of gallic acid is the hydrolysis of (-)-epicatechin gallate (Boulton et al., 1996). White wine contains about 7 mg/L gallic acid (Frankel et al., 1995). Cantos et al. (2002) analysed four red and three white table grape cultivars and did not detect any benzoic acids.

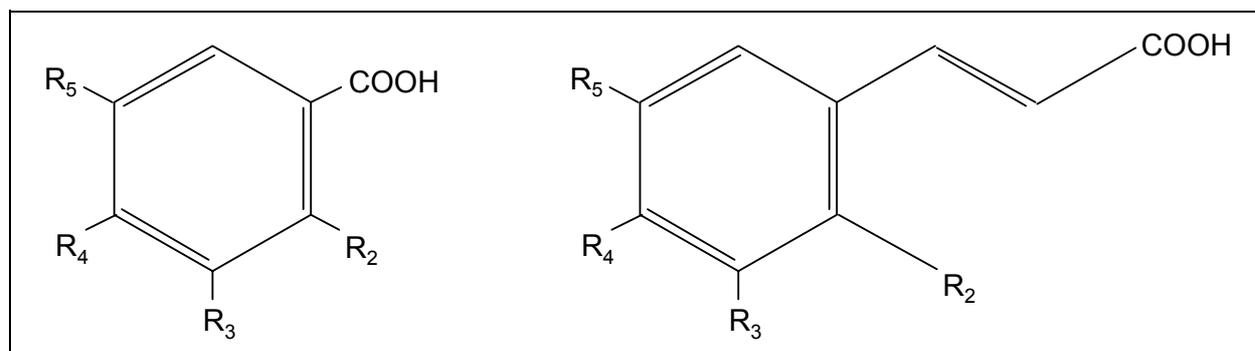


Figure 2.1 Phenolic acids: Benzoic acid (Left) and Cinnamic acid (Right) (Ribéreau-Gayon et al., 2000).

Table 2.1 Structures of phenolic acids (Ribéreau-Gayon et al., 2000).

Benzoic acid	R ₂	R ₃	R ₄	R ₅	Cinnamic acid
<i>p</i> -Hydroxybenzoic acid	H	H	OH	H	<i>p</i> -Coumaric acid
Protocatechuic acid	H	OH	OH	H	Caffeic acid
Vanillic acid	H	OCH ₃	OH	H	Ferulic acid
Gallic acid	H	OH	OH	OH	
Syringic acid	H	OCH ₃	OH	OCH ₃	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentisic acid	OH	H	H	OH	



2.2.1.1.2 Hydroxycinnamic acids

The hydroxycinnamic acids (C₆-C₃) (Figure 2.1), caffeic, *p*-coumaric and ferulic acids are mainly esterified to tartaric acid – forming respectively caftaric (caffeoyltartaric acid), coutaric (*p*-coumaroyltartaric acid) and fertaric acids (Boulton et al., 1996). Only small quantities are found in the free form and some may also be bound to glucose (Ribéreau-Gayon et al., 2000).

Singleton et al. (1978) only found *cis*- and *trans*-coutaric acids and *trans*-caftaric acids in Müller-Thurgau white wine grapes. The other cinnamates, such as the fertaric acids and free cinnamic acids were absent. Cantos et al. (2002) identified caftaric and *p*-coumaric acid in four red table grape (Redglobe, Flame Seedless, Crimson Seedless, Napoleon) and three white table grape (Superior Seedless, Dominga, Moscatel Italica) cultivars. Caftaric acid contributed to 60% of the total hydroxycinnamic acids in all the cultivars except for Superior Seedless where it was only 40%. The total amount of hydroxycinnamic acids ranged from 48 mg/kg fresh weight in Flame Seedless to

8.4 mg/kg fresh weight in Redglobe (only the skin was used for extraction; it was assumed that the skin forms 10% of the total berry weight). The total hydroxycinnamic acids represented 13% and 4% of the total phenols in Redglobe and Flame Seedless, respectively. There were no significant differences between the amount in red and white grapes.

Postharvest hydrolysis frees a part of these hydroxycinnamic acids from the tartaric acid. Caftaric and coumaric acids will form the same quinone when they are oxidized by polyphenoloxidase (PPO). This and other quinones from vicinal diphenols react with sulfhydryl derivatives. The first product is 2-S-glutathionyl caftaric acid. This product forms very rapidly, thus extreme care must be taken during preparation of samples to make sure that the original content of the grape is reflected (Boulton et al., 1996).

2.2.1.2 Flavonoids

The flavonoids can be divided into four main groups: flavan-3-ols, proanthocyanidins, flavonols and anthocyanins. The different structures differ in the degree of oxidation of the heterocyclic ring. See Figure 2.2 for the basic structure of a flavonoid.

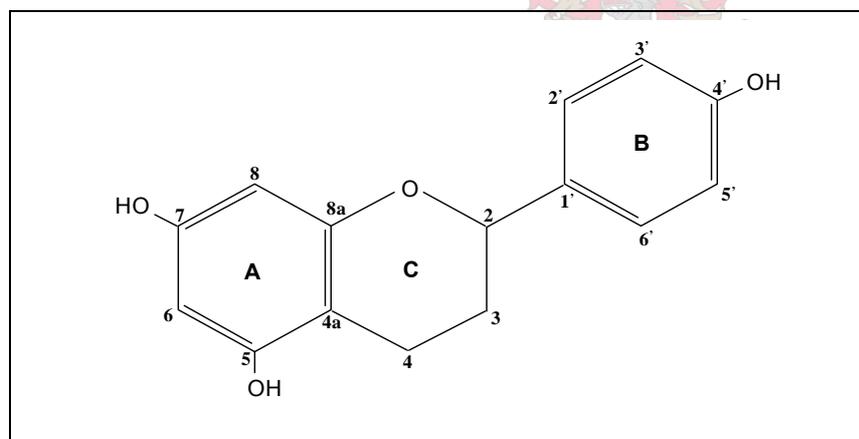


Figure 2.2 Basic structure of a flavonoids (<http://www.herbalchem.net>).

2.2.1.2.1 Monomeric flavan-3-ols and proanthocyanidins

The primary flavan-3-ols are (+)-catechin and (-)-epicatechin (Figure 2.3, Table 2.2). These two compounds can also be esterified to gallic acid. The total content in ripe berries of seeded *Vitis vinifera* wine varieties is about 500 mg/kg fresh weight for each of the two flavan-3-ols (Boulton et al., 1996). The gallocatechins and epicatechin gallates are present in smaller amounts. The flavan-3-ols occur free or polymerise to form dimers, trimers, higher oligomers and polymers through interflavan (C_4-C_6/C_4-C_8) linkages. These polymeric flavan-3-ols are called proanthocyanidins (Boulton et al.,

1996). Proanthocyanidins form the largest part of the total phenolics in the grape (Cantarelli & Peri, 1964).

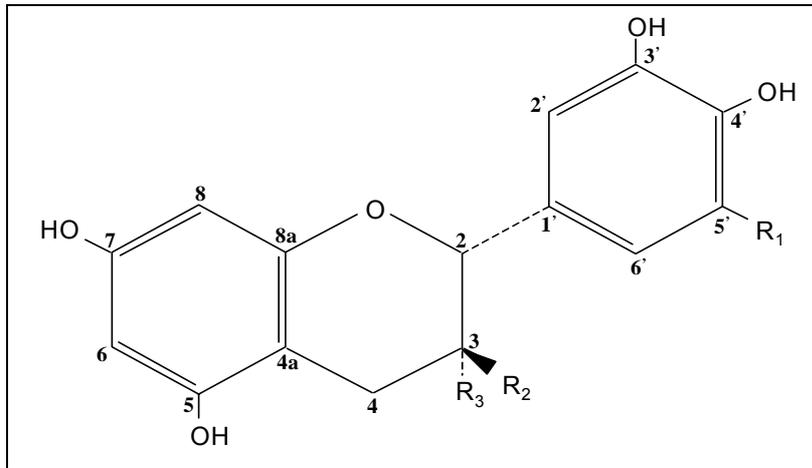


Figure 2.3 Basic structure of flavan-3-ols (Ribéreau-Gayon et al., 2000).

Table 2.2 Structures of flavan-3-ols (Ribéreau-Gayon et al., 2000).

R_1	R_2	R_3	
H	OH	H	(+) – catechin
OH	OH	H	(+) – gallocatechin
H	H	OH	(-) – epicatechin
OH	H	OH	(-) – epigallocatechin

Flavan-3-ols are located in the solid parts of the berry of both red and white cultivars (Lea et al., 1979; Singleton & Trousdale, 1983). The highest concentrations are present in the seeds (Cantarelli & Peri, 1964; Fernández de Simón et al., 1993), with lower concentrations in the skins and it is basically absent in the grape juice (Boulton et al., 1996). Fernández de Simón et al. (1992b) reported that the flavan-3-ols ((+)-catechin, (-)-epicatechin, catechin-catechin gallate and epicatechin gallate) are mainly present in the seeds of Cencibel red wine grapes. These compounds were totally absent in the must.

Dimeric proanthocyanidins can be divided into two groups, identified by a letter and a number (Weinges et al., 1968; Thompson et al., 1972); type A and B. Trimeric proanthocyanidins can also be divided into two categories; type C and D. Only the proanthocyanidin dimers and some trimers have been completely identified. It is possible to isolate and separate (+)-catechin, (-)-epicatechin, dimeric, trimeric,

oligomeric and condensed procyanidins. All the dimers are present in the seeds while procyanidin dimers B4, B7 and B8 are absent in the skins (Ribéreau-Gayon et al., 2000).

Oligomeric proanthocyanidins consist of three to ten flavanol units linked by C₄-C₈ or C₄-C₆ bonds. Condensed proanthocyanidins (tannins) have more than ten flavan units and a molecular weight greater than 3000 (Ribéreau-Gayon et al., 2000). The condensed tannins or proanthocyanidins in skins can be divided into procyanidins and prodelphinidins. The procyanidins consist of (epi)catechin units while the prodelphinidins contain both (epi)catechin and (epi)galocatechin units (Ribéreau-Gayon et al., 2000). When (epi)catechin and (epi)galocatechin units are heated in an acid medium, unstable carbocations are released and form respectively red cyanidin and delphinidin.

There are tannins in the vacuoles, forming dense clusters in the cells close to the epidermis (skin) and diffuse granules in the internal cells of the mesocarp (pulp). Some tannins are very strongly bound to the proteophospholipidic membrane (tonoplast) (Amrani-Joutei et al., 1994) and others are integrated in the cellulose-pectin walls (Ribéreau-Gayon et al., 2000). The distribution of these molecules is consistent with their antifungal properties.

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

This group of compounds is able to form stable complexes with proteins and polysaccharides. One of the results of this property is astringency where the glycoproteins in the saliva react with the tannins (Ribéreau-Gayon et al., 2000).

2.2.1.2.2 Flavonols

The flavonols are found in both red and white grapes in the glycoside form in the vacuoles of epidermal tissue. The flavonol aglycones (non-glycosylated form) have fifteen carbon atoms (C₆-C₃-C₆) and differ from each other with respect to both the number and the type of substituents on the B-ring (Figure 2.4), producing kaempferol, quercetin and myricetin (Ribéreau-Gayon et al., 2000). Flavonols have been found in

grape skins (Fernández de Simón et al., 1993; Price et al., 1995) but apparently no flavonols are present in the pulp or seeds (Singleton & Esau, 1969). However, Fernández de Simón et al. (1993) detected myricetin and quercetin in the must, but in very low quantities. Flavonol glycosides were detected in greater amounts in the skin than the must. All three flavonol aglycones (kaempferol, quercetin, myricetin) are present in red wine grapes while white wine grapes only have the first two compounds (Ribéreau-Gayon et al., 2000). In contrast to this finding, Fernández de Simón et al. (1992a) detected kaempferol, quercetin and myricetin in white wine grapes (Var. Airén) as well as isorhamnetin in samples around maturity. Quercetin-3-glucoside and quercetin-3-glucuronide are the most prominent in Pinot Noir grape berries (Price et al., 1995).

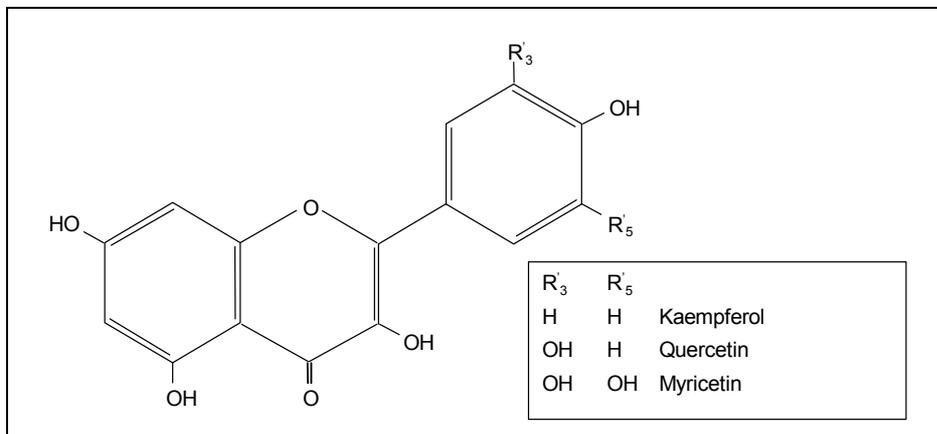


Figure 2.4 Structures of flavonols (Ribéreau-Gayon *et al.*, 2000).

The main flavonols in different table grape cultivars were quercetin-3-glucuronide, quercetin-3-glucoside and quercetin-3-rutinoside (Cantos et al., 2002). Quercetin-3-glucuronide was the main flavonol in Flame Seedless and Napoleon while quercetin-3-glucoside and quercetin-3-rutinoside were dominant in Redglobe and Dominga. Cantos et al. (2002) also discovered other flavonols in trace amounts, namely kaempferol-hexosides and isorhamnetin-3-glucoside. The flavonol myricetin was not found in any of the seven table grape cultivars. However, there are previous reports that describe the presence of myricetin and its derivatives in table grapes (Fernández de Simón et al., 1992a). This could be due to different extraction methods used. The total flavonol content ranged from 13 (Crimson Seedless) to 64 (Superior Seedless) mg/kg of fresh weight. The contribution of flavonols to the total phenolic content was higher for the white than red cultivars (Cantos et al., 2002).

2.2.1.2.3 Anthocyanins

Anthocyanins are the red pigments in red grapes located mainly in the vacuoles of the skin cells (Boulton et al., 1996; Ribéreau-Gayon et al., 2000). In the grape cultivars known as 'teinturiers', anthocyanins are also present in the flesh producing grapes that are rich in colour (Ribéreau-Gayon et al., 2000).

These molecules are more stable in the glycoside (anthocyanin) than the aglycone (anthocyanidin) form (Ribéreau-Gayon et al., 2000). Malvidin-3-glucoside (Figure 2.5) is the most abundant in the red cultivars, representing about 40% of the total anthocyanins (Boulton et al., 1996). Though, in Grenache and Sangiovese malvidine-3-glucoside varies from respectively 90% to under 50% of the total anthocyanins (Ribéreau-Gayon et al., 2000). Anthocyanin accumulation begins at véraison (Boulton et al., 1996). The colour of these molecules depends on the conditions in the medium (SO_2 and pH), the molecular structure and the environment (Ribéreau-Gayon et al., 2000). The molecules are also present in the leaves, mainly at the end of the growing season (Darné & Glories, 1988). Regal Seedless is a white table grape cultivar and contains no anthocyanins. The contribution of these flavonoids will thus be ignored during the rest of the discussion.

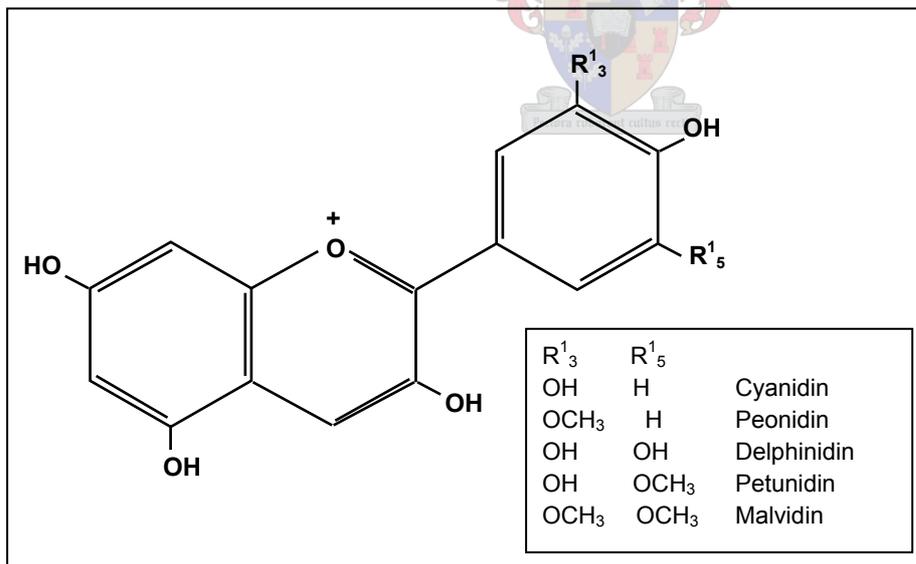


Figure 2.5 Structures of anthocyanins (Ribéreau-Gayon et al., 2000).

2.2.2 DEVELOPMENT OF PHENOLS DURING MATURATION

Maturation is a complex process where physical and biochemical changes take place within the berry from véraison (onset of ripening) to physiological maturity. The development of the grape berry follows a typical double sigmoid growth pattern which can be divided into three growth stages (Coombe & Hale, 1973; Coombe, 1992; Ribéreau-Gayon et al., 2000). Stage I is an initial rapid growth phase occurring after berry set and consists firstly due to cell division. During stage I, chlorophyll is the predominant pigment and the berries have high respiration rates (Winkler et al., 1974). The acid concentration is high while the sugar concentration remains almost constant. Stage II is a lag phase during which the chlorophyll content decreases, maximum acid levels are reached and sugars start to accumulate. The level of total phenolics is low during stage I and II (Pirie & Mullins, 1980). Stage III is the second growth (ripening) phase and is entirely due to enlargement of the pericarp cells (Coombe & Hale, 1973). During stage III there is an increase in berry weight and volume, increase in deformability (softening), decrease in the concentration of organic acids, increase in sugar concentration, loss of chlorophyll, accumulation of anthocyanins and a decrease in respiration rate (Winkler et al., 1974). The inception of this growth phase (boundary between stages II and III) is called véraison (Coombe & Hale, 1973). Pirie & Mullins (1980) confirmed that the soluble sugars in the pulp and the skin started to increase from stage III but the total phenolics and anthocyanins in the skin only started to increase rapidly a week after véraison. Figure 2.6 and 2.7 illustrate the biosynthesis of phenolic compounds via the shikimic acid pathway.

A few studies have been done to investigate sugar and phenolic relationship. Singleton & Esau (1969) were unable to find a correlation between berry °Brix and polyphenol content. Pirie & Mullins (1977) also found no relationship between the total soluble solids in the berry and the total phenols in the skins of ripening Shiraz and Cabernet Sauvignon grapes. There was, however, a positive correlation between the sugar content in the skin of the fruit and the levels of phenolic substances in both the cultivars. This indicated that the sugars in the skins play a regulatory role in the production and rate of accumulation of total phenolics (Pirie & Mullins, 1976, 1980).

Seasonal, regional and environmental factors influence the quantity, rate of accumulation and maximum amount of phenolics (Singleton & Trousdale, 1983; Lee & Jaworski, 1989; Ribéreau-Gayon et al., 2000). Cultivation system and harvest time also play a role.

Cultivar differences, with regards to phenolic composition, occur in grapes. Singleton & Trousdale (1983) studied in detail the patterns of phenolic compounds and concluded that it is influenced by the genetics of the grapevine. The qualitative phenolic composition is usually similar within a grape cultivar. The rate of accumulation of total phenolics differed between cultivars (Pirie & Mullins, 1977). Cantarelli & Peri (1964) reported that some white cultivars had a high concentration of proanthocyanidins while others have a very low concentration. The decrease in seed phenolic concentration with maturation as well as the initial or end concentration differed between cultivars (Ribéreau-Gayon et al., 2000).

There are differences between red and white grapes in terms of specific phenols that are present within the grape. The main difference is of course the fact that red grapes contain anthocyanins while they are absent in white grapes. White and red grapes show the same phenomena; the phenolics increase in the skins, while the concentrations in the seeds decrease regularly (Ribéreau-Gayon et al., 2000). Lee & Jaworski (1987) found that *trans*-caffeoyl tartaric acid (*t*-caftaric acid), *cis*-coumaroyl tartaric acid (*c*-coumaric acid) and *trans*-coumaroyl tartaric acid (*t*-coumaric acid) are the major non-flavonoids while catechin, epicatechin, and procyanidin dimers B1, B2 and B3 are the major phenolic compounds in the flavonoid group in white wine grapes. They also found three unknown compounds, which were later isolated and quantified as catechin-gallate and two isomers of catechin-catechin gallate. Red and white grapes have similar distributions of proanthocyanidins in the seeds. Ribéreau-Gayon et al. (2000) found that procyanidin B2 is in the highest concentration in red grape skins, while absent from white grapes. In white grapes procyanidin B1 is in the majority. This is contradictory to the previous study of Lee & Jaworski (1987) who did find procyanidin B2 in white grapes while the concentration of procyanidin B1 was very low and definitely not the major proanthocyanidin. The reason for these differences might be that Ribéreau-Gayon et al. (2000) only investigated the skin while Lee & Jaworski (1987) studied the whole berry.

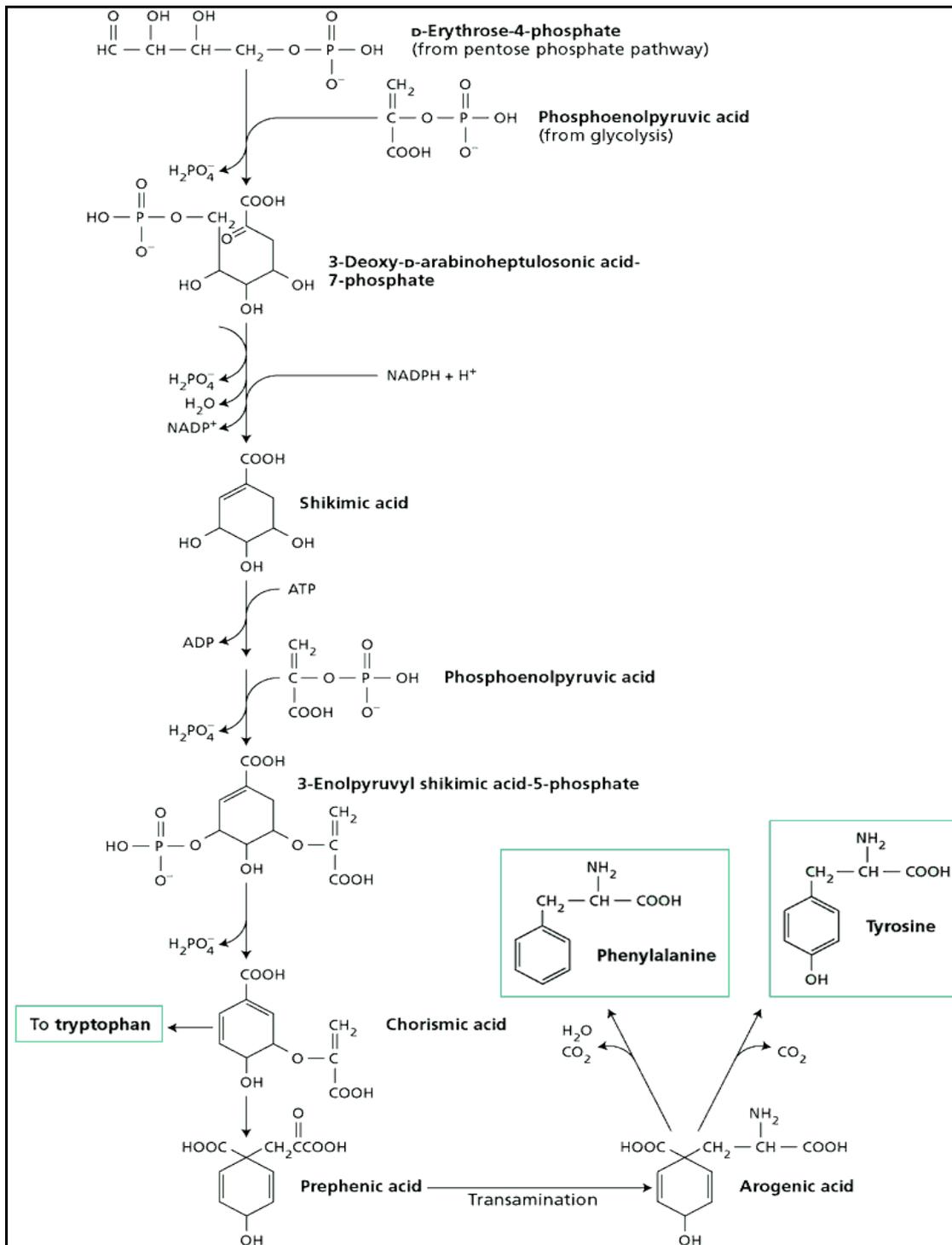


Figure 2.6 In the shikimic acid pathway, the aromatic amino acids are synthesized from carbohydrate precursors derived from the pentose phosphate pathway (D-erythrose-4-phosphate) and glycolysis (phosphoenolpyruvic acid) (Taiz & Zeiger, 2002).

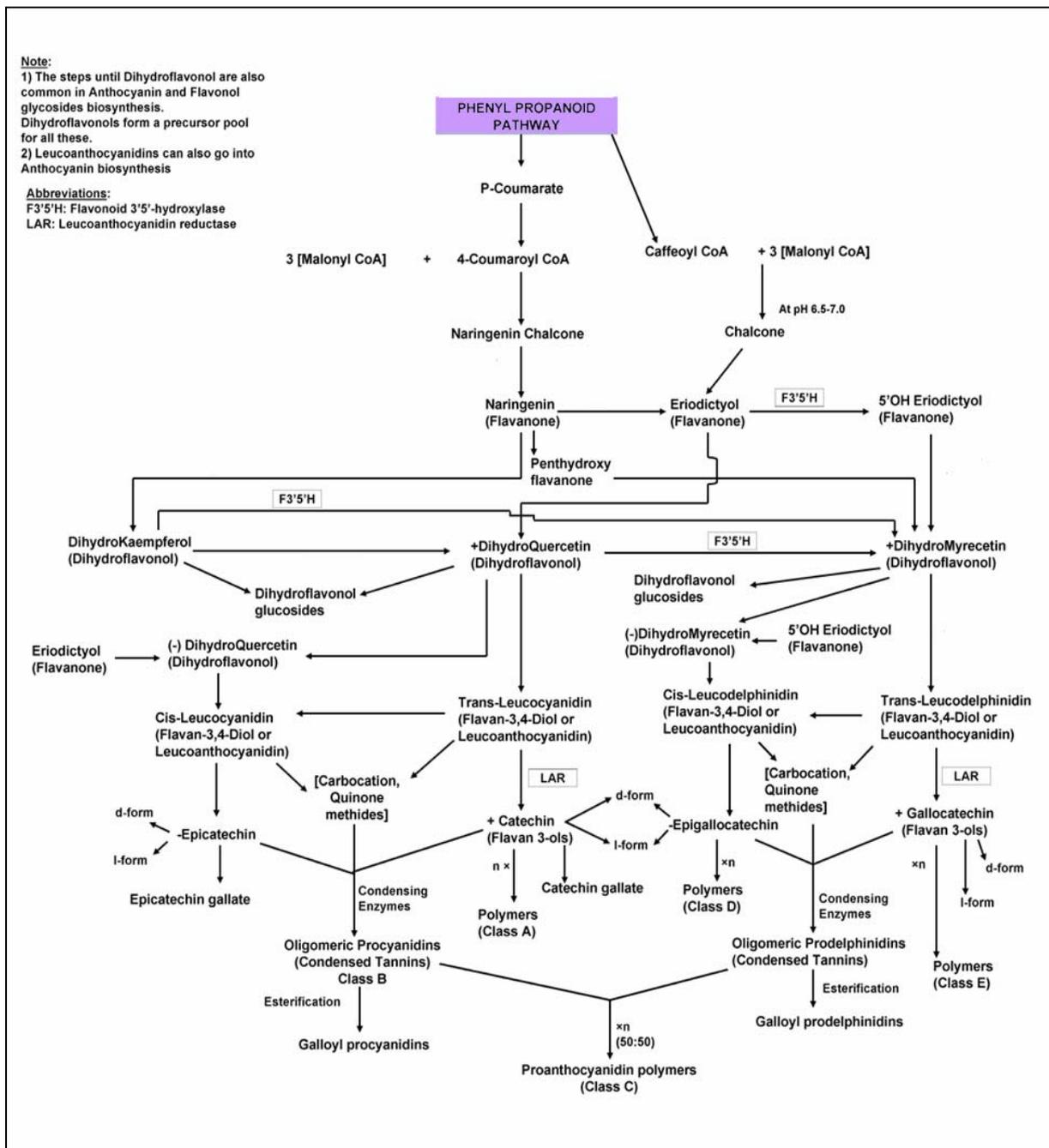


Figure 2.7 Outline of phenolic biosynthesis from the phenyl propanoid pathway to form many plant phenolics (<http://mtngrv.missouristate.edu>).

The evolution of total phenolics and individual phenolics in the whole berry during maturation has been studied extensively. In earlier research, for example a trial by Cantarelli & Peri (1964), it appeared that the phenolic concentration was the highest in the immature grape clusters which then started to decrease with maturity. Singleton (1966) however proved differently and research after that confirms it.

Singleton (1966) found that the total extractable phenols per berry weight decreased during ripening but the downward trend was irregular. He suggested that the decrease was the result of a greater increase in berry weight which diluted the

accumulation and probable net synthesis of phenolic substances in the berry. This hypothesis was confirmed by Boulton et al. (1996). There was however a rapid increase in the total extractable phenols per berry throughout maturation until the sugar content was well developed. During the last month of ripening the total phenolic content per berry is quite constant, but during high ripeness it may decrease (Boulton et al., 1996). These results indicated that the total phenols do not always decrease at the end of ripening as was suggested before. It was observed that the phenolic compounds had greater biochemical activity than realised, due to the net synthesis or accumulation in the berry as well as the irregular increases and decreases during short periods. It is important to keep in mind that Singleton (1966) studied the whole berry including the seeds and used the Folin-Denis method for determination of the total phenolics. More recent research uses spectrophotometric methods using absorbance at 280 nm for estimation of total phenolics.

The highest concentration of flavan-3-ols was present around véraison as determined by a modified vanillin-HCl method. The level then decreased to a more or less steady level. The flavanols (+)-catechin and (-)-epicatechin experienced little fluctuation over time. The amount of (-)-epicatechin 3-O-gallate showed a definite decrease from véraison (Czochanska et al., 1979). Lee & Jaworski (1989) confirmed that the flavan-3-ols, proanthocyanidins and their gallates increased sharply at véraison and then decreased to their lowest concentration at harvest.

Ong & Nagel (1978) reported that caftaric acid in White Riesling wine grapes rapidly declined from 7 to 11°Brix and rose only slightly in riper samples. Romeyer et al. (1983) found caftaric acid decreased in concentration during ripening in Grenache (red wine grape). Ong & Nagel (1978) and Romeyer et al. (1983) agreed that coutaric and fertaric acids followed the same changes than caftaric acid during ripening. However, Singleton et al. (1986) found that the concentration of caftaric and related acids stayed relatively constant throughout berry maturation. If the content is high, the concentration per berry might fall but the amount per berry rises as the berry enlarges during ripening. Thus it appears that there was a net synthesis of caftaric acid, but the berry growth was greater. Lee & Jaworski (1989) agreed with Romeyer et al. (1983) and reported that the concentrations of four hydroxycinnamic acid-tartaric acid esters, namely cis- and trans-caffeoyl tartrate (caftaric) and cis- and trans-coumaroyl tartrate (coutaric) are high early in the season then continuously decrease to a low concentration at harvest.

Fernández de Simón et al. (1993) found that when the different phenolic compounds over the ripening season are plotted, a series of maxima and minima are

found. These results show that the grapes change from a composition rich in hydroxycinnamic tartaric acids, gallic acid and flavan-3-ols at véraison to another at maturity where these phenolic compounds are lower. At maturity the grapes are a lot richer in benzoic and cinnamic acids and their aldehydes and flavonol aglycones and glucosides. The maximum concentrations of the benzoic and cinnamic acids at the end of ripening did not coincide with the minimum concentrations of the hydroxycinnamic tartrates and flavan-3-ols (Fernández de Simón et al. 1992b, 1993). These data together with data on acidity and sugar content should be taken into account when choosing the optimum harvest time.

Phenolic development during ripening in the skin, pulp and seeds are well documented. Evolution of phenolics in the seeds will not be reviewed due to the fact that Regal Seedless is a seedless cultivar. The concentration of catechin in the skin decreased rapidly from véraison (Kennedy et al., 2002; Downey et al., 2003), while there was an increase in the level of epicatechin (Downey et al., 2003). The mean degree of polymerization of skin proanthocyanidins increased steadily during fruit maturation (Kennedy et al., 2001, 2002). Lee & Jaworski (1989) found a large amount of hydroxycinnamic tartrates in the skin while Fernández de Simón et al. (1992b) detected nothing in the skins. A possible reason is the different extraction solutions. Fernández de Simón et al. (1992b) used diethyl ether as an extractant which might cause a lower extraction yield of these components in comparison to Lee & Jaworski (1989) who used ethanol. The benzoic and cinnamic acids and their aldehydes in the skins, followed a rising trend, ending on a maximum at maturity (Fernández de Simón et al., 1992a, 1992b, 1993). Flavonol aglycones and glycosides in the skin increase slightly in concentration at véraison, with a large increase at maturity (Fernández de Simón et al., 1992a, 1993; Kennedy et al., 2002). The total phenolic content in the skin increases from véraison through maturation until full ripeness (Pirie & Mullins, 1977, 1980; Ribéreau-Gayon et al., 2000). This pattern is true for many cultivars and most vineyards.

The concentration of hydroxycinnamic tartaric acids in the pulp showed a declining trend through maturation (Fernández de Simón et al., 1992b, 1993). There was a substantial decline at véraison, followed by a slower decline and levelling off around maturity (Fernández de Simón et al., 1992b). Lee & Jaworski (1989) found that the pulp contained a large amount of flavan-3-ols (catechin, epicatechin) and procyanidins (procyanidin B2) while Ricardo da Silva et al. (1992) did not find any monomeric or polymeric flavan-3-ols in the pulp.

2.2.3 ANALYSIS AND QUANTIFICATION OF PHENOLS

Analysis of white and red wine phenols has been accomplished by high-performance liquid chromatography (HPLC) (Singleton & Trousdale, 1983; Revilla & Ryan, 2000; Rodríguez-Delgado et al., 2002). A HPLC method was developed to separate most monomeric anthocyanins from the pigmented polymers in Pinot Noir wines (Price et al., 1995; Waterhouse et al., 1999). It is also an effective and accurate technique for the analysis of catechins and oligomeric procyanidins in grape seeds (Ricardo da Silva et al., 1991; Fuleki et al., 1997). This technique, however, did not allow the exact quantification of the polymeric procyanidins (tannins), since they were only recognized as a broad peak. With this in mind, Peng et al. (2001, 2002) developed a RP-HPLC method specifically for the quantitative analysis of polymeric phenols in grape seeds and wine. Ultrafiltration, protein precipitation and Sephadex LH20 chromatography combined with electrospray ionization mass spectrometry confirmed the polymeric nature of the polymeric phenol peak (Peng et al., 2001, 2002).

The concept of HPLC is simple, easy to use and convenient, but there are a lot of variables including sample preparation, column, solvent, etc. that play a role in the optimum quantitative and qualitative measurement of phenols. Two types of columns can be used in HPLC namely a C₁₈ column (Singleton & Trousdale, 1983; Peng et al., 2001; Rodríguez-Delgado et al., 2002; Pomar et al., 2005) or a polystyrene/divinylbenzene reversed phase column (PLRP-S) (Price et al., 1995; Waterhouse et al., 1999; Peng et al., 2002). The PLRP-S column is more effective to quantify the polymeric peak. Separation can be accomplished by using an isocratic solvent system or a gradient elution (Price et al., 1995; Katalinić, 1997). Waterhouse et al. (1999) used a multilinear gradient with three solvents instead of the usual two solvents.

HPLC has also been used in the analysis of specific phenolic compounds in both white and red wine grapes (Oszmianski & Lee, 1990; Price et al., 1995; Pomar et al., 2005). Different solvents and concentrations of solvents are used for the extraction of phenolic compounds from the grapes, for example methanol (Oszmianski & Lee, 1990), ethanol (Fernández de Simón et al., 1993; Price et al., 1995) and acetone (Kennedy et al., 2002). Price et al. (1995) studied the anthocyanin and flavonol content of Pinot Noir grapes and skin. Pomar et al. (2005) composed anthocyanin profiles for red table grapes with reverse phase HPLC analysis. In this study, anthocyanin extracts were also separated on descending paper chromatography (PC) and ascending thin layer chromatography (TLC).

Mass spectrometry (MS) detectors coupled to high-performance liquid chromatography (HPLC-MS) have been commonly employed for structural characterization of phenolics (Cantos et al., 2002; Alonso Borbalán et al., 2003). Electrospray ionization mass spectrometry (ESI/MS) has been used for structural confirmation of phenolics in grape seeds and red wine (Peng et al., 2001, 2002).

There are a few spectrophotometric methods for estimating the total anthocyanin and phenol content of grapes and wine. In earlier studies the Folin-Denis or Folin-Ciocalteu analytical methods have been preferred for red and white wine (Singleton & Rossi, 1965; Singleton & Esau, 1969; Amerine & Ough, 1974; Alonso Borbalán et al., 2003), usually with a standard such as gallic acid. However, problems have been identified related to this method since the non-phenolics (SO_2) interact with the phosphomolybdate-tungstate, giving an incorrect estimate (Somers & Ziemelis, 1980). This effect is only significant in white wine and the interaction with SO_2 can be removed with acetaldehyde. A close relationship was found between A_{280} and the measure of total phenolics in red wines with the Folin-Ciocalteu method (Somers & Evans, 1977).

Iland et al. (2000) described a method for the determination of red pigments and total phenolics of grape berries. This method is based on the extraction of these compounds, with 50 % ethanol, from a known weight of macerated whole berries. A portion of the ethanol extract is diluted with 1 M HCl and the absorbance of the solution is measured with a spectrophotometer at 520 nm. The extinction coefficient of malvidin-3-glucoside is used in the calculation of the total anthocyanins and the results are expressed as equivalents of this anthocyanin. A measurement of this solution at 280 nm provides an estimate of the amount of total phenolics in the diluted extract in absorbance units.

The concentration of flavonoids and hydroxycinnamic acids in white wine or juices can be estimated spectrophotometrically at 280 nm (A_{280}) and 320 nm (A_{320}), respectively (Iland et al., 2000). An absorbance measure at 420 nm (A_{420}) gives an estimate of the concentration of yellow brown pigments in the wine. The following spectral measures were defined for the phenolic composition of white wine/juice:

1) Total phenolics (A.U) = $A_{280} - 4$. This gives a measure of the concentration of all the phenolic compounds present in the juice and wine. The subtraction of 4 allows for the absorbance of non phenolic material (Somers & Ziemelis, 1972; Somers & Evans, 1977; Bakker et al., 1986; Iland et al., 2000).

2) Total hydroxycinnamates (A.U) = $A_{320} - 1.4$. This gives a measure of the concentration of hydroxycinnamates. The subtraction of 1.4 allows for the absorbance of non phenolic material.

3) Total flavonoids (A.U) = $(A_{280} - 4) - (0.66) * (A_{320} - 1.4)$. This gives a measure of the concentration of flavonoids. The factors are correction factors allowing for the contribution of hydroxycinnamic acids to the absorbance at 280 nm (Somers & Ziemelis, 1985, Iland et al., 2000).

The data from Somers & Ziemelis (1985) supported the concept that $(A_{280} - 4)$ is a better measurement of total phenolics than the Folin-Ciocalteu assay. Gorinstein et al. (1993) used ultraviolet (UV) and infrared (IR) spectroscopy to detect and identify phenolic compounds in white grapes and wine. The IR spectra were measured by Fourier Transformation Infrared Spectroscopy (FTIR).

2.2.4 THE ROLE OF PHENOLS

Phenolic compounds are very important components in wines and grapes. They contribute to the sensorial properties such as colour, flavour, taste and mouth-feel characteristics like astringency, both directly or by interaction with proteins, polysaccharides or other phenolic compounds (Haslam, 1974; Robichaud & Noble, 1990). It has been observed that these phenols might have a positive effect on health due to their antioxidant properties (Frankel et al., 1995). Each group of phenols has a unique contribution. The contribution of the phenolic compounds to astringency perception will be discussed in section 2.3.2.

2.2.4.1 Phenolic acids

Phenolic acids are responsible for the browning reactions in wines. Lee & Jaworski (1988) discovered that the browning of acidic phenolics (hydroxybenzoic and hydroxycinnamic tartrates) were very low. Noble (1990) and Ribéreau-Gayon et al. (2000) found the opposite, namely that these compounds (cinnamic acids) are highly oxidizable and are responsible for the browning of white must. Hydroxybenzoic acids are not very abundant in grapes, but they are important in organoleptic perception of the fruit (Singleton & Esau, 1969). Gallic acid, for example, contributes to the bitterness of wine (Robichaud & Noble, 1990). Phenolic acids are precursors of volatile phenols which enhance the wine aroma (Rapp et al., 1977) and are considered to be important in the preservation and ageing of wine (Nagel & Wulf, 1979). Frankel et al. (1995) found

that gallic acid was the phenol with the highest relative antioxidant activity in Californian wine, followed by catechin > myricetin > quercetin > caffeic acid.

2.2.4.2 Monomeric flavan-3-ols and proanthocyanidins

Monomeric and polymeric flavan-3-ols are important contributors to bitterness (Noble, 1990). As the degree of polymerization increased from monomers (e.g. epicatechin) to trimers, the bitterness intensity and total duration decreased (Peleg et al., 1999). Earlier Arnold et al. (1980) discovered that, on a weight basis, bitterness intensity increased with an increase in molecular weight. A possible reason for the different findings is that Peleg et al. (1999) studied molecules of known configuration while Arnold et al. (1980) compared different phenolic fractions of grape seeds. Arnold & Noble (1978) studied three levels of increased total phenolic concentration and also found an increase in bitterness from level one to two, but no further increase in bitterness was observed at higher phenolic concentrations. This finding might be due to the masking effect of high astringency levels.

Individual phenolic compounds have different degrees of browning. The monomeric and dimeric proanthocyanidins brown more intensely than the other phenolics (Simpson, 1982). In agreement, Lee & Jaworski (1988) found that catechin and epicatechin had the fastest rate of browning in white grapes, reaching a maximum within six hours. Procyanidin B2 and B3 were slow at the beginning but increased with time, reaching a maximum at 48 hours. These results showed that it is possible to predict the browning potential of the white grape juice or wine, if you determine the individual phenolics in the grapes after harvest.

2.2.4.3 Flavonols

Flavonols have an inherent yellow colour and may, to some extent, have an impact on wine colour. A quercetin solution of 30 mg/L, equivalent to the concentration in wine made from sun-exposed berries, was visibly yellow with significant absorbance between 400 and 420 nm (Price et al., 1995). Quercetin appears to elicit a bitter taste with weak astringency in alcohol solutions and in beer (Dadic & Belleau, 1973). Resveratrol, quercetin and other phenols have been shown to elicit positive biological effects which help to protect living cells against free radicals (Calabrese, 2003).

2.3 ASTRINGENCY

Unripe fruit is usually astringent, but during fruit maturation it generally disappears while the fruit is still on the tree. There are some fruit, for example many cultivars of persimmon and some of banana which do not lose their astringency with ripening. The acceptability and palatability of fruit and fruit products like wine and the stability of certain fruit products, is dependent on the type and concentration of astringents present (Bate-Smith, 1954). A balance between sugar, acid and astringency is essential in wine. The sugar-acid ratio and the amount of astringents are both of equal importance to juices, like apple, cherry and grape juice. Thus, its removal is necessary for the fruit to be edible.

2.3.1 PERCEPTION OF ASTRINGENCY

The word 'astringent' comes from the Latin word *ad* (to) and *stringere* (bind) (Joslyn & Goldstein, 1964a). Astringency is commonly described as a 'drying', 'puckering' and 'roughing' sensation perceived throughout the oral cavity. There has been a lot of controversy on whether astringency is a taste or tactile sensation. Moncrief (1944) defined astringency as a 'contracting or drying taste'. Psychophysical evidence supports the hypothesis that astringency is a tactile sensation and not a taste. There is a linear relationship between perceived intensity and concentration (psychophysical function) of various phenols across a wide concentration range (Robichaud & Noble, 1990) while these functions are non linear for the all other taste substances either decreasing negatively with concentration (Bartoshuk, 1978) or logarithmically (McBride, 1983). Breslin et al. (1993) argued that the chorda tympani (a nerve with special sensory fibers providing taste sensation) is known to contain mechano-receptive fibres and therefore it cannot be ignored that astringency might be a tactile sensation. He showed that astringency is a tactile rather than gustatory stimulus since the sensation was elicited on non-gustatory surfaces like the upper lip.

The sensation of astringency does not only take place in certain areas of the mouth or tongue like sweetness or sourness; it is more like a diffuse stimulus. Hinreiner et al. (1955) reported that astringency might have a long persistence and be carried over. Guinard et al. (1986) confirmed that astringency is characterized by a tendency to increase in intensity with repeated ingestion. It is not instantaneous or adaptable like the other taste sensations but requires time to develop (Joslyn & Goldstein, 1964a; Noble, 1990).

Saliva contains proteins, glycoproteins, glycolipids, carbohydrates and inorganic ions (Wu et al., 1994). Each of these groups has specific activities in the mouth. One of these functions is the lubrication of the mouth surface which arises from the presence of mucoproteins and proline-rich proteins. Proline-rich proteins, which form $\pm 70\%$ of the human saliva protein, can strongly bind phenolics, suggesting a crucial role in astringency perception (Ozawa et al., 1987; Luck et al., 1994).

The astringency phenomenon is thought to be due to the association of the salivary proteins and the phenols through hydrogen bonding and hydrophobic effects (Haslam, 1974; Oh et al., 1980; McManus et al., 1981; Artz et al., 1987). This will result in the formation of insoluble phenol-protein complexes that precipitate, obstructing the lubrication of the palate, increasing friction with the mouth surfaces and causing dryness (Bate-Smith, 1973; Noble, 1990). Guinard et al. (1986) proposed that the reactions with epithelial proteins may take place after the complexation with salivary proteins and after the mucus layers which cover the epithelium have been stripped away. More recently Baxter et al. (1997) found that polyphenols are self-associated when bound to proline-rich proteins, indicating that polyphenol-polyphenol cross linking might be an extra factor in protein precipitation and thus astringency perception. Astringency can be removed by salivation which cleanses the mouth of phenols or provides new proteins to replace the precipitated ones (Joslyn & Goldstein, 1964a). The complex formation between phenols and proteins are influenced by molecular structure, size and mass of the substrates and their concentration. The pH, temperature, ionic and ethanol concentration of the medium also affect the interaction (Gawel, 1998).

Astringency perception varies greatly between individuals (Fischer et al., 1994). Since salivary lubrication is important in astringency perception, research has focused on the relationship between saliva flow rate and composition, and the intensity and duration of astringency. Fischer et al. (1994) found that individuals with a low flow rate perceived higher maximum intensities and slower increase or decrease rates of tannic acid in wine. This result was supported by Ishikawa & Noble (1995). It has been proposed by Lee & Lawless (1991) that individuals who have higher levels of salivary protein in their mouth perceive substances as less astringent since astringency is mainly attributed to the binding of salivary proteins. In contrast with these previous reports, Peleg et al. (1999) found that the persons with the high saliva flow rate rated the maximum intensity of astringency higher than the low-flow group of people. However, in agreement with previous studies, the low-flow subjects took longer to reach the maximum intensity than the high-flow group and astringency had a longer

persistence with the medium and low-flow group but no significant differences were found between the groups.

2.3.2 THE CONTRIBUTION OF PHENOLS TO ASTRINGENCY

Astringency is caused primarily by the flavonoids. The polymeric flavan-3-ols also referred to as the proanthocyanidins are mainly responsible for the astringency of red wine (Singleton, 1992). The monomers catechin and epicatechin are not defined as astringents since polyphenols with molecular weights (MW) below 500 do not precipitate proteins (Joslyn & Goldstein, 1964a; Bate-Smith, 1973).

The following studies, however, contradict this statement illustrating that both of these monomers have astringent properties. This might be because of alterations of the protein that they interact with. Thorngate & Noble (1995) studied the sensory properties of the monomeric flavan-3-ols and found that epicatechin had a higher maximum and a longer total duration for astringency than catechin. Several authors concluded that both monomers (+)-catechin and (-)-epicatechin are astringent (Kallithraka et al., 1997; Peleg et al., 1999). High concentrations of (-)-epicatechin were significantly more astringent than the equal concentration of (+)-catechin in a model wine solution (Kallithraka et al., 1997). A report by Peleg et al. (1999) pointed out that epicatechin was insignificantly higher in astringency than catechin in an aqueous ethanol solution (1% v/v). De Freitas & Mateus (2001) studied protein-polyphenol interactions and discovered that (+)-catechin had a higher tannin specific activity (TSA) for proline-rich proteins than (-)-epicatechin. Thus, one would expect (+)-catechin to be more astringent than (-)-epicatechin, which is inconsistent with the findings of the previous reports.

The relative astringency of the flavonoids has been reported to increase with an increase in the degree of polymerisation (Joslyn & Goldstein, 1964a; Bate-Smith, 1973; Haslam, 1974; Arnold et al., 1980; Peleg et al., 1999). A possible reason for this is the more extensive formation of phenol-protein complexes via hydrogen binding between the hydroxyl group of the phenol and the carbonyl group of the peptide linkages in the protein (Bate-Smith, 1973; Porter & Woodruffe, 1984; Peleg et al., 1999). Gawel (1998) pointed out that as the concentration of monomers and polymers increases, the maximum intensity and duration of perception of astringency increases. In red wine the monomer reaches a lower maximum intensity, reaches it more quickly and persists for a shorter time than the polymer. It is important to keep in mind that as the degree of polymerisation increases the estimated detection threshold decreases, but there are studies that differ from this perception.

Arnold et al. (1980) compared different phenolic fractions and found that the polymeric fractions are more astringent on a weight basis than the smaller fractions. As the phenolic concentration was increased for each of the fractions, significant differences were found in astringency for the dimeric (fraction II) and trimeric and tetrameric (fraction III) fraction. Peleg et al. (1999) agreed that the monomeric flavan-3-ols (epicatechin and catechin) had a lower maximum intensity of astringency throughout the onset and decay of the sensation than the dimers (procyanidin B3, B4 and B6) and trimers (C and C2). No significant differences were found in the total duration of astringency between the polymeric fractions. This shows that molecular size is a major factor influencing astringency.

The structural differences among the compounds of the same molecular size also have a significant effect on perceived astringency. These structural properties include the stereochemistry of the monomeric units (catechin or epicatechin) and the specific bond site of the dimers (C_4-C_6 or C_4-C_8). Peleg et al. (1999) discovered that the dimer B3 (catechin-(4-8)-catechin) was lower in astringency than either dimer B6 (catechin-(4-6)-catechin) or B4 (catechin-(4-8)-epicatechin). The fact that B6 had a higher astringency than B3 is in agreement with studies done on chemical astringency of dimers (Hagerman, 1989). The trimer C (catechin-(4-8)-catechin-(4-8)-epicatechin) was more astringent than C2 (catechin-(4-8)-catechin-(4-8)-catechin), though not significantly. The difference in astringency between B4 and B3 and the trimer C and C2 might be due to the more planar conformation of the C ring of epicatechin than catechin (Haslam, 1982). As a result of this planarity, the C ring position hydroxyl group in epicatechin has reduced interaction with the lipophilic methylene group at C ring position 4. This increases epicatechin's overall lipophilicity (Porter, 1988) and may promote epicatechin's greater astringency since the hydroxyl group will be more available for hydrogen bonding. Haslam et al. (1992), however, noted that hydrophobic interactions play the primary role in complexation of phenolic compounds and the hydroxyl groups that take part in hydrogen bonding are not the major factor. De Freitas & Mateus (2001) confirmed that the specific linkages play a role in protein binding. The procyanidin dimers with a C_4-C_8 interflavanoid bond had greater tannin specific activity (TSA) than the dimers with a C_4-C_6 linkage. In other words, these compounds will cause more protein precipitation and one would expect higher astringency.

Substituted benzoic acids have been found to elicit an astringent taste (Peleg & Noble, 1995). It has been postulated that these small phenolic acids induce astringency due to the precipitation of, or strong binding with proteins because of the presence of

one 1,2-dihydroxy or 1,2,3-trihydroxy groups (McManus et al., 1981). Confirming this, gallic acid has been found to elicit an astringent property (Robichaud & Noble, 1990). It was found that only protocatechuic acid and gentisic acid as well as all hydroxycinnamates have astringent qualities (Dadic & Belleau, 1973; Singleton & Noble, 1976). The flavonol quercetin also exhibited weak astringency.

2.3.3 FACTORS INFLUENCING ASTRINGENCY IN FRUIT

The factors that influence the levels of phenolic compounds will also consequently affect the astringent taste. As the phenolic concentration increase or decrease, it is suspected that astringency perception will follow the same pattern.

The composition and concentration of phenols within a cultivar are genetically controlled (Lee & Jaworski, 1987, 1989; Katalinić & Males, 1997; Ramos et al., 1999). Environmental factors that affect the levels of phenolic compounds in fruits include fruit maturity (Kennedy et al., 2001, 2002), locality, climate, seasonal conditions (Lee & Jaworski, 1989), vine water status (Kennedy et al., 2002) and mineral nutrition of soil (Garcia et al., 1993; Jackson & Lombard, 1993; Keller & Hrazdina, 1998). Temperature and water availability are also important (Jackson & Lombard, 1993). Some fruits are astringent when they are unripe, but during maturation or in storage the astringency decreases or disappears, as for example in persimmons. The change may entail an increase or decrease in the concentration of individual phenolics (see section 2.2.2) (Joslyn & Goldstein, 1964a). A lot of research has also been done to investigate the effect of cultural and viticultural practices on phenolic levels. These include: canopy management (Price et al., 1995; Dokoozlian & Kliewer, 1996; Keller & Hrazdina, 1998), irrigation, growth regulators, pruning, crop load and rootstocks (Jackson & Lombard, 1993).

There are secondary treatments that can affect the level of astringency, for example, treatment with carbon dioxide, nitrogen gas, ethylene; precipitation of the phenols with acetic acid, formaldehyde or *n*-propyl aldehyde; or oxidation with ozone (see section 2.3.4 for more detail). Astringency might also be lowered with air drying specifically in persimmons. If the fruit is then chilled the astringency may rise again (Joslyn & Goldstein, 1964a).

2.3.4 THE LOSS OR REMOVAL OF ASTRINGENCY

Researchers postulated a few processes that might cause the loss in astringency during ripening. When unripe fruit is eaten the cells containing the tannins tear easily and the

tannins diffuse into the mouth. When ripening fruit loses its astringency, the tannin cells shrink and the tannin coagulates. Some speculated that the tannin bound to cellulose or an aldehyde. It was observed that with a 50% increase of methanol-extractable material, there was a 100% decrease in the methanol-soluble phenolic fraction. Thus, a binding action causes the loss in astringency (Joslyn & Goldstein, 1964a). It is also possible that the loss in the astringency during ripening may be due to the production of significant amounts of water-soluble fragments of the pectin structure as the cell wall structure softens (Bonner & Varner, 1965). A lot of other mechanisms are also proposed (see section 2.4).

If the de-astringency process does not take place during ripening, there are external applications that have been found to decrease or remove astringency. These methods include treatments with warm water, carbon dioxide, ethanol or acetaldehyde (see section 2.4), freezing, drying and coating with resin films. It was also found that sun dried, lightly- or un sulphured fruit would lose its astringency while fruit with high sulphur content would retain their astringency (Joslyn & Goldstein, 1964a).

2.4. ANAEROBIC CONDITIONS

Anaerobic respiration occurs due to insufficient oxygen. There are two types of fermentation: lactic acid fermentation and alcoholic fermentation. During lactic acid fermentation lactate dehydrogenase (LDH) uses NADH to reduce pyruvate to lactate and releases NAD⁺. The accumulation of lactate causes the pH to decrease, which then inhibits LDH activity and stimulates enzymes involved in ethanol production. This type of fermentation is common in animals but is also found in some plants (like potatoes). As soon as aerobic conditions are restored, the lactate is converted back to pyruvate using NAD⁺. In some plants like apples, only a small amount of lactate forms during anaerobic conditions due to the already low pH. In this case alcoholic fermentation (common to plants, but mainly in yeasts) occurs. The enzyme pyruvate decarboxylase converts pyruvate to acetaldehyde releasing CO₂, while alcohol dehydrogenase reduces acetaldehyde to ethanol, oxidising NADH to NAD⁺. The result is small amounts of acetaldehyde which is toxic to plant tissue and large amounts of ethanol (in other words fermentation starts) (Taiz & Zeiger, 2002).

The production of anaerobic metabolites, acetaldehyde (AA) and ethanol, on the tree and during the postharvest storage period e.g. in modified (MA) or controlled atmosphere (CA), has a great effect on fruit ripening. These atmospheres include

combinations of carbon dioxide, nitrogen and oxygen levels. The optimum compositions of controlled and modified atmospheres (CA and MA) for fresh produce depend on the species, its maturity stage, the temperature and the duration of exposure. These conditions should be applied very carefully. Every fruit and vegetable, exposed to low O₂ has a certain threshold below which fermentation damage can occur. Fermentation damage includes off-flavours. There is a relation between these off-flavours and the production of acetaldehyde and ethanol. Both climacteric and non-climacteric fruit produce a lot of acetaldehyde and ethanol depending on their genetic characteristics and storage conditions (Pesis, 2005).

It has been found that the application of acetaldehyde or ethanol might be beneficial for postharvest fruit quality, for example on persimmons to remove astringency or on grapes to increase anthocyanins. Both of these metabolites are used for sterilisation and for their fungicidal and insecticidal properties (Pesis, 2005).

Different scientists have hypothesized about the mechanism which is responsible for the loss in astringency. The astringency in persimmon fruit is due to the methanol-extractable phenol content, which decreases with loss of astringency (Joslyn & Goldstein, 1964b), presumably due to polymerisation into insoluble tannin (Ito & Oshima, 1962). Tannin molecules can also react with cellular materials during astringency loss (Joslyn & Goldstein, 1964b). Kitagawa (1969) proposed another concept that the astringency loss during warm water treatment is due to protoplast gel formation in tannin cells rather than the polymerisation reaction with acetaldehyde. Matsuo & Ito (1982) disagreed and demonstrated that the loss of astringency in persimmons is due to the immobilisation of the tannin caused by the reaction with acetaldehyde produced during the maturation process forming an insoluble gel. Kays (1991) found that the existing tannins also polymerise to form larger water-insoluble molecules. Fukushima et al. (1991) concentrated on the osmotic dehydration of the tannin cell during ethanol treatment. This will be caused by binding free water to hydrophilic surfaces of breakdown products, of the cell wall. They speculated that the formation of large insoluble polymers might be due to the high concentration of tannins in the tannin cells brought about by water loss from the cell. Ittah (1993) suggested the formation of a glycoside bond between the soluble tannin molecules and soluble sugars, which would reduce their ability to bind to proteins, during treatment with carbon dioxide. Oshida et al. (1996) investigated the chemical properties of the insoluble polymers after ethanol treatment and found that the first step of tannin coagulation may

be the formation of noncovalent chemical bonds such as hydrogen bonding. Es-Safi et al. (2002) demonstrated that acetaldehyde is able to polymerise phenolic compounds.

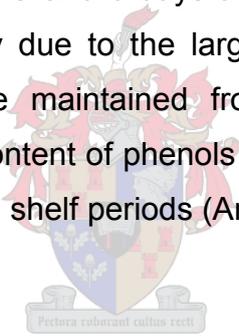
2.4.1 CARBON DIOXIDE (CO₂) AND NITROGEN (N₂)

The use of carbon dioxide and nitrogen gas in astringency removal cause anoxic or hypoxic conditions, which induce fermentative metabolism. The removal of astringency with carbon dioxide (Ben-Arie & Guelfat-Reich, 1976; Matsuo & Ito, 1977) and nitrogen enriched atmospheres has become common commercial practice in persimmon fruit. Postharvest application of high CO₂ has great effects on fruit quality. This includes influences on ethylene, chlorophyll (colour), phenols (astringency), volatiles (Kader, 1997; Beaudry, 1999), fruit firmness and internal or external browning (caused by polyphenol oxidase activity) (Arnal & Del Río, 2003).

A few studies compared the effectiveness of CO₂ and N₂ during different times on the de-astringency process (Zavrtanik et al., 1999; Arnal & Del Río, 2003). It is important to note that the results of the two reports might differ since there was a difference in length of treatment, storage temperature and time after which quality assessment was done. These two studies agreed that CO₂ treatments were more efficient in removing astringency than the N₂ treatments. Astringency removal was dependant on the length of the treatment, best achieved at 27 h. The ethanol and acetaldehyde content was significantly higher in CO₂-treated fruit than those of N₂, with the highest amount at the 27 h-period. However, Zavrtanik et al. (1999) reported that N₂ treated fruit showed greater amounts of ethanol after two days. Fruit firmness and colour were not affected by the different atmosphere treatments but the exposure time showed differences. Fruit at 27 h had lower firmness but a higher colour index than fruit at the 18 h-period. The total soluble solids content and polyphenol oxidase (PPO) activity were not affected by the enriched atmospheres (Arnal & Del Río, 2003). Zavrtanik et al. (1999) found that internal browning occurred after 10 days of storage at 1°C. There was a positive correlation between the rate of astringency removal and acetaldehyde (AA) content (Matsuo & Ito, 1977; Arnal & Del Río, 2003). The CO₂ treatments had higher AA and a higher astringency removal than the N₂ treatments (Arnal & Del Río, 2003). This might be explained by the polymerization of the soluble tannin by the AA to get non-astringent fruit (Matsuo & Ito, 1982). The anaerobic conditions decreased the ratio of oligomeric to polymeric forms of tannins (correlated with astringency) in the fruit as well as the total phenols. This ratio was also lower after treatment with CO₂ than with N₂ (Zavrtanik et al., 1999).

It has been demonstrated in various persimmon cultivars that CO₂ treatment is more effective in reducing the tannin content and thus astringency, than the application of ethanol (EtOH) vapour. The soluble tannin content was significantly less after the CO₂ treatment than after the ethanol treatment (Yamada et al., 2002).

Different postharvest gaseous treatments have been used for table grapes not to remove astringency but to maintain quality during storage life. Fumigation with SO₂ is the most common method to control decay during cold storage (Crisosto et al., 1994). However, SO₂ is corrosive to metals, dangerous to people allergic to sulfites and causes injury to the rachis and berries when used excessively. The use of modified atmosphere packaging (MAP), controlled atmosphere (CA) (Basiouny, 1998; Crisosto et al., 2002) and ozone (O₃) (Palou et al., 2002) have been tested as alternative techniques on table grapes. A MAP of 5 kPa of O₂ + 15 kPa of CO₂ + 80 kPa of N₂ which is the easiest and cheapest technique, maintained the quality of the grapes at harvest. The use of 8 ppm O₃ and CA (5 kPa of O₂ + 15 kPa of CO₂ + 80 kPa of N₂) during cold storage (38 days at 0°C and 6 days at 15°C) increased the total stilbenoid content after shelf life, especially due to the large increase in resveratrol, while the amounts of total flavonols were maintained from harvest. These were the best treatments to enhance the total content of phenols (antioxidants) for cv. Napoleon table grapes after both cold storage and shelf periods (Artés-Hernández et al., 2003).



2.4.2 ETHANOL

Ethanol is a GRAS (generally recognized as safe) product and is therefore considered safe for use with food. The use of ethanol has become a very common practice since acetaldehyde is considered carcinogenic (see section 2.4.3). Ethanol can be applied in many different ways, as a vapour or as a liquid (spraying or dipping) (Pesis, 2005).

The astringency of persimmon fruit is generally removed by the treatment with ethanol. The only downside to this method is that a long time is required. Thus it is commercially impractical. Persimmons treated with ethanol (10 – 20%) vapours followed by ethylene (1, 3, 5, 10 nl C₂H₄/ml) reduced the soluble tannin concentration and the fruit became non-astringent after 6 – 9 days. Even if no ethylene was used the astringency still decreased over time but was not totally removed. The application of ethylene in combination with ethanol is necessary to induce ripening, since some of the cultivars have to be harvested very early due to market demands (Kato, 1990). In bananas, the postharvest application of ethanol vapour (25 – 75%) removed astringency, as measured by the total phenolic content (Esguerra et al., 1992).

It has been shown that there is an effect of ethanol application on ripening and anthocyanin development in grape berries on the vine. An aqueous solution of ethanol was sprayed onto grapes at véraison. It was found to enhance the internal ethylene concentration as well as an increase in the colour intensity of the wine (Chervin et al., 2001).

The ability of ethanol to suppress decay development was shown relatively recently in scientific research. Lichter et al. (2002) showed that dipping grape bunches in 50, 40 or 33% , but not 20% ethanol, inhibited berry decay as well as or better than SO₂ released from generator pads. Decay control was feasible for a cold storage period of 4 – 5 weeks and sometimes even longer. Ethanol had no negative effect on the appearance of the bunch or the firmness and the ethanol treated berries had higher organoleptic scores than the SO₂ treated ones.

2.4.3 ACETALDEHYDE

Acetaldehyde (AA) has been used as a flavour additive and is listed as generally recognized as safe (GRAS) by the US Food and Drug Administration (Pesis, 2005). AA contributes to the freshness, fruitiness and/or nuttiness of many foods such as fruit juice, dairy products, alcoholic beverages, etc. (Wecker & Zall, 1987). The application of AA must be done with care, in a closed system flushing air over liquid AA to avoid human exposure to the compound (Pesis & Frenkel, 1989). Recently, AA has been added to the list of carcinogenic materials and this has reduced the opportunities to apply it commercially.

The postharvest application of AA to non-climacteric fruit has been reported to cause induction of CO₂ production in grapes (Pesis & Mariniansky, 1992). The postharvest treatment of 'Sultanina' and 'Perlette' grapes with AA vapours caused an increased %TSS, decreased acidity and enhanced sensory preference after the grapes initially had a low sugar concentration (13 – 14% TSS) and high acidity (Pesis & Frenkel, 1989).

Acetaldehyde is well known for its fungicidal and insecticidal properties. Several reports have illustrated its effectiveness in controlling the development of postharvest rots like *Botrytis cinerea* and *Rhizopus stolonifer* on grapes (Avissar et al., 1989; Avissar & Pesis, 1991). Acetaldehyde has some adverse effects. Acetaldehyde was applied to Sultanina grapes at high temperatures; it penetrated via the stem and caused rind damage around the stem (Pesis & Frenkel, 1989).

2.5 CONCLUSIONS

Regal Seedless is a white, seedless, South African bred table grape. This cultivar has a lot of positive properties but the sporadic, unacceptable occurrence of astringency can result in a decrease in the demand of this grape from international markets. Astringency is seen as a negative mouth-feel sensation in fresh fruit and is caused by the presence of a group of secondary metabolites called phenolic compounds. The proanthocyanidins are specifically responsible for this astringent sensation. The astringent taste is thought to be due to the interaction of phenols and salivary proteins in the mouth through hydrogen bonding and hydrophobic effects. This result in the 'drying', 'puckering' and 'roughing' sensation perceived throughout the mouth which is not preferable.

Phenolic compounds are divided into two main groups: non-flavonoids and flavonoids. The non-flavonoids can be divided into subgroups namely the hydroxybenzoic acids and hydroxycinnamic acids while the flavonoids are separated into monomeric flavan-3-ols and proanthocyanidins, flavonols and anthocyanins. Each of these groups has a unique contribution to the sensory properties and quality of grapes and wine. It includes colour, flavour, taste and mouth-feel characteristics like the astringent taste.

Environmental factors, viticultural practices and postharvest treatments have an influence on the quality and quantity of phenols in the grape. Environmental factors include maturity, region, climate, seasonal conditions and mineral nutrition in the soil. Cultural practices that are of importance are canopy management, irrigation, growth regulators, pruning, crop load and rootstocks. The de-astringency of fruit have been achieved by postharvest treatments with carbon dioxide, nitrogen gas and anaerobic metabolites, ethanol and acetaldehyde.

A correlation between levels of phenolic compounds, environment, viticultural and postharvest practices was suspected, which led to this investigation. The manipulation of the taste of Regal Seedless has been studied intensively at the ARC Infruitec-Nietvoorbij. Solving this problem was identified by the table grape industry as a very high priority. The producers would benefit from the results by applying the recommended practices to improve grape taste and keep access to certain international markets.

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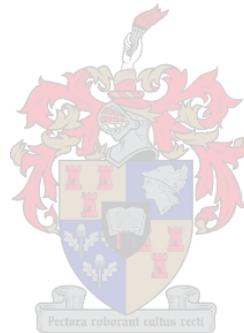
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RESEARCH RESULTS

THE EFFECT OF FRUIT MATURITY ON THE PHENOLIC CONTENT AND TASTE OF REGAL SEEDLESS (*VITIS VINIFERA* L.)

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THE EFFECT OF FRUIT MATURITY ON THE PHENOLIC CONTENT AND TASTE OF REGAL SEEDLESS (*VITIS VINIFERA* L.)

ABSTRACT

The astringent taste of Regal Seedless (*Vitis vinifera* L.) table grapes is negatively affecting its acceptability by table grape industries and consumers. The phenolic composition of Regal Seedless berries was determined during maturation and ripening by spectrophotometric and HPLC analyses. Berries were analysed from véraison for a five week period, over three consecutive seasons. Total soluble solids (TSS), total titratable acidity (TTA) and the sugar-acid ratio were determined. A tasting panel evaluated the berries for bitterness, astringency, skin tenacity and eating quality. Thirty-four different phenolic compounds were identified throughout the trial. Flavanols, which are responsible for the astringent perception, were the most abundant phenolics in Regal Seedless. TSS was identified as the most important physical measurement for the table grape producer to determine maturity. The following significant correlations were found: TSS vs. astringency ($r = -0.54$, $p < 0.01$), TSS vs. eating quality ($r = 0.66$, $p < 0.0005$), TSS vs. total phenolics per berry ($r = 0.86$, $p < 0.0001$) and TSS vs. total flavanols per berry weight ($r = -0.73$, $p < 0.0001$). The influence of seasonal conditions was evident in the results. From this study the optimal maturity level for the harvesting of Regal Seedless could be determined.

KEYWORDS: Astringency; maturation; flavanols; flavonols; total phenolics; HPLC; table grapes; Regal Seedless; *Vitis vinifera*

3.1 INTRODUCTION

Regal Seedless (*Vitis vinifera* L.) is currently one of the major new table grape cultivars in South Africa. The white cultivar has a few good characteristics: seedless with inherent large berries, very fertile vines, requires minimal bunch preparation and has good storage ability. However, the acceptability of the cultivar on local and international markets is affected by a sporadic, unacceptable astringent taste. Astringency is a negative mouth-feel characteristic in fresh fruit like persimmons and table grapes, while it is an important organoleptic property in beverages like wine.

Astringency has been defined as 'the complex of sensations due to shrinking, drawing or puckering of epithelium as result of exposure to substances such as alums or tannins' (1). Astringency perception is a mouth feel or tactile sensation (2), most commonly described as a 'drying', 'roughing' and 'puckering' feeling (3). The astringent sensation is due to the presence of phenolic compounds (4) in especially the group called the flavonoids (5). Monomeric and polymeric flavan-3-ols (also called the proanthocyanidins) induce both astringent and bitter sensations and have been reviewed extensively (6-9). The relative astringency of the flavonoids has been reported to increase with the degree of polymerisation (4, 10–12).

Fruit maturation is a complex process where physical and chemical changes take place within the berry from véraison until physiological maturity. The development of the grape berry follows a typical double sigmoid growth pattern which can be divided into three stages. Stage I is an initial rapid growth phase occurring after berry set and consists mainly of cell division. Stage II is a lag phase during which véraison occurs. Stage III is the second growth (ripening) phase and is entirely due to enlargement of the pericarp cells (13–15). During the ripening phase the total titratable acids decrease, while berry weight, pH and sugar concentration increase. The levels of the different phenolic compounds also undergo changes during the ripening process.

In the wine industry, the concept of phenolic maturity has become a talking point in the last few years. The evolution of phenolic compounds during ripening in the skin, berry and seeds of red and white wine grapes is well documented (16–18). It was found that the concentration of total phenolics in the skin increases from véraison (15, 17, 19) while the phenol concentration in the seeds decreases (15). The concentration of phenolics in the pulp is very low (20). Singleton (16) found that the total phenol content increased on a per berry basis (entire berry, including seeds) during maturation. This suggests that the increase in skin phenolics is substantial since the phenols in the seeds decrease. In the last month of ripening the total phenols per berry is quite constant, but it may decrease during advanced ripeness (20). Singleton (16) discovered that the total phenol content per berry weight decreased during maturation and he and Boulton et al. (20) suggested that the greater increase in berry weight diluted the accumulation and probable net synthesis of phenols in the berry.

The individual phenolic compounds also change in concentration during maturation. The monomeric flavan-3-ols and the polymeric proanthocyanidins showed declining trends from the onset of ripening until maturation (15, 21, 22). The concentration of catechin in the skin decreased rapidly from véraison (22, 23), while there was an

increase in the level of epicatechin (22). The mean degree of polymerisation of skin proanthocyanidin increased steadily during fruit maturation (23, 24). The concentration of hydroxycinnamic-tartaric acids in the must decreased during ripening while the benzoic and other cinnamic acids and their aldehydes in the skin demonstrated an increase (21, 25, 26). The flavonols in the skin also increased from véraison, reaching a maximum around maturity, after which there was a decline (23, 26).

The objective of this trial was to determine the optimum maturity level of Regal Seedless grape berries where the phenolic content and specifically the concentration of flavanols, will be the lowest and the astringent taste the least.

3.2 MATERIALS AND METHODS

3.2.1 EXPERIMENTAL SITE

Grape samples were collected from a three-year-old *Vitis vinifera* L. cv. Regal Seedless commercial table grape vineyard on Moselle, a farm situated in the Hex River Valley in the Western Cape, South Africa. This is a Mediterranean area with typical warm, dry summers and cool, wet winters. Figure 1 shows the monthly temperature and rainfall for the De Doorns experimental farm weather station (the closest weather station to experimental site) for the three seasons over which the trial was conducted. From the period November to February the mean maximum temperature was 30.5°C, the mean minimum temperature was 13.6°C and the mean rainfall was 15.5 mm for the three years during which the study was done. The vineyard consists of Regal Seedless as scion cultivar grafted on Ramsey (*Vitis champinii*). The vines were spaced 3.0 m x 1.5 m on a loamy soil with a slightly acidic pH and trained onto a gable trellis system. The rows were planted in an east-west direction.

3.2.2 SAMPLE COLLECTION

Regal Seedless fruit samples were harvested at weekly intervals from approximately véraison until a few weeks after commercial harvest during the 2002/03, 2003/04 and 2004/05 seasons for a five week period. This was done mid-morning when it was still cool. Bunches were taken randomly from different vines, alternately from both sides of the rows at varying heights and distances from the trunk. Only clusters in the shade were harvested. The block had a one-row and one-panel buffer to avoid end effects. After collection the grapes were packed into six separate 2 kg cartons according to

commercial export standards and placed in a cold storage room for four weeks at -0.5°C (simulating shipping period overseas) followed by one week at 15°C (simulating shelf life period). As part of standard procedures, the cartons were evaluated separately after the cold storage period for defects. A random sub-sample of 100 berries was taken at each harvest date from the Regal Seedless vineyard, to monitor the increase in total soluble solids during the ripening period.

3.2.3 SENSORY EVALUATION

A sensory evaluation was conducted on each carton after the cold storage evaluations. A panel consisting of six people tasted the grapes for four properties, namely bitterness, astringency, skin tenacity and overall eating quality. The panel rated the properties on an 11-point unstructured line scale (scale from 0 – 10). A score of zero meant no bitterness, no astringency, soft skin and not favourable to eat, and a score of ten meant high in bitterness and astringency, high skin tenacity and very favourable to eat. The judges ate unsalted cream crackers between samples to clean their palates to prohibit the carry over-effect of astringency.

3.2.4 PHYSICAL MEASUREMENTS

A fresh berry sample (100 berries per sample) was taken from each replicate after storage and was used for berry mass and juice analyses. The total soluble solids (TSS) was measured with a bench refractometer and expressed as $^{\circ}\text{Brix}$. The pH and total titratable acidity (TTA) were determined by titrating a 25 mL sample with 0.333 M NaOH to a pH end point of 7, using a Mettler DL21 titrator (Mettler Toledo, Switzerland). TTA was expressed in g/L.

3.2.5 SPECTROPHOTOMETRIC ANALYSIS

A random sample of 50 berries was taken from each of the cartons and stored at -20°C until further analysis. These berries were removed from the bunches with the pedicel still intact, without damaging the skin. Total phenolics of whole berries were determined by a spectrophotometric method described by Iland *et al.* (27). A few modifications were, however, made by us. The grapes were removed from the -20°C freezer, thawed in a refrigerator overnight, destemmed, weighed and then homogenized with an Ultra-Turrax T25 disperser (IKA[®], Germany) at 24000 rpm for 1 min. The weight of a scoop of approximately five grams of the homogenate was recorded and then extracted with 10 mL of 50% aqueous ethanol adjusted to pH 2.0. The contents were mixed

periodically by inverting the tube every 10 minutes for one hour. Thereafter, the samples were centrifuged at 1100 x g for 30 min in an ALC[®] 4235A centrifuge (Milan, Italy) and the volume of the extract (supernatant) determined. A Helios Gamma UV-Vis spectrophotometer (Model no. 9423UVG1702E) (Thermo Electron Corporation, Johannesburg, South Africa) was used to measure the absorbance at 700 nm, 420 nm, 365 nm, 320 nm and 280 nm. This determined, respectively, the sample turbidity, oxidation state, flavonol, cinnamic acid and total phenol content of the sample. Excessive turbidity is an indication of insufficient clarification during centrifugation. The A_{700} should be < 0.01. The oxidation state indicates if any problem occurred during preparation of the samples. These are the main absorbance areas of the different phenolic groups. The total phenols per berry (1) and total phenols per gram berry weight (2) were determined as follows:

1) $A_{280} \times [\text{final extract volume (mL)} / 100] \times [\text{weight of 50 berries (g)} / \text{weight of homogenate for extraction (g)}] \times [1 / 50]$ expressed as absorbance units (AU) per berry (27)

2) $A_{280} \times [\text{final extract volume (mL)} / 100] \times [\text{weight of 50 berries (g)} / \text{weight of homogenate for extraction (g)}] \times [1 / \text{weight of 50 berries (g)}]$ expressed as absorbance units (AU) per gram berry weight (27).

A sample of the extract was filtered through a 0.45 μm Cameo Nylon filter, transferred to a 1.8 ml HPLC auto-sampler vial and analyzed with high-performance liquid chromatography using diode array detection (HPLC-DAD).

3.2.6 CHROMATOGRAPHIC ANALYSIS

3.2.6.1 HPLC-DAD analysis

A Hewlett Packard Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used with a binary pump and a diode array detector. Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100 \AA , 250 \times 4.6 mm, 5 μm) from Polymer Laboratories (Ltd) (Shropshire, UK) protected with a guard cartridge (PLRP-S, 10 \times 4.6 mm) (Polymer Laboratories (Ltd), Shropshire, UK) with the same packing material.

The mobile phase consisted of Solvent A: 1.5% aqueous ortho-phosphoric acid (Sigma-Aldrich, Kempton Park, South Africa) and Solvent B: 80% HPLC grade acetonitrile (Sigma-Aldrich, Kempton Park, South Africa) / 20% of 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. Similar conditions had been previously employed with satisfactory results (28, 29). An injection volume of 100 μ L was used. A blank and known standard mix were run after every ten samples, the column temperature was held at 35°C and the system was equilibrated for 15 min at the starting solvent conditions between samples to ensure a stable baseline, consistent retention times and validity of the standard curves. The spectra were recorded from 250 nm to 400 nm.

3.2.6.2 HPLC-MS analysis

LC-MS was performed using a Waters API Quattro Micro and Waters API Q-TOF Ultima connected to a Waters UPLC (Waters Corporation, Massachusetts, USA) system. Chromatographic separation was carried out on a PLRP-S column (100Å, 250 \times 4.6 mm, 5 μ m, Polymer Laboratories (Ltd), Shropshire, UK) using Solvent A: 1% formic acid/water solution (Merck) and Solvent B: 80% HPLC grade acetonitrile (Sigma-Aldrich, Kempton Park, South Africa) / 20% of solvent A as mobile phases. Elution was performed with the same gradient conditions as described before. The phenols were detected with an electrospray ionization (ESI) system in the negative mode. A cone voltage of 25 V and 35 V were respectively used with the Quattro Micro and Q-TOF Ultima MS systems. A capillary voltage of 3.5 kV with a desolvation temperature of 400°C was also employed. UV chromatograms were recorded at 360 nm, 320 nm and 280 nm. The LC-MS analyses were performed to identify the unknown phenolic compounds detected by HPLC-DAD.

3.2.7 PHENOLIC IDENTIFICATION AND QUANTIFICATION

Chromatograms were recorded at 280 nm, 316 nm and 360 nm. The absorbance at 280 nm was used for the quantification of the benzoic acids and flavanols, 316 nm for the cinnamic acids and 360 nm for the flavonols.

Identifications were carried out by comparing the retention times and UV spectra of the relative peaks found at chromatograms of Regal Seedless to published data (28–32) as well as authentic standards. The following authentic standards were analysed on the HPLC: gallic acid, epigallocatechin, galocatechin, catechin, procyanidin B1 and B2, epicatechingallate, *p*-coumaric acid, caffeic acid, kaempferol, quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-rutinoside and myricetin.

Benzoic acids, flavanols, cinnamic acids and flavonols were quantified respectively, as gallic acid, catechin, *p*-coumaric acid and quercetin-3-rutinoside (rutin) equivalents.

These four commercial standards were bought from Sigma-Aldrich (Kempton Park, South Africa) and were of the highest quality available. Concentrations of gallic acid, catechin, *p*-coumaric acid and rutin equivalents were calculated from linear standard curves prepared from the injection of four different concentrations of each of the four commercial standards. Phenolic content was expressed as mg per berry and mg per gram berry fresh weight. Some of the compounds were present in very low concentrations and the phenolic content was then expressed in μg .

LC-MS analysis enabled us to identify a gallodimer, a dimermonogallate, kaempferol-glucoside/glucuronide and quercetin-glucoside. Other phenolic compounds were also identified, but the data are not reported in this paper.

3.2.8 PRECISION STUDY AND LIMIT OF QUANTIFICATION

Repeatability was assessed using six determinations at 100% of the test concentration and the relative standard deviation (RSD) calculated. The acceptance criteria for precision were at a level of 3% RSD. The limit of quantification was taken to be the lowest amount giving a signal-to-noise ratio of approximately 7:1 or more.

3.2.9 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was completely randomised. There were five treatments (harvest dates) and six replications with a single carton constituting an experimental unit. The statistical software programme, SAS version 8.2 (33) was used for all the statistical analyses. The data were subjected to an analysis of variance (ANOVA) using the General Linear Means procedure. The Shapiro-Wilk test (34) 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.

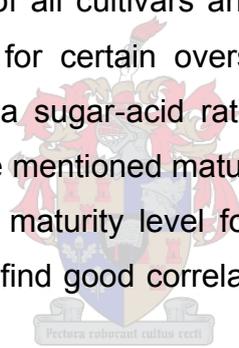
3.3 RESULTS AND DISCUSSION

3.3.1 JUICE ANALYSIS

Figure 2 shows a typical pattern of change in chemical composition of Regal Seedless berries during maturation and ripening. There was a linear increase ($R^2 = 0.88 - 0.99$, $p < 0.0001$) in total soluble solids (TSS) during ripening. Significant differences were found between the harvest dates, which would make it possible to attain an optimum maturity. The changes in pH followed the same linear pattern ($R^2 = 0.93 - 0.96$,

$p < 0.0001$) as TSS with significant differences, while the total titratable acidity (TTA) of the berries decreased significantly over time, also following a linear curve ($R^2 = 0.75 - 0.99$, $p < 0.0001$). The sugar-acid ratio increased linearly ($R^2 = 0.92 - 0.98$, $p < 0.0001$) with fruit ripening. All the treatments were significantly different from each other. The same significant differences and tendencies were observed over all three years. It is well known that these changes in sugar, pH and acid take place during fruit maturation (14, 35, 36). The ripening season was about two weeks earlier in 2004/05. We therefore missed the first two weeks and were not able to harvest the grapes from exactly véraison.

The measurement of TSS is the best parameter for the table grape producer to calculate the ideal maturity to harvest. The sugar-acid ratio is generally used as a maturity criterion for table grapes. The sugar content on its own is, however, preferred due to easy determination (37). László & Saayman (38) also confirmed that TSS was a more reliable indicator of harvest maturity than the sugar-acid ratio. Export companies provide the producer with a list of all cultivars and recommended sugar levels and/or sugar-acid ratios that are ideal for certain overseas markets. The minimum export standard for Regal Seedless is a sugar-acid ratio of 30:1 and TSS of 16°Brix. The cultivar must comply with both the mentioned maturity indices or with a minimum TSS of 17°Brix (39). The recommended maturity level for Regal Seedless is 18°Brix (40). It would thus be ideal, if one could find good correlations between sugar content and the other parameters measured.



3.3.2 SENSORY EVALUATION

During 2002/03 and 2004/05 the score for astringency was inconsistent throughout the whole sampling period (Table 1). No significant differences were found between the different harvest dates. There was, however, a linear decrease ($R^2 = 0.76$, $p < 0.01$) in astringency over the harvest period of 2003/04, but only the last harvest date was rated significantly lower than the rest. Astringency correlated positively with TTA ($r = 0.45$, $p < 0.05$) and negatively with TSS ($r = -0.54$, $p < 0.01$) and the sugar-acid ratio ($r = -0.55$, $p < 0.005$) during 2003/04, but similar results were not found in the other seasons (data not shown). These relationships in 2003/04 are in accordance with the findings of Tiitinen et al. (41) who studied the sensory quality of buckthorn varieties.

The eating quality (EQ) score increased linearly with increasing harvest time for both 2002/03 ($R^2 = 0.85$, $p < 0.01$) and 2003/04 ($R^2 = 0.78$, $p < 0.01$) (Table 1). Significant differences, according to the P-value, were only found in 2003/04, the first

harvest date being significantly lower than the rest. No trends or significant differences were found in the 2004/05 season. There was a positive correlation between EQ and TSS and a negative correlation between EQ and TTA in 2002/03 and 2003/04 (Figure 3 and 4, respectively) but the same correlations were not found in 2004/05. Similar results were obtained by Sonogo et al. (42) who found that TSS gave the best correlation with overall EQ, while neither TTA nor sugar-acid ratio showed a good correlation with EQ. It is generally accepted that the grapes will become more edible as the sugar increases and the acid decreases, but one can assume that there will be a turning point where the sugar levels become too high and are not preferred by the tasters. This was seen in the 2004/05 season where the EQ started to decrease when the sugar level reached $\pm 20^\circ$ Brix and higher. It is interesting to note that in all the seasons the lowest astringency score resulted in the highest eating quality rating. A negative correlation between astringency and EQ was found in the 2003/04 ($r = 0.84$, $p < 0.0001$) (Figure 5) and 2004/05 season ($r = 0.80$, $p < 0.0001$).

The organoleptic scores for skin tenacity were only significantly different in 2002/03, where the highest skin tenacity was accompanied by the highest astringency (Table 1), but no correlation was found between skin tenacity and the juice analysis (data not shown). No specific trend could be found for the three years investigated. There was, however, a positive correlation between skin tenacity and astringency in all three seasons: 2002/03 ($r = 0.54$, $p < 0.01$), 2003/04 ($r = 0.62$, $p < 0.01$) and 2004/05 ($r = 0.74$, $p < 0.0001$) (data not shown). Since high concentrations of phenols are present in the skin, a thicker skin can cause the taster to chew the grape more, releasing more phenols into the mouth. The phenols bind to the proline-rich proteins in the oral cavity, leading to an increase in astringency perception (43, 44). Significant correlations were found between skin tenacity and eating quality in 2003/04 ($r = -0.50$, $p < 0.05$) and 2004/05 ($r = -0.78$, $p < 0.0001$) (data not shown). This is an indication that a thick and hard skin is not preferred by the tasters. The sensory property, bitterness, will not be discussed since the data might not be that relevant due to the fact that only a percentage of people are sensitive for this taste. Another concern is that many people have difficulty to distinguish between bitterness and astringency. The data might have been more accurate if each of the tasters' sensitivity to bitterness had been determined beforehand.

Sensory evaluation is a subjective assessment. This may contribute to the inconsistent data of some seasons. The differences between individual tasters may have an influence on the sensory evaluations. These differences include gender, age,

physiological state (salivary flow rate), genetics and psychology. Other factors that may affect tasting ability includes adaptation, fatigue and genetic predisposition (45). For these reasons it is important to use an objective, reproducible, non-destructive and quantitative measurement.

3.3.3 SPECTROPHOTOMETRIC ANALYSIS

Figures 6 and 7 show the development of total phenols per berry and berry weight respectively, as a function of the total soluble solids content. The total phenols per berry followed the same pattern in all the seasons; an increase followed by a decrease. The total phenols per berry weight, however, showed no specific pattern. The total phenols per berry will probably correlate better with the sensory analysis, while per berry weight will correlate better with the other analytical data. Significant differences were found between the means of total phenolics (A_{280}) per berry and flavonols (A_{365}) per berry during the three seasons of the trial (Table 2). A linear increase (2002/03: $R^2 = 0.65$, $p < 0.001$; 2003/04: $R^2 = 0.87$, $p < 0.0001$) was observed for the total phenols per berry during the first two seasons, while a quadratic trend ($R^2 = 0.76$, $p < 0.01$) was found in 2004/05. We suspect that the first two seasons would also follow a quadratic trend if the sampling period was extended. An increase in total phenols on a berry basis may indicate an increase in phenolic concentration of the existing phenols or the formation of new phenols that have a higher 280 nm absorbance (larger extinction coefficient) than the original phenols. The opposite is also true. A decrease in total phenols per berry may indicate the breakdown of phenols later in the season or the formation of phenols with a lower 280 nm absorbance (smaller extinction coefficient) than the original phenols. The rapid increase per berry is in accordance with the findings of Singleton (16), Boulton et al. (20) and Kennedy et al. (23). They, however, studied red wine grapes which mean that the increase is probably associated with an increase in anthocyanins. Meyer et al. (46) pointed out that white varieties are rich in flavanols to compensate for the absence of anthocyanins. The development of total phenolics throughout ripening in table grapes has not been published previously. Since only two unidentified cinnamic acids with very low concentrations were detected with HPLC the A_{320} reading will not be discussed.

On a weight basis, significant differences were found for the total phenolics and flavanols during 2002/03 and 2004/05, while no significant differences were recorded in 2003/04 (Table 3). In contrast with the per berry unit, different trends were found for the total phenols on a weight basis. There was a quadratic trend in 2002/03 ($R^2 = 0.66$,

$p < 0.0001$) and a linear increase in 2004/05 ($R^2 = 0.86$, $p < 0.0001$). This is conflicting to the results of Singleton (16) who found a decrease in total phenols on a weight basis. These discrepancies between per berry and per berry weight are due to the increase in berry size during the season which does not have an influence on the per berry measurements.

The highest values for total phenolics on per berry and per berry weight basis were found in 2003/04, while the lowest values were in 2002/03. This is an indication of how much the phenolic levels can differ due to seasonal variability (21, 47, 48). The influence of weather conditions on the grape ripening process is well known and is one of the main reasons for seasonal differences (49–51). The average rainfall during the period November to February was the lowest in 2003/04 and the highest in 2002/03 (Figure 1). It has been reported that a drier season and thus vine water deficit gives rise to higher phenol levels (23, 52, 53). It is, therefore, important to take seasonal differences into consideration when choosing the optimum maturity. It is again important to point out that the sampling period started later during 2004/05, when harvesting only began at 16.9°Brix instead of approximately 15°Brix. One of the reasons for an earlier season was the higher temperatures from November 2004 until harvest. This resulted in the sampling period stretching to 23.4°Brix in comparison to 19.0°Brix or 19.7°Brix in the other two seasons. Climatic conditions and different windows of the harvest period might be the reason for different trends between seasons.

When seeking correlations between TSS and total phenols per berry and per berry weight, each season differed from another. In 2002/03 and 2003/04 positive linear correlations were found between TSS and total phenols per berry ($r = 0.66$, $p < 0.001$ and $r = 0.86$, $p < 0.001$), while there was no relationship found in 2004/05. However, on a per weight basis no correlations were found with TSS during the first two seasons, while there was a linear correlation ($r = 0.76$, $p < 0.001$) in 2004/05. This contradicts the findings of Pirie & Mullins (19) and Singleton & Esau (49) who did not find any relationship between TSS in the berry and total phenols in the skins. If they analyzed the whole berry for total phenolics, similar results might have been found.

There were no correlations found between total phenols per berry or per berry weight and the sensory data. This is partly due to the fact that the absorbance reading at 280 nm provides only an estimate of the concentration of total phenolics present in the grape. Astringency, however, is caused by a specific group of phenols called the flavonoids (5). In contrast to this, Condelli et al. (54) and Amarowicz et al. (55) found a significant correlation between astringency and total phenol content.

Analysis of individual phenols with HPLC would firstly expand our knowledge of phenol development in Regal Seedless and secondly enable us to identify the specific phenols that are responsible for the astringent sensation and monitor their change in concentration through maturation.

3.3.4 HPLC-DAD ANALYSIS

Thirty-four different compounds were identified throughout the whole trial. The levels of these phenols in the grapes differed between the seasons. Some of the phenols were not observed during all the seasons, while others could not be quantified due to the fact that they were present in amounts below the limit of quantification (LOQ). The LOQ of each of the standards were: gallic acid, 0.2 mg/L; catechin, 1.0 mg/L; *p*-coumaric acid, 0.1 mg/L and quercetin-3-rutinoside, 0.2 mg/L.

Flavanols: Twenty-seven different flavanols were identified, although not all were quantifiable in all three seasons. The flavanols formed the largest percentage of the phenolic composition of Regal Seedless grapes. The polymeric fraction (include trimers, oligomers and polymers), a gallodimer (gallodimer1) and a dimermonogallate (dimermonogallate2) were present in the highest concentrations throughout the trial. Epicatechin, catechin, procyanidin B1, procyanidin B2 and gallodimer2 were the other main flavanols (data not shown). A few of the above mentioned compounds have been previously identified in white table grapes (Superior, Dominga and Moscatel Italica) via LC-MS (56). The rest of the flavanols were present in small concentrations and/or unidentified. The total flavanols were calculated by adding all the identified and unidentified flavanols detected.

The total flavanols, polymeric fraction and dimermonogallate2 followed decreasing linear trends during ripening on a per berry (data not shown) and berry weight basis (Table 4). These results are in accordance with Lee & Jaworski (21), Fernández de Simón et al. (26) and Downey et al. (57). Downey et al. (22) found that the proanthocyanidin levels in the skin decreased between véraison and harvest. Since a decrease in the concentration of the main flavanols was observed, a decrease in astringency perception might be expected. The gallodimer1 levels varied a lot between the seasons. Significant differences were observed between the means of the treatments for the three main compounds in all three seasons (Figure 8).

The total amount of flavanols ranged between 0.20 – 1.01 mg/g berry weight in 2002/03, 0.59 – 1.56 mg/g berry weight in 2003/04 and 0.40 – 0.54 mg/g berry weight in

2004/05. Figure 9 shows the relationship between TSS and total flavanols. In 2002/03 and 2003/04 the flavanols decreased up to 19 and 18.5°Brix, respectively. In 2004/05 the flavanols remained relatively constant and markedly lower than in previous seasons. Good correlations were found between TSS and total flavanols in 2002/03 ($r = -0.77$, $p < 0.0001$) and 2003/04 ($r = -0.73$, $p < 0.0001$), but in 2004/05 a weak correlation ($r = -0.39$, $p < 0.0356$) was found. The same observations were found for dimermonogallate2 and the polymeric fraction (Figure 8). The concentration of gallodimer1 was the lowest between 17 and 18.5°Brix. The contribution of flavanols to the total phenolics ranged from 80 – 92%. The polymeric fraction formed 58 – 86% of the total phenols and the rest of the flavanols a mere 11 to 25%. These percentage-ranges cover the three consecutive seasons. The differences observed between total phenols determined spectrophotometrically and by HPLC is a function of the phenolic concentration and their individual extinction coefficients. It is possible that an apparent decrease in the concentration during maturation is due to the fact that the phenolic compounds change to compounds with smaller extinction coefficients than catechin, when the absorbance at 280 nm stayed constant. If the concentration decreased, it could mean that phenolics with higher extinction coefficients make up a larger percentage of the phenolic content. The development of individual phenolic compounds in table grapes has not previously been published.

No correlations were found between the sensory data and any of the individual flavanols or group of flavanols, whether expressed as per berry or per berry weight. This is possibly due to the fact that the total observed astringency is a function of the matrix, the concentration of the individual flavanols as well as the flavanol composition of the sample. Astringency increases with the concentration of a specific flavanol and generally with an increase in polymerisation. The astringency of an individual phenol depends on its ability to interact with the saliva proteins. The continual building and degradation reactions of the flavanol oligomers could constantly influence the astringency observed.

Of the three most abundant flavanols, only the polymeric fraction and dimermonogallate2 were highly correlated with TSS: TSS vs. dimermonogallate2 (2002/03: $r = -0.75$, $p < 0.0001$; 2003/04: $r = -0.76$, $p < 0.0001$; 2004/05: $r = -0.71$, $p < 0.0001$) and TSS vs. polymeric fraction (2002/03, 2003/04: $r = -0.76$, $p < 0.0001$). TSS also correlated with the concentration of total flavanols (2002/03: $r = -0.77$, $p < 0.0001$; 2003/04: $r = -0.73$, $p < 0.0001$). TTA also showed good correlations with dimermonogallate2 (2002/03: $r = 0.64$, $p < 0.01$; 2003/04: $r = 0.85$, $p < 0.0001$; 2004/05:

$r = 0.60$, $p < 0.005$), polymeric fraction (2002/03: $r = 0.73$, $p < 0.0001$; 2003/04: $r = 0.87$, $p < 0.0001$; 2004/05: $r = 0.43$, $p < 0.05$) and total flavanols (2002/03: $r = 0.74$, $p < 0.0001$; 2003/04: $r = 0.86$, $p < 0.0001$). The sugar-acid ratio showed good relationships with: dimermonogallate2 (2002/03: $r = -0.77$, $p < 0.0001$; 2003/04: $r = -0.75$, $p < 0.0001$; 2004/05: $r = -0.59$, $p < 0.005$), polymeric fraction (2002/03: $r = -0.80$, $p < 0.0001$; 2003/04: $r = -0.76$, $p < 0.0001$; 2004/05: $r = -0.40$, $p < 0.05$) and total flavanols (2002/03: $r = -0.81$, $p < 0.0001$; 2003/04: $r = -0.74$, $p < 0.0001$).

Flavonols: Seven flavonol glycosides were identified and were present in the grape in all three seasons. The main flavonols found in Regal Seedless were quercetin glycosides: quercetin-3-glucoside, quercetin-3-galactoside and quercetin-3-rhamnoside. Quercetin-3-glucoside was also found to be the dominant flavonol in Dominga, Superior and Moscatel Italica white table grape varieties (56). The other flavonols were detected in smaller amounts. They were quercetin-3-rutinoside, myricetin-3-glucoside, kaempferol-3-galactoside and kaempferol-3-glucoside or kaempferol-3-glucuronide.

The total flavonol content ranged between 16 – 26 $\mu\text{g/g}$ berry weight in 2002/03, 28 – 35 $\mu\text{g/g}$ berry weight in 2003/04 and 43 – 100 $\mu\text{g/g}$ berry weight in 2004/05. Figure 10 illustrates the relationship between total soluble solids and total flavonols over the three years. The concentration of total flavonols ($\mu\text{g}/\text{berry}$ and $\mu\text{g}/\text{berry weight}$) was constant in 2002/03 and 2003/04. In 2004/05 there was a substantial increase from $\pm 19^\circ\text{Brix}$. This was also found for the individual flavonols. Downey et al. (58), however, observed that the total flavonols per weight was fairly constant from véraison till harvest. The increase on a per berry basis found in 2004/05, agrees with the findings by Kennedy et al. (23), Fernández de Simón et al. (26) and Downey et al. (58). The same increase may have been observed in the other seasons if the sampling period was extended. This increase is possibly partly a result of the grapes being more exposed to sunlight due to a more open canopy (28, 59). Thus, the flavonols were acting as UV protectants. Flavonol synthesis also takes place in grapes during this ripening period (57).

The contribution of flavonols to the total phenolic content ranged from 2 to 20% over the three seasons. It is evident that the maturity levels of the grapes and seasonal conditions have a great influence on flavonol development. There were poor correlations in 2002/03 and 2003/04 between juice analysis and the different individual or total flavonols. In 2004/05 good correlations were found eg. TSS vs. total flavonols ($r = 0.78$, $p < 0.0001$), TSS vs. quercetin-3-glucoside ($r = 0.82$, $p < 0.0001$). A possible

reason for this is the fact that the sampling period started later in the season and the increase in sugar was accompanied by the expression of the flavonol synthase genes (57). No correlations were found between the four sensory properties and the different flavonols or total flavonols, whether expressed as per berry or per fresh berry weight.

Quercetin appears to elicit a bitter taste with weak astringency in alcohol solutions and in beer (60). There are no studies on the threshold values of the rest of the flavonols. It is possible that the flavonols can develop a synergistic effect between themselves or the other phenolic compounds within the grape and then contribute to astringency.

Non-flavonoids: Only one unknown benzoic acid and two unknown cinnamic acids were detected by HPLC with very low concentrations. Thus they will not be discussed.

Total phenolics: The total phenolic content via HPLC was determined as the sum of the flavanols, flavonols, cinnamic acids and benzoic acids. The total phenolics decreased during maturation for the first two years (data not shown) and were fairly constant in 2004/05. Since the group of flavanols contribute such a large percentage towards the total phenols, the same pattern was found for both parameters. No correlation was found between the spectrophotometric value at 280 nm and the total phenolics measured with HPLC. A possible reason is the fact that all phenolic compounds have an absorbance at 280 nm when measured with the spectrophotometer, while with HPLC we calculated the total phenolics by adding the amounts determined at each of the phenolic group's maximum absorbance which is respectively 280 nm, 316 nm and 365 nm in catechin, *p*-coumaric acid and quercetin-3-rutinoside units.

3.4 CONCLUSIONS

The role of seasonal differences is evident in this study. The influence of climatic conditions on the grape ripening process and in particular the phenolic composition is well known and gives rise to differences between harvests. It is also responsible for fluctuations within a harvest period.

The eating quality (EQ) of Regal Seedless improved from 17°Brix and upwards. The total flavanols (HPLC), which are mainly responsible for astringency, were the lowest between 18 – 19°Brix. Certain practical implications like the occurrence of browning of

the berries during cold storage at high harvest maturities and the fact that certain overseas markets prefer a more greenish grape than a yellow one prohibit us to harvest the grapes at high sugar levels. Thus, the recommended harvest maturity for Regal Seedless is 17 – 18°Brix.

Future recommendations are to determine a minimum value of acceptability for astringency and eating quality before the sensory evaluation are conducted. Since the grapes on the vine do not ripen at the same tempo, each berry and cluster differ in their sugar content. Grape berries can be separated with sucrose solutions after cold storage into groups with the same sugar levels (61). The best correlation between TSS and the rest of the parameters are possible if sensory evaluation, juice and phenolic analysis are conducted on berries with the same sugar level.

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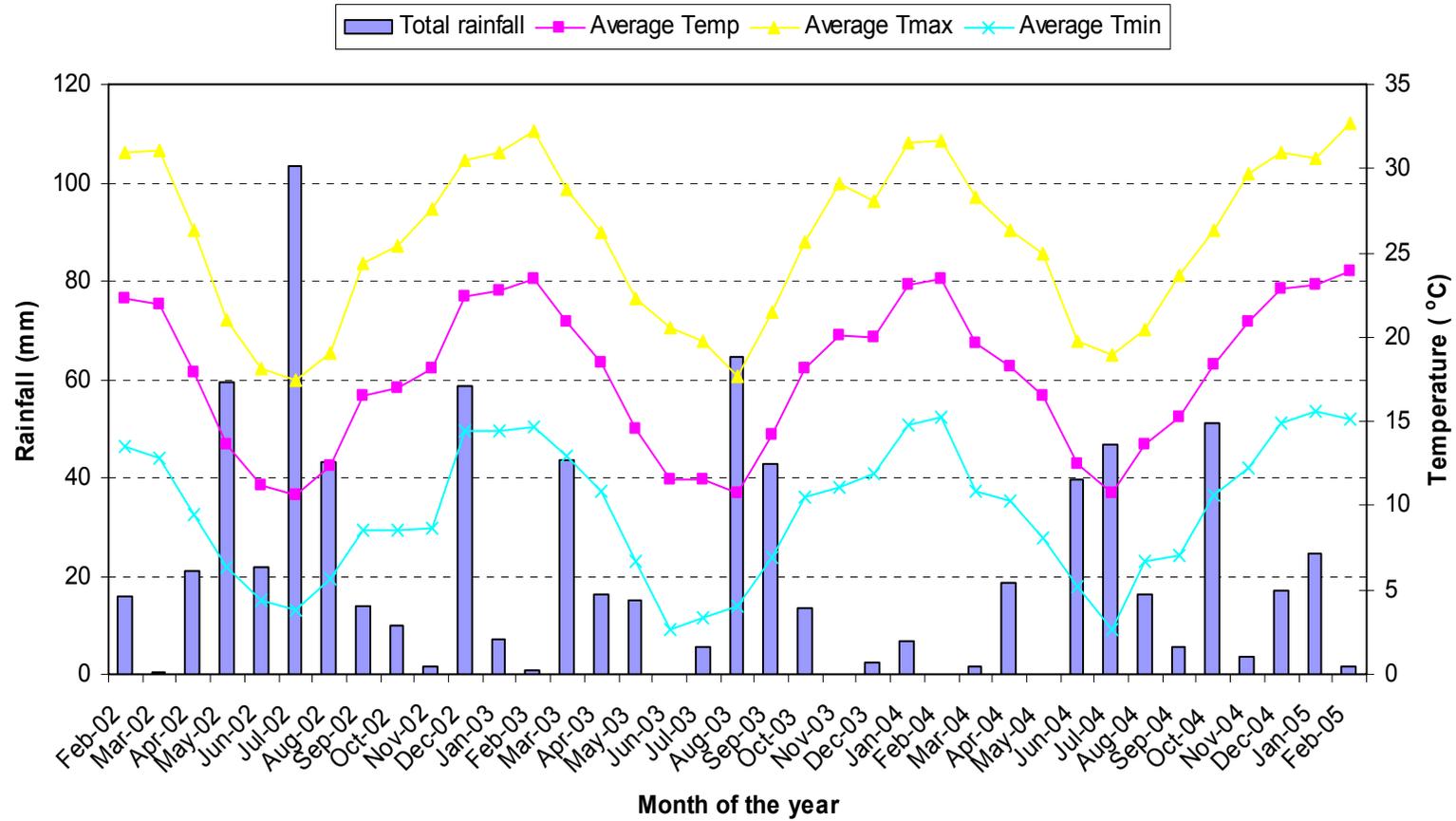


Figure 1 Monthly temperature and rainfall for the De Doorns Experimental Farm, Hex River Valley, South Africa (Source: ARC-ISCW).

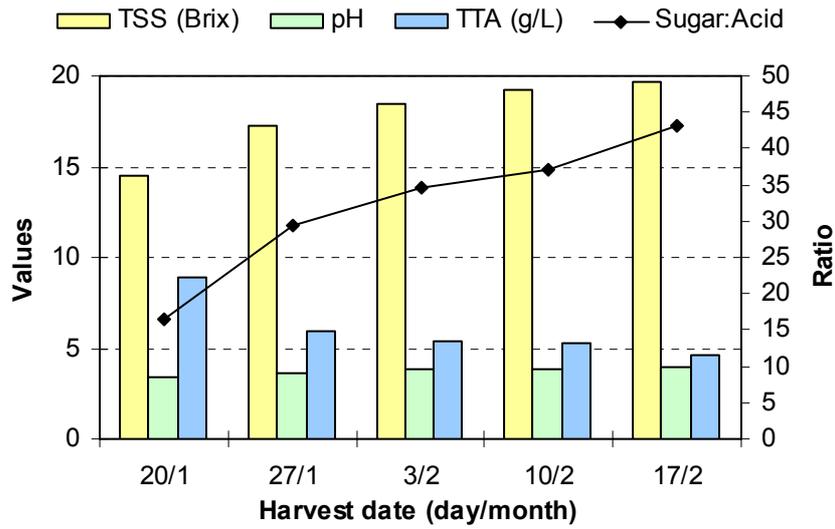
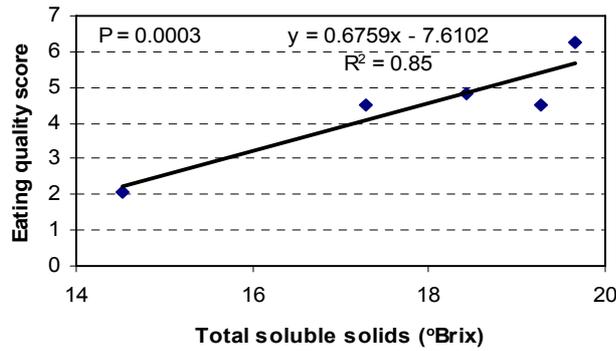


Figure 2 The change in berry composition for 2003/04 ($P < 0.0001$ for all variables).



Pectera roburant culius recti

Figure 3 The relationship between eating quality and total soluble solids for 2003/04.

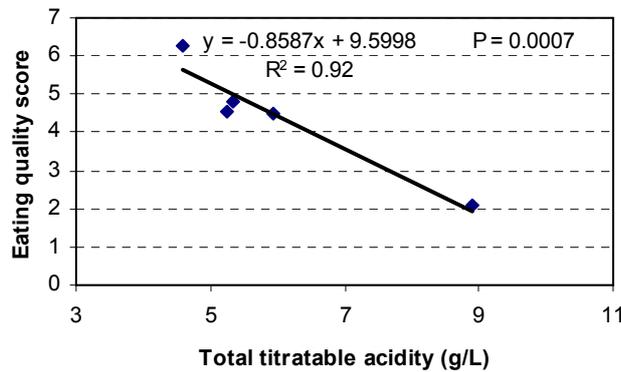


Figure 4 The relationship between eating quality and total titratable acidity for 2003/04.

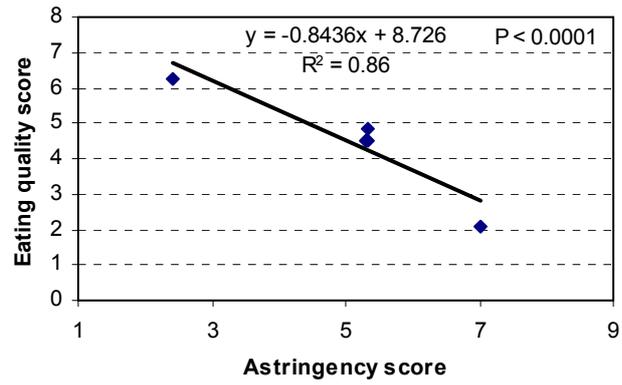


Figure 5 The relationship between eating quality and astringency for 2003/04.

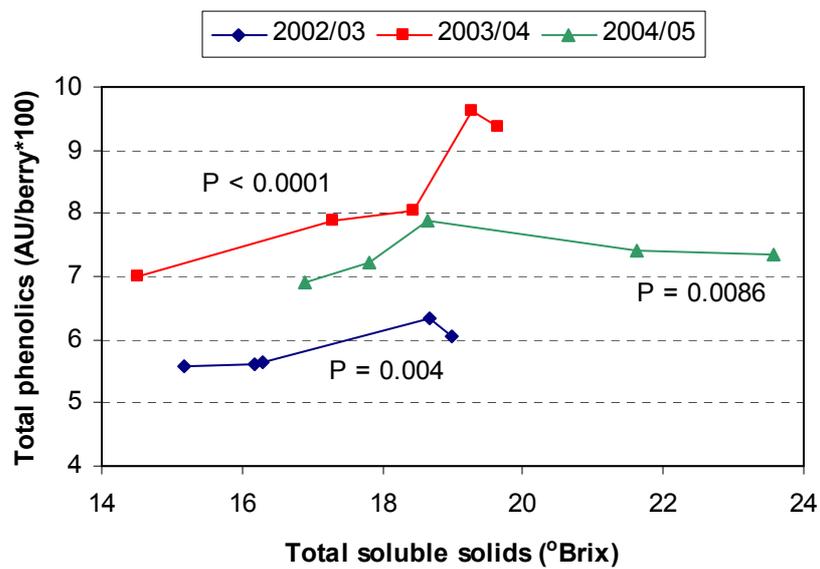


Figure 6 The relationship between total soluble solids and total phenolics (AU/berry) in Regal Seedless grapes over three seasons.

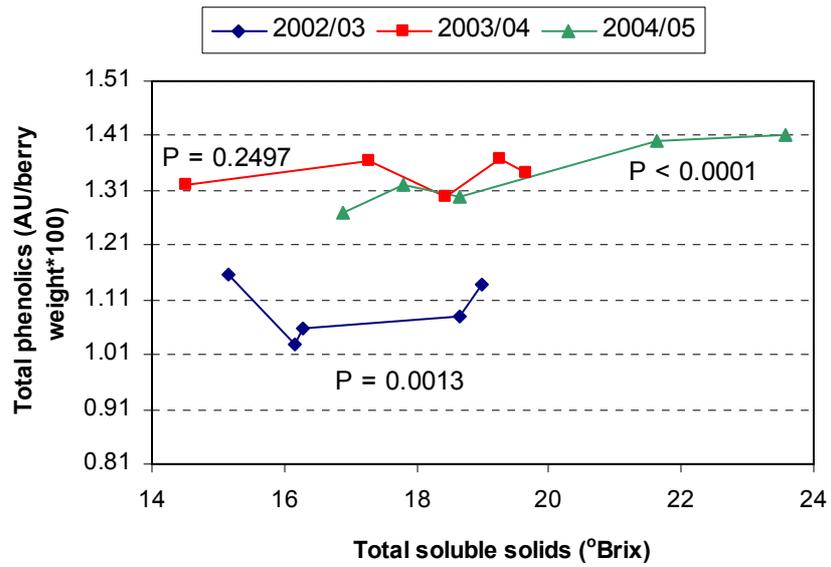
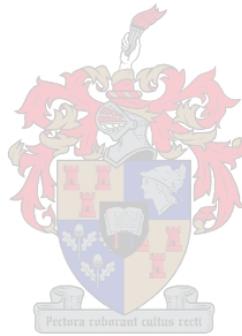


Figure 7 The relationship between total soluble solids and total phenolics (AU/gram berry weight) in Regal Seedless grapes over three seasons.



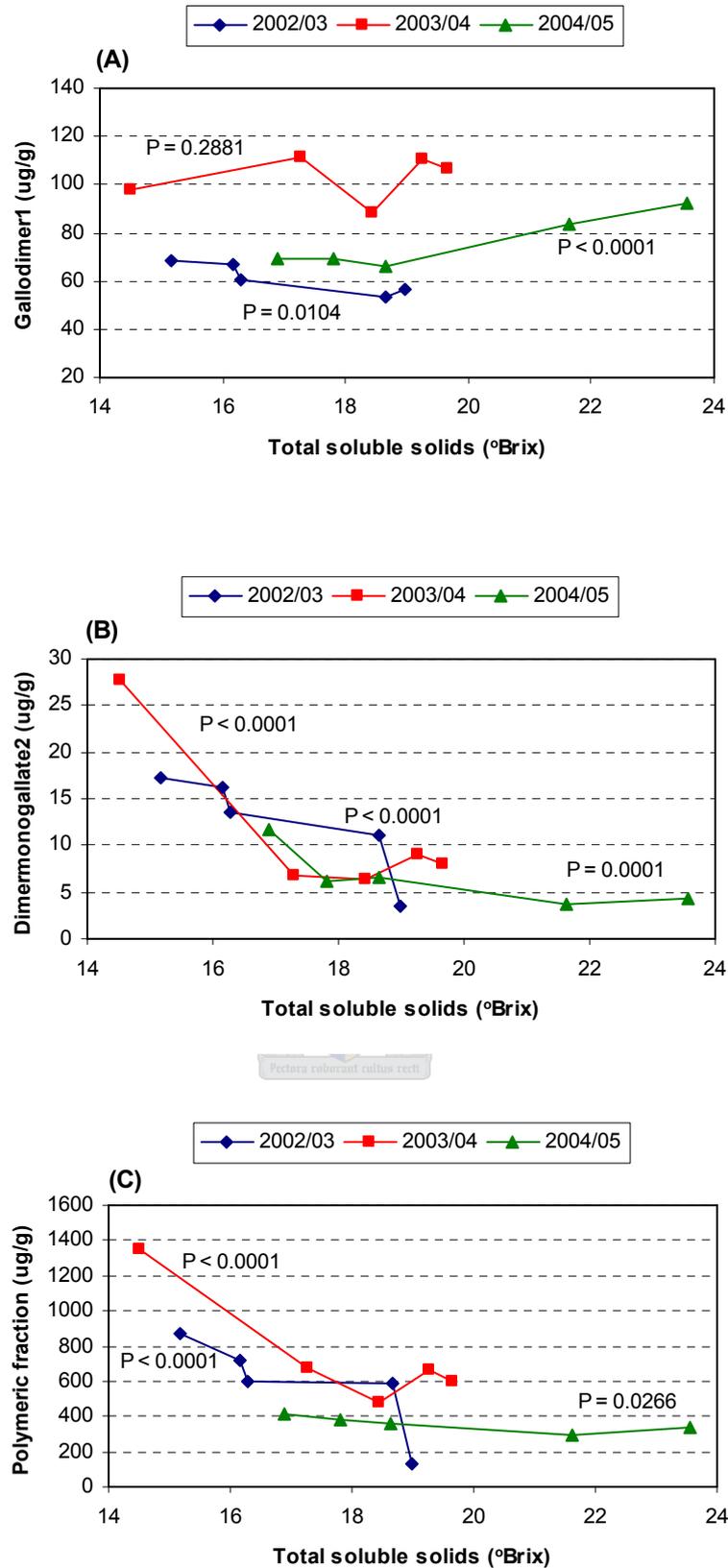


Figure 8 The relationship between total soluble solids and (A) gallodimer1, (B) dimermonogallate2 and (C) polymeric fraction (µg/gram berry weight) in Regal Seedless grapes over three seasons.

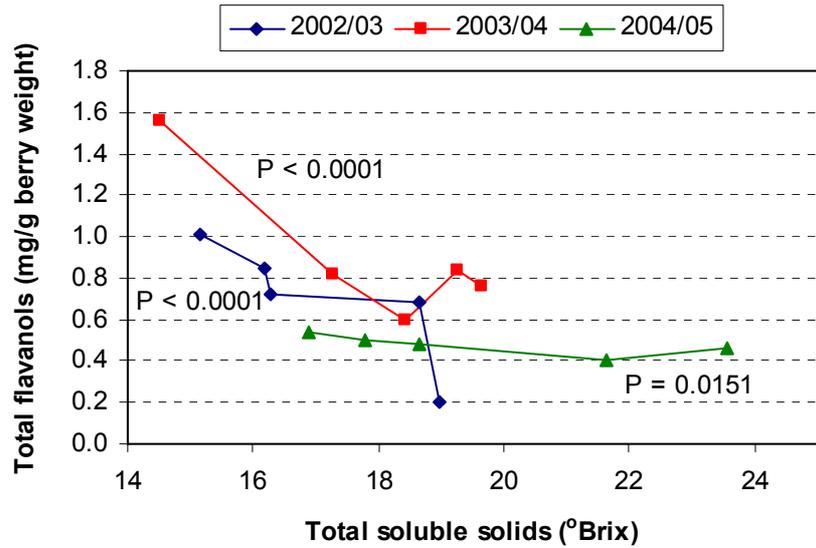


Figure 9 The relationship between total soluble solids and total flavanols (mg/gram berry weight) in Regal Seedless grapes over three seasons.

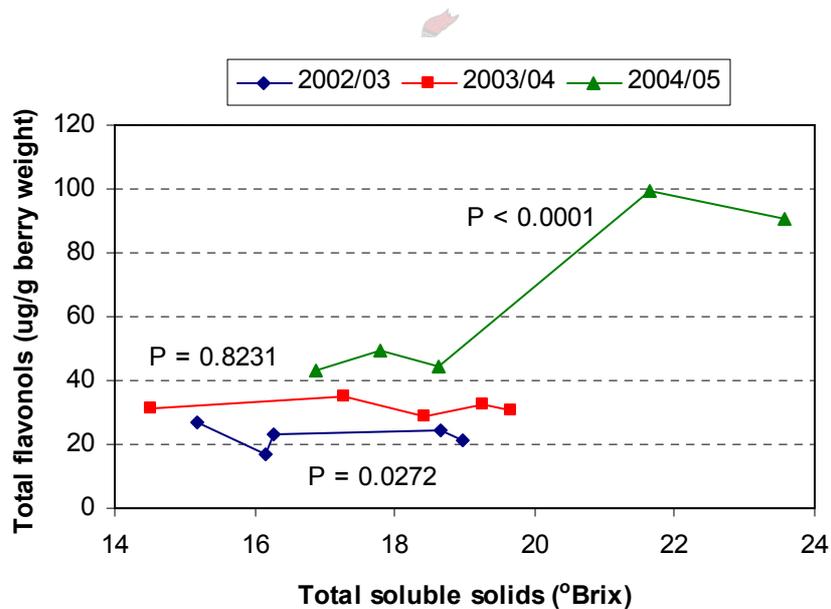


Figure 10 The relationship between total soluble solids and total flavonols (µg/gram berry weight) in Regal Seedless grapes over three seasons.

Table 1 Effect of different harvest dates (maturity levels) on the sensory evaluation of Regal Seedless grapes for the three seasons.

Season	Date	Astringency ^z	Skin tenacity ^z	Eating quality ^z
2002/03	20/1	3.19 a	4.88 ab	3.59 c
	24/1	4.71 a	6.16 a	4.21 bc
	31/1	3.19 a	4.76 ab	4.93 abc
	7/2	2.43 a	3.21 b	6.19 a
	14/2	3.73 a	4.08 b	5.74 ab
LSD^y		2.35	1.88	1.83
P^x (p ≤ 0.05)		0.3812	0.0378	0.1133
2003/04	20/1	7.02 a	4.89 a	2.07 b
	27/1	5.30 a	4.44 a	4.51 a
	3/2	5.34 a	4.41 a	4.83 a
	10/2	5.33 a	5.60 a	4.53 a
	17/2	2.41 b	4.09 a	6.27 a
LSD		2.41	2.56	2.23
P (p ≤ 0.05)		0.0015	0.2895	0.0217
2004/05	12/1	4.48 a	4.00 a	4.55 a
	19/1	3.43 a	3.52 a	5.56 a
	26/1	4.23 a	4.03 a	5.32 a
	3/2	3.68 a	3.48 a	5.06 a
	9/2	4.17 a	4.79 a	4.84 a
LSD		4.12	2.78	3.06
P (p ≤ 0.05)		0.9711	0.0668	0.9269

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z Scores per variable – scale 0-10, 0 – no astringency, soft skin, not favourable to eat; 10 – high astringency and skin tenacity, very favourable to eat.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

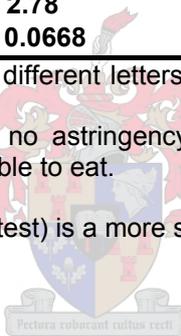


Table 2 Effect of different harvest dates (maturity levels) on the phenolic content (AU/berry) of Regal Seedless grapes.

Season	Date	TSS	A280 ^z	A365 ^z	A320 ^z
2002/03	20/1	15.2 c	0.0557 c	0.1012 b	0.1612 bc
	24/1	16.2 bc	0.0560 c	0.0888 c	0.1469 d
	31/1	16.3 b	0.0565 bc	0.0951 bc	0.1481 cd
	7/2	18.7 a	0.0633 a	0.1160 a	0.1784 a
	14/2	19.0 a	0.0606 ab	0.1123 a	0.1671 ab
LSD^y		1.02	0.0042	0.0103	0.0136
P^x (p ≤ 0.05)		<0.0001	0.0040	<0.0001	0.0004
2003/04	20/1	14.5 d	0.0701 c	0.1338 c	0.2099 b
	27/1	17.3 c	0.0789 b	0.1622 b	0.2229 b
	3/2	18.4 b	0.0804 b	0.1604 b	0.2250 b
	10/2	19.3 ab	0.0962 a	0.1960 a	0.2868 a
	17/2	19.7 a	0.0937 a	0.1858 a	0.2703 a
LSD		0.89	0.006	0.0227	0.0301
P (p ≤ 0.05)		<0.0001	<0.0001	<0.0001	<0.0001
2004/05	12/1	16.9 e	0.0690 c	0.1722 c	0.2300 b
	19/1	17.8 d	0.0723 bc	0.1930 bc	0.2430 ab
	26/1	18.6 c	0.0789 a	0.2032 b	0.2635 a
	3/2	21.6 b	0.0741 ab	0.2407 a	0.2688 a
	9/2	23.6 a	0.0736 bc	0.2341 a	0.2670 a
LSD		0.36	0.0051	0.0291	0.0284
P (p ≤ 0.05)		<0.0001	0.0086	0.0003	0.0332

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z A280 = total phenol content; A365 = flavonols; A320 = cinnamic acids.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

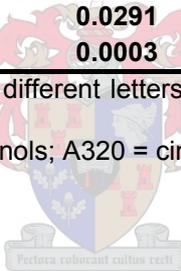


Table 3 Effect of different harvest dates (maturity levels) on the phenolic content (AU/gram berry weight of Regal Seedless grapes.

Season	Date	A280 ^z	A365 ^z	A320 ^z
2002/03	20/1	0.0116 a	0.0210 a	0.0334 a
	24/1	0.0103 c	0.0163 c	0.0270 c
	31/1	0.0106 c	0.0179 bc	0.0278 c
	7/2	0.0108 bc	0.0198 ab	0.0305 b
	14/2	0.0114 ab	0.0211 a	0.0314 ab
LSD^y		0.0006	0.0021	0.0027
P^x (p ≤ 0.05)		0.0013	0.0002	0.0002
2003/04	20/1	0.0132 a	0.0252 a	0.0395 ab
	27/1	0.0136 a	0.0280 a	0.0386 ab
	3/2	0.0130 a	0.0259 a	0.0364 b
	10/2	0.0137 a	0.0279 a	0.0407 a
	17/2	0.0134 a	0.0266 a	0.0387 ab
LSD		0.0007	0.0032	0.0036
P (p ≤ 0.05)		0.2497	0.3022	0.1410
2004/05	12/1	0.0127 b	0.0318 b	0.0423 b
	19/1	0.0132 b	0.0351 b	0.0442 b
	26/1	0.0130 b	0.0337 b	0.0437 b
	3/2	0.0140 a	0.0455 a	0.0508 a
	9/2	0.0141 a	0.0451 a	0.0514 a
LSD		0.0005	0.0059	0.0054
P (p ≤ 0.05)		<0.0001	<0.0001	0.0030

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z A280 = total phenol content; A365 = flavonols; A320 = cinnamic acids.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 4 Linear regressions of flavanol levels on a per berry weight basis for Regal Seedless grapes over three seasons.

Season	gallodimer1		di-mo-gal2 ^z		polymer		total flavanols	
	R ²	P-value	R ²	P-value	R ²	P-value	R ²	P-value
2002/03	0.82	0.0015	0.91	< 0.0001	0.85	< 0.0001	0.87	< 0.0001
2003/04	-	-	0.42	< 0.0001	0.48	< 0.0001	0.45	< 0.0001
2004/05	0.71	< 0.0001	0.75	< 0.0001	0.7	0.0066	0.66	0.0285

^z di-mo-gal2 = dimermonogallate2.

RESEARCH RESULTS

THE EFFECT OF POSTHARVEST TREATMENTS ON THE PHENOLIC CONTENT AND TASTE OF REGAL SEEDLESS (*VITIS VINIFERA* L.)

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THE EFFECT OF POSTHARVEST TREATMENTS ON THE PHENOLIC CONTENT AND TASTE OF REGAL SEEDLESS (*VITIS VINIFERA* L.)

ABSTRACT

'Regal Seedless' (*Vitis vinifera* L.) table grapes have a quality problem namely the seasonal occurrence of an unacceptable, astringent taste. Three different postharvest trials, namely zero-oxygen conditions, ethanol dip and cold storage duration, were applied to 'Regal Seedless'. Each trial was conducted over two seasons. During the zero-oxygen trial, grapes were stored at 0°C for 4, 8, 16, 32 and 64 hours, in air or 100% N₂. The ethanol dip trial consisted of five ethanol concentrations (0%, 10%, 20%, 40% and 80%) in combination with two SO₂ pad treatments (with and without). In the cold storage trial grapes were stored at -0.5°C for 0, 4 and 7 weeks, with or without an additional week at 15°C. The phenolic composition was determined spectrophotometrically and by HPLC analyses. Other quality parameters, namely total soluble solids (TSS), pH and total titratable acidity (TTA) were also measured. The grapes were evaluated for bitterness, astringency, skin tenacity and eating quality by a tasting panel. The use of 100% nitrogen decreased the astringent taste and improved eating quality, irrespective of the exposure time. The nitrogen treatment also decreased the total phenolics (A₂₈₀) per berry but an increase in individual and total flavanols was observed when compared to the air treatment. In 2003/04, the astringent taste decreased and the eating quality improved with an increase in ethanol concentration. All the ethanol concentrations caused a significant decrease in the concentration the polymeric fraction and total flavanols as well as individual and total flavonols, but it was only seen in one season. No effect was, however, seen in the spectrophotometric data. Four weeks of cold storage with one week of shelf life resulted in lower astringency and higher eating quality. This period also resulted in lower total phenolics (A₂₈₀) per berry weight and lower concentrations of individual and total flavanols and flavonols, when compared to the other cold storage periods.

KEYWORDS: Astringency; flavanols; total phenolics; ethanol; nitrogen; storage; HPLC; 'Regal Seedless'; *Vitis vinifera*

4.1 INTRODUCTION

Table grapes are an important crop produced in different regions in South Africa. 'Regal Seedless' (*Vitis vinifera* L.) is an early mid-season, white seedless cultivar obtained from a complex hybrid crossing in 1988 at the ARC Infruitec-Nietvoorbij, South Africa and was released in 1997 as a supplementary cultivar to Sultanina. This cultivar brings the benefits of earlier harvest and larger inherent berry size. However, the acceptability of the cultivar on local and international markets is affected by a sporadic, unacceptable astringent taste. Astringency is seen as a quality problem in fresh fruit like table grapes and persimmons, while it is an important organoleptic property in beverages like wine.

Astringency perception is a mouth feel or tactile sensation (Breslin et al., 1993), most commonly described as a 'drying', 'roughing' and 'puckering' feeling perceived as a diffuse stimulus (Joslyn & Goldstein, 1964; Lawless et al., 1994) which takes time to develop (Kallithraka et al., 1997). Phenolic compounds, especially monomeric and polymeric flavan-3-ols are responsible for the astringent sensation (Joslyn & Goldstein, 1964; Robichaud & Noble, 1990) and have been reviewed extensively (Gawel, 1998). The oligomeric to polymeric flavan-3-ols, also referred to as the proanthocyanidins, become more astringent with an increase in degree of polymerisation (Joslyn & Goldstein, 1964; Bate-Smith, 1973; Haslam, 1974; Arnold et al., 1980) until it becomes so large that it influences the solubility and interaction with the saliva proteins.

Different methods such as keeping fruit under anoxic or hypoxic conditions or exposure to products that enhance anaerobic respiration have been used successfully in the de-astringency process of persimmons (Pesis et al., 1986; Ben-Arie & Sonogo, 1993). These methods induce alcoholic fermentation. The enzyme pyruvate decarboxylase converts pyruvate to acetaldehyde releasing CO₂, while alcohol dehydrogenase reduces acetaldehyde to ethanol oxidising NADH to NAD⁺ (Taiz & Zeiger, 2002). Acetaldehyde (AA) is much more toxic to the fruit cell than ethanol. The cell transforms AA into the less toxic ethanol (Hribar et al., 2000) resulting in large amounts of ethanol. It is been proven that the excessive production of these anaerobic metabolites leads to off-flavours.

The postharvest application of carbon dioxide (CO₂), nitrogen (N₂), ethanol (EtOH) vapour and ethylene have proved to be effective in removing astringency in persimmons (Ben-Arie & Guelfat-Reich, 1976; Matsuo & Ito, 1977; Kato, 1990; Zavrtnik et al., 1999; Yamada et al., 2002; Arnal & Del Río, 2003). It has been demonstrated that CO₂

treatments were more efficient in the de-astringency of persimmons than N₂ treatments and ethanol vapour (Zavrtanik et al., 1999; Yamada et al., 2002; Arnal & Del Río, 2003).

The mechanism by which the loss in astringency takes place, has been previously proposed. There was a positive correlation between astringency removal and AA content in the fruit (Matsuo & Ito, 1977; Arnal & Del Río, 2003). Water-soluble tannins responsible for astringency are polymerised by AA, produced under these anaerobic conditions, to form water-insoluble tannins which are less or non-astringent (Matsuo & Ito, 1982; Taira et al., 1997).

It is important to note that although astringency might be removed, postharvest applications of high CO₂ or N₂ concentrations have other positive and negative effects on fruit quality. This includes influences on ethylene, chlorophyll (colour), volatiles (Kader, 1997; Beaudry, 1999), fruit firmness and internal and/or external browning (caused by polyphenol oxidase activity) (Arnal & Del Río, 2003). Exogenous application of anaerobic metabolites, ethanol and AA, have also been used on table grapes to maintain quality and retard decay development during storage life (Avissar et al., 1989; Avissar & Pesis, 1991; Lichter et al., 2002; Chervin et al., 2003; Gabler et al., 2005).

Phenolic composition of fresh products seems to be influenced by certain postharvest treatments. Therefore, the objective of this study was to investigate (1) zero-oxygen conditions, (2) an ethanol dip and (3) cold storage duration as postharvest treatments to determine which of these will decrease the phenolic content (specifically the flavanols) and astringent taste the most effectively in 'Regal Seedless' grapes. To the best of our knowledge no similar studies on the influence of these treatments on the phenolic composition of this cultivar, have been reported.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL SITE

The grapes used for the zero oxygen and ethanol trials came from the ARC Hex River Valley Experimental Farm in the Western Cape, South Africa. The vineyard consists of a rootstock trial with *Vitis vinifera* L. cv. 'Regal Seedless' as scion cultivar on a sandy soil. Grapes for these trials were only sampled from vines grafted on Ramsey (*Vitis champinii*). The experimental vines were planted 3.0 m x 2.0 m apart in an east-west direction and trained onto a gable trellis system.

Grape samples for the cold storage trial were collected from a three-year-old *Vitis vinifera* L. cv. 'Regal Seedless' commercial table grape vineyard on Moselle, a farm situated in the Hex River Valley in the Western Cape, South Africa. The vines were grafted on Ramsey (*Vitis champinii*), spaced 3.0 m x 1.5 m apart on a clay-loam soil with a slightly acidic pH and trained onto a gable trellis system. The rows were planted in an east-west direction.

The climate in the Hex River Valley is typically Mediterranean with warm, dry summers and cool, wet winters. From the November to February the mean maximum temperature was 30.5°C, the mean minimum temperature was 13.6°C and the mean rainfall was 15.5 mm over the three years that the study was done.

4.2.2 SAMPLE COLLECTION AND TREATMENTS

4.2.2.1 Zero-oxygen conditions (Trial 1)

The trial was conducted during the 2002/03 and 2003/04 seasons. Clusters were harvested during mid-morning. Bunches were taken randomly from different vines, alternately from both sides of the rows at varying heights and distances from the trunk to represent the entire plot. Only clusters in the shade were harvested. After collection the grapes were packed into 4.5 kg cartons according to commercial export standards. The boxes were transported about 100 km by car to the Department of Horticultural Sciences, University of Stellenbosch. The grapes were pre-cooled at -0.5°C for two hours before the commencement of the treatments.

The clusters were sealed in 20 L plastic containers (four clusters per container; two clusters per replicate) through which either air from a compressor (used as control) or 100% N₂ (nitrogen gas) were passed. The containers were connected via flow boards to the respective gases. The duration of the treatments was 4, 8, 16, 32 and 64 (only in 2003/04) hours. The treatments were carried out in a cold room at -0.5°C. After the treatments the grapes were placed back into the 4.5 kg cartons according to commercial export standards and placed in cold storage for four weeks at -0.5°C (simulating shipping period overseas) followed by a shelf life of one week at 15°C (as a typical retail temperature). The replicates were evaluated separately after the shelf period for postharvest defects.

4.2.2.2 Ethanol dip (Trial 2)

The trial was conducted during the 2003/04 and 2004/05 seasons. The treatments were five ethanol concentrations (0%, 10%, 20%, 40% and 80%) in combination with two SO₂ pad treatments (with and without).

During 2003/04, the sample collection was the same as for the zero-oxygen trial. The trial was performed in a building on the farm. The whole bunches were dipped briefly (ca. 10 s) in ethanol solutions at the above mentioned concentrations at ambient temperature. Bunches were then dried in the shade for 60 – 90 min. During 2004/05 the same treatments were done while the grapes were still on the vines. The treatments were applied randomly per vine. The clusters were left to dry for 60 – 90 min, after which the grapes were harvested.

The storage procedures were the same for both years: the grapes were packed in polycote (paper) bags and arranged in 2 kg cartons (with and without Uvasys SO₂ pads). Paper pads were placed on top of the bunches, under the SO₂ pad. A non-perforated outer bag was used. The boxes were placed in cold storage for four weeks at -0.5°C, followed by one week of shelf life at 15°C. Standard quality control evaluations were done after the shelf life period.

4.2.2.3 Cold storage duration (Trial 3)

The trial was carried out during the 2002/03 and 2003/04 seasons. Bunches were harvested at an average maturity of 18°Brix (commercial maturity for 'Regal Seedless'). Sample collection was the same as for the zero-oxygen trial. After collection the grapes were packed into separate 2 kg cartons according to commercial export standards. Two factors were evaluated: three cold storage times at -0.5°C (W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks) and two shelf life periods (S0 = no shelf life, S1 = an additional week at 15°C). Fruit quality evaluations were done after each storage period for postharvest defects.

4.2.3 SENSORY EVALUATION

A sensory evaluation was conducted on each replicate after the cold storage evaluations. Six panellists (3 men and 3 women; aged 23 – 46 years) conducted the sensory analysis. Four properties, namely bitterness (data not shown), astringency, skin tenacity and overall eating quality were evaluated on an 11-point unstructured line scale (scale from 0 – 10). In the ethanol trial, the grapes were also evaluated for any off-flavours. The data will not be reported since no off-flavours were found. A score of zero

meant no bitterness, no astringency, soft skin and not favourable to eat, and a score of ten meant high in bitterness and astringency, high skin tenacity and very favourable to eat. The judges ate unsalted cream crackers between samples to clean their palates to prohibit the carry over-effect of astringency.

4.2.4 PHYSICAL MEASUREMENTS

A fresh berry sample (50 berries per sample in trial 2 and 3; 30 berries per sample in trial 1) was taken from each replicate after storage and was used to record the berry mass and for juice analyses. The total soluble solids (TSS) was measured with a bench refractometer and expressed as °Brix. The pH and total titratable acidity (TTA) were determined by titrating a 25 mL sample with 0.333 M NaOH to an end point of pH 7, using a Mettler DL21 titrator (Mettler Toledo, Switzerland). TTA was expressed in g/L.

4.2.5 SPECTROPHOTOMETRIC ANALYSIS

A random sample of 50 berries was taken from each of the cartons and stored at -20°C until further analysis. In trial 1 and 2 the sample was taken after shelf life, while in trial 3 it was collected after cold storage or shelf life, depending on the treatment. These berries were removed from the bunches with the pedicel still intact, without damaging the skin. Total phenolics of whole berries were determined by a spectrophotometric method described by Iland *et al.* (2000). A few modifications were, however, made by us. The grapes were removed from the -20°C freezer, thawed in a refrigerator overnight, destemmed, weighed and then homogenized with an Ultra-Turrax T25 disperser (IKA®, Germany) at 24000 rpm for 1 min. The weight of a scoop of approximately five grams of the homogenate was recorded and then extracted with 10 mL of 50% aqueous ethanol adjusted to pH 2.0. The contents were mixed periodically by inverting the tube every 10 minutes for one hour. Thereafter, the samples were centrifuged at 1100 x *g* for 30 min in an ALC® 4235A centrifuge (Milan, Italy) and the volume of the extract (supernatant) determined. A Helios Gamma UV-Vis spectrophotometer (Model no. 9423UVG1702E) (Thermo Electron Corporation, Johannesburg, South Africa) was used to measure the absorbance at 700 nm, 420 nm, 365 nm, 320 nm and 280 nm. This determined, respectively, the sample turbidity, oxidation state, flavonol, cinnamic acid and total phenol content of the sample. Excessive turbidity is an indication of insufficient clarification during centrifugation. The A_{700} should be < 0.01. The oxidation state indicates if any problem occurred during preparation of the samples. These are the

main absorbance areas of the different phenolic groups. The total phenols per berry (1) and total phenols per gram berry weight (2) were determined as follows:

1) $A_{280} \times [\text{final extract volume (mL)} / 100] \times [\text{weight of 50 berries (g)} / \text{weight of homogenate for extraction (g)}] \times [1 / 50]$ expressed as absorbance units (AU) per berry (Iland et al. (2000))

2) $A_{280} \times [\text{final extract volume (mL)} / 100] \times [\text{weight of 50 berries (g)} / \text{weight of homogenate for extraction (g)}] \times [1 / \text{weight of 50 berries (g)}]$ expressed as absorbance units (AU) per gram berry weight (Iland et al. (2000)).

A sample of the extract was filtered through a 0.45 μm Cameo Nylon filter, transferred to a 1.8 ml HPLC auto-sampler vial and analyzed with high-performance liquid chromatography (HPLC) using diode array detection (DAD).

4.2.6 CHROMATOGRAPHIC ANALYSIS

4.2.6.1 HPLC-DAD analysis

A Hewlett Packard Agilent 1100 series high-performance liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) was used with a binary pump and a diode array detector. HPLC separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100 \AA , 250 \times 4.6 mm, 5 μm) (Polymer Laboratories (Ltd), Shropshire, UK) protected by a guard cartridge (PLRP-S, 10 \times 4.6 mm).

A gradient elution of 1.5% aqueous ortho-phosphoric acid (Sigma-Aldrich, Kempton Park, South Africa) (solvent A) and 80% HPLC grade acetonitrile (Sigma-Aldrich, Kempton Park, South Africa) / 20% of solvent A (solvent B) was used. The following gradient was established: 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. Similar conditions had been previously employed with satisfactory results (Price et al., 1995; Peng et al., 2002). An injection volume of 100 μL was used. A blank and known standard mix were run after every ten samples, the column temperature was held at 35 $^{\circ}\text{C}$ and the system was equilibrated for 15 min at the starting solvent conditions between samples to ensure a stable baseline, consistent retention times and validity of the standard curves. The spectra were recorded from 250 nm to 400 nm.

4.2.6.2 HPLC-MS analysis

Liquid chromatography-mass spectrometry (LC-MS) was performed using a Waters API Quattro Micro and Waters API Q-TOF Ultima connected to a Waters UPLC (Waters

Corporation, Massachusetts, USA) system. Chromatographic separation was carried out on a PLRP-S column (100Å, 250 × 4.6 mm, 5 µm, Polymer Laboratories (Ltd), Shropshire, UK) using Solvent A: 1% formic acid/water solution (Merck) and Solvent B: 80% HPLC grade acetonitrile (Sigma-Aldrich, Kempton Park, South Africa) / 20% of solvent A as mobile phases. Elution was performed with the same gradient conditions as described before. The phenols were detected with an electrospray ionization (ESI) system in the negative mode. A cone voltage of 25 V and 35 V were respectively used with the Quattro Micro and Q-TOF Ultima MS systems. A capillary voltage of 3.5 kV with a desolvation temperature of 400°C was also employed. UV chromatograms were recorded at 360 nm, 320 nm and 280 nm. The LC-MS analyses were performed to identify the unknown phenolic compounds detected by HPLC-DAD.

4.2.7 PHENOLIC IDENTIFICATION AND QUANTIFICATION

Chromatograms recorded at 280 nm were used for the quantification of the benzoic acids and flavanols, at 316 nm for the cinnamic acids and at 360 nm for the flavonols.

The following standards were used for identification on the HPLC: gallic acid, epigallocatechin, galocatechin, catechin, procyanidin B1 and B2, epicatechingallate, *p*-coumaric acid, caffeic acid, kaempferol, quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-rutinoside and myricetin. Retention times and UV spectra from published data were used additionally (Lamuella-Raventós & Waterhouse, 1994; Price et al., 1995; Betés-Saura et al., 1996; Bartolomé et al., 1996; Peng et al., 2002).

Benzoic acids, flavanols, cinnamic acids and flavonols were respectively quantified as gallic acid, catechin, *p*-coumaric acid and quercetin-3-rutinoside (rutin) equivalents. All commercial standards were bought from Sigma-Aldrich (Kempton Park, South Africa) and were of the highest quality available. Concentrations of gallic acid, catechin, *p*-coumaric acid and rutin equivalents were calculated from external calibration curves constructed from the injection of the four different commercial standards at four different concentrations. Phenolic content was expressed as mg per berry and mg per gram fresh berry weight, except at very low concentrations where µg per berry and µg per fresh berry weight was rather used as unit.

LC-MS analysis enabled us to identify a gallodimer, procyanidin B2, a dimermonogallate, kaempferol-glucoside/glucuronide and quercetin-glucoside. Other phenolic compounds were also identified, but the data are not reported in this paper.

4.2.8 PRECISION STUDY AND LIMIT OF QUANTIFICATION

Repeatability was assessed using six determinations at 100% of the test concentration and the relative standard deviation (RSD) calculated. The acceptance criteria for precision were at a level of 3% RSD. The limit of quantification was taken to be the lowest amount giving a signal-to-noise ratio of approximately 7:1 or more.

4.2.9 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Zero-oxygen: The experimental design was completely randomised. The treatment design was a 4 x 2 factorial with 8 treatments (2002/03) and a 5 x 2 factorial with 10 treatments (2003/04) and six replications of two bunches each.

Ethanol dip: The experimental design was completely randomised. An experimental unit consisted of a single 2 kg carton. The treatment design was a 5 x 2 factorial with 10 treatments and 4 replicates.

Cold storage: A completely randomised design. There were six treatments and six replications with a single 2 kg carton constituting an experimental unit. Statistical analysis was separately performed on the cold storage and shelf life periods. The changes in quality variables from the end of cold storage to the end of shelf life were determined and also compared statistically.

The statistical software programme, SAS version 8.2 (SAS, 1999) was used for all the 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-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 ZERO OXYGEN CONDITIONS

In the 2002/03 season the boxes with the grapes of the air treatment at 32 hours were misplaced by the company who evaluated the grapes after cold storage. Thus there are no data available for this treatment. When data at 32 hours are presented, they only includes the average of the six values of the nitrogen application.

4.3.1.1 Sensory evaluation

In the 2002/03 season no significant differences occurred in astringency, skin tenacity and eating quality between the air and nitrogen gas treatments (Table 1). By extending the exposure period, the average astringency score increased (non-significantly) and a significant decrease in eating quality (EQ) was observed by the tasters. In 2003/04 the nitrogen exposure significantly decreased the astringent taste and increased the eating quality (Table 1). The different exposure times did not improve the results significantly. Thus, nitrogen was effective irrespective of the exposure time. The astringency scores were clearly lower in 2003/04 when compared to 2002/03. A possible reason is that the sugar content was 20.6°Brix in 2002/03, while in 2003/04 it was 23.6°Brix. It has been proved that sugar content delays or camouflages the effect of astringency (Walsh, 1998). The more sugar, the longer the delay. A positive correlation was found between astringency and skin tenacity (2002/03: $r = 0.80$, $p < 0.0001$; 2003/04: $r = 0.54$, $p < 0.0001$) and a negative correlation between astringency and EQ (2002/03: $r = -0.52$, $p < 0.005$; 2003/04: $r = -0.44$, $p < 0.0005$). A consumer preference analysis of Thompson Seedless after controlled atmospheres (CA) indicated that the preference for the grapes was negatively affected when treated with 100% CO₂ or 55% CO₂ + 0.5 O₂ at 20°C, but not by any of the treatments at 5°C (Ahumada et al., 1996). The anaerobic metabolites, acetaldehyde and ethanol, can influence the preference or total eating quality of the grapes. Off-flavour was induced by CA treatments when grapes were stored in 15% CO₂ or higher (Crisosto et al., 2002a) while Artés-Hernández et al. (2004) did not detect off-flavours for any of the CA or MAP treatments. In our study, taste evaluation did not include off-flavour detection.

4.3.1.2 Spectrophotometric analysis

There was a tendency for the nitrogen treatment to lower the total phenolics (A_{280}) per berry in 2002/03 (data not shown) and 2003/04 (Table 2), but the means did not differ significantly. Kader et al. (1997) pointed out that reduced levels of O₂ and elevated levels of CO₂ decrease total phenolic content of horticultural products. Pesis & Ben-Arie (1984, 1986) and Zavrtnik et al. (1999) showed that acetaldehyde production was significantly higher in a CO₂ atmosphere than in a N₂ atmosphere, suggesting CO₂ will be more effective and needs shorter exposure time (24 – 48h) to lower the phenolic content and remove astringency. It is important to keep in mind that Pesis & Ben-Arie (1984) did not determine the phenolic content before and after the treatments, and that the degree of astringency was determined by the FeCl₃ paper test. When N₂ was used,

72 h was needed to obtain non-astringent fruit. Matsuo et al. (1976) found a substantially decrease in soluble tannin content (determined by the micro-Löwenthal method) between 24 to 48 h after CO₂ treatment. Zavrtnik et al. (1999) also found that the total phenolic content (measured via Folin-Ciocalteu) decreased after persimmons were treated with CO₂ and N₂. CO₂ was faster in removing astringency than the N₂ treatment. Maybe the use of CO₂ should be considered to remove astringency from 'Regal Seedless' grapes.

A significant interaction AN (air-nitrogen) x T (time) (P = 0.0358) was found for the flavonols (A₃₆₅) per berry in 2002/03 (data not shown), while in 2003/04 no significant differences occurred between air and nitrogen application (Table 2). During 2002/03 exposure time of 32 hours significantly increased the total phenolics on a berry (data not shown) and weight basis (Table 3). This finding is supported by the sensory evaluations where the highest astringency score and lowest eating quality was found at 32 hours (Table 1). It is important to keep in mind that the means only include the nitrogen treatment. If the air treatment formed part of the mean a different answer might have been found. No meaningful trends were observed. The A₃₂₀ reading will not be discussed in detail since only two cinnamic acids at very low concentrations were detected with HPLC.

In 2002/03 the use of nitrogen significantly decreased the total phenolics (A₂₈₀) and flavonols (A₃₆₅) per gram berry fresh weight (Table 3). Statistical analysis indicated a significant AN x T interaction for the total phenolics and the flavonols on a weight basis in 2003/04 (Fig. 1a and 1b). The total phenolics and flavonols were lowered significantly by the nitrogen treatment for 64 hours, compared to the air treatment for the same duration of time. Except for the treatment of air and nitrogen for four hours, a similar tendency was observed for the other times, but it was not significantly different. Although nitrogen was effective at 64 hours, the absorbance units (A₂₈₀) were still higher than the treatments at 4, 8 and 16 hours. No relationships were found between the sensory evaluation and the spectrophotometric analysis. This differs from the results obtained by Condelli et al. (2006) and Amarowicz et al. (2004) who found a significant correlation between astringency and total phenol content.

4.3.1.3 HPLC-DAD analysis

HPLC analyses, to determine the flavanol and flavonol content of fruit, have not previously been performed in studies that concentrated on astringency removal.

Flavanols. Sixteen flavanols were detected with HPLC. This included identified and unidentified compounds. The four most abundant flavanols found in 'Regal Seedless' was the polymeric fraction (includes trimers, oligomers and polymers), a gallodimer¹, a dimermonogallate² and procyanidin B2. The dimermonogallate² was only present in large concentrations in 2002/03 (Table 4). The total flavanols were calculated as the sum of the concentrations of all sixteen compounds.

In 2002/03 the nitrogen applications slightly increased the concentrations of all the mentioned flavanols and total flavanols, except for the dimermonogallate² that exhibited a slight decrease (Table 4). It was, however, not significantly different from the air treatment, whether expressed per berry (data not shown) or per berry weight. The polymer concentration and total flavanols increased significantly with extended exposure time (Table 4). This increase agrees with the increase in astringent taste. The increase may be partly influenced by the fact that all flavanols are quantified in catechin monomeric units while the new acetaldehyde linked phenols may have totally different absorbance characteristics (extinction coefficients) which would influence quantification. The same observations were found on a per berry (data not shown) and per berry weight basis.

In 2003/04 a significant AN x T interaction was found for the three main compounds and the total flavanols on a per berry (data not shown) and per berry weight basis (Fig. 2). The nitrogen treatment for 4, 16 and 64 hours decreased the concentration of the gallodimer¹, polymeric fraction, and total flavanols non-significantly in 2003/04 when compared to the air treatment, while at 8 and 32 hours, an increase was observed in contrast to the air treatment. The increase and decreases may result from the continuous building and degradation reactions of phenolics that take place during the treatments (Prieur et al., 1994; Dallas et al., 1996). The sensory evaluation confirms that the eight hour treatment resulted in a significantly higher astringency score. This suggests that the grapes used in this treatment did contain more phenolics and it is probably due to sampling error. The procyanidin B2 concentration was significantly higher with the 32 hours nitrogen treatment than with the air application. The same pattern was observed at 8 and 16 hours.

No statistical trends were found for any of the compounds. The polymeric fraction and the total flavanols followed the same pattern. The reason is that the polymeric fraction contributes 77 – 85% of the total flavanols. No correlations were found between sensory and HPLC data (data not shown). Good correlations were, however, found between the spectrophotometric and HPLC analyses in 2002/03: A_{280} per berry vs. total

phenolics per berry (via HPLC) ($r = 0.72$, $p < 0.0001$), A_{280} per berry weight vs. total phenolics per berry weight as measured by HPLC ($r = 0.58$, $p < 0.0005$).

Flavonols. The four main flavonols that were identified with HPLC were quercetin-3-galactoside, quercetin-3-glucoside, kaempferol-3-glucoside/glucuronide and quercetin-3-rhamnoside. Other flavonols were detected in trace amounts (1.0 – 4.0 $\mu\text{g/g}$). The total flavonols were calculated as the sum of the concentrations of all the individual flavonols.

The application of nitrogen decreased the concentration of the flavonols in both seasons, but only quercetin-3-glucoside decreased significantly in 2003/04 (Table 5). The results were the same on a per berry and berry weight basis. The duration of the treatments significantly influenced the amount of flavonols in both seasons (Table 5). In 2002/03 the concentrations of all flavonols, thus also the total flavonols increased significantly as exposure time increased (Fig. 3). A linear trend was observed ($R^2 = 0.99$, $p < 0.05$) (data not shown). This increase may be due to the fact that all the flavonols are quantified as quercetin-3-rutinoside units, while the new acetaldehyde complexes that form may have totally different extinction coefficients. At 32 hours the amount of flavonols was significantly higher than at four hours, where the concentration was the lowest. The next season the concentrations of all four main flavonols at 8 hours were significantly lower than at 16 and 32 hours. Kaempferol-3-glucoside/glucuronide and quercetin-3-rhamnoside were also significantly lower after the 8 hour treatment than after the 64 hour treatment. Treatments exposed for 4 and 8 hours resulted in the lowest amounts of all four main flavonols. There were no significant trends found for any of the compounds. The results for flavonol concentrations over exposure times were the same on a per berry and per berry weight basis. During 2003/04 the eight hour treatment resulted in a significantly lower concentration of total flavonols than 16 to 64 hours (Fig. 3).

Non-flavonoids: Only one unknown benzoic acid and two unknown cinnamic acids were detected by HPLC. Their concentrations were very low and will not be discussed.

Total phenolics: The total phenolics content was determined as the sum of the flavanols, flavonols, cinnamic acids and benzoic acids. The use of air or nitrogen did not have a significant effect on the total phenolics in 2002/03. Since the total flavanols contribute such a large percentage towards the total phenols, the same pattern was

found for both parameters. An extended exposure time (air and nitrogen) resulted in a significant increase in total phenolics (data not shown). In 2003/04 a significant AN x T interaction was found. Both treatments decreased the total phenolics with an increase in exposure time (data not shown). It has been shown by Zavrtnik et al. (1999) that persimmons can lose their astringency by storing in air. The use of N₂ or CO₂ only increases the rate of de-astringency.

4.3.1.4 Quality evaluations

It is important that quality parameters are taken into account when the use of CA treatments is considered, since it is the visual appearance and taste of table grapes that convince consumers to purchase a specific cultivar. In previous studies CA treatments had no influence on TSS, TTA, sugar-acid ratio, berry shatter and berry browning (Crisosto et al. 2002b; Retamales et al. 2003). It was only rachis browning that was negatively affected. In our present study neither air nor nitrogen significantly affected TSS, TTA (only in 2003/04), sugar-acid ratio, berry shatter and rachis browning (data not shown).

The removal of astringency with carbon dioxide (CO₂) or nitrogen (N₂) enriched atmospheres have become a common commercial practice in persimmon fruit (Ben-Arie & Guelfat-Reich, 1976; Matsuo & Ito, 1977). Both of these gases induce fermentative metabolism/anaerobic conditions. It is believed that acetaldehyde that forms under these conditions is involved in the de-astringency process. The use of modified atmosphere packaging (MAP), controlled atmosphere (CA) (Basiouny, 1998; Crisosto et al., 2002a; Retamales et al, 2003) and ozone (O₃) (Palou et al., 2002) have been tested on table grapes to extend the storage life period and maintain quality. There is however no study that focused on the effect of these CA treatments on the astringent taste and phenolic content of table grapes.

Ramos et al. (1993) found that the hydroxycinnamic acids in wine grape skins decreased during anaerobic treatment under CO₂ (0 – 10 days) at 30°C, reproducing the first stage of carbonic maceration. In persimmons anaerobic treatments are usually conducted at 20°C (Zavrtnik et al., 1999). We conducted the treatments at 0°C. A higher temperature might have been more effective, but table grapes are prone to physical damage and the appearance of table grapes is of utmost importance. Ahumada et al. (1996), however, found that controlled atmospheres at 20°C and 5°C did

not affect the weight loss, firmness, TA, berry browning or berry shattering of Thompson Seedless grapes.

4.3.2 ETHANOL DIP

Higher organoleptic scores for ethanol treated berries in comparison to berries treated with SO₂ (Lichter et al., 2002), as well as the success of the de-astringency of persimmons with ethanol in literature urged us to investigate the possibility of ethanol having an effect on the astringent taste and therefore the phenolic content of 'Regal Seedless' table grapes.

4.3.2.1 Sensory evaluation

There were no significant differences between the means of the two SO₂ pad treatments (with and without) for all of the attributes tasted in both seasons (data not shown). This is in agreement with Lichter et al. (2002) who did not find any significant differences in taste between SO₂ treated fruit and untreated fruit. In 2003/04 the astringent taste decreased and the eating quality (EQ) improved with an increase in ethanol concentration (Table 6). The astringency score of grapes treated with 20, 40 and 80% ethanol was significantly lower than the control. Grapes treated with 40 and 80% ethanol received significantly higher scores in terms of eating quality, when compared to the control (according to the t-test). Both of these attributes followed linear trends (astringency: $R^2 = 0.64$, $p < 0.001$; EQ: $R^2 = 0.54$, $p < 0.05$). Good relationships were found between astringency and skin tenacity ($r = 0.49$, $p < 0.005$) and between astringency and EQ ($r = -0.56$, $p < 0.0005$) in 2003/04. Chervin et al. (2003) and Lurie et al. (2006) found that none of the ethanol or SO₂ pad treatments influenced the overall taste (hedonic scale) rating of the non-specialist panel, while Lichter et al. (2002) found that the tasting scores of grapes treated with ethanol were significantly higher than that of untreated berries. The skin tenacity score varied between the treatments and no significant differences were found. During 2004/05 no significant differences between ethanol treatments were found for astringency, EQ and skin tenacity, and no trends were observed. It is, however, notable that the lowest astringency score was associated with the highest EQ score. The astringency score was already low and EQ score high at the control level. These scores were acceptable throughout all of the treatments. No undesirable off-tastes were detected in either season (data not shown). When only sensory evaluation is considered, it will be recommended to use an ethanol

concentration of 40% and higher to improve the taste of the grapes (based on 2003/04 results).

4.3.2.2 Spectrophotometric analysis

During 2003/04 no significant differences in absorbance measurements were found between the means of the two SO₂ pad treatments (with and without), nor was there an interaction between SO₂ and ethanol concentration ([E]) (data not shown). No significant differences or trends were found between the ethanol treatments for all the absorbance measurements, whether it was expressed on per berry or per berry weight basis for the 2003/04 season (Table 7). Yamada et al. (2002) found that the soluble tannin content in persimmons (measured via Folin-Denis method) decreased after the use of ethanol vapour.

In 2004/05, there was a significant difference between the phenolic content of fruit with a SO₂ pad and without. A significant interaction between main effects (SO₂ x [E]) was found at some of the absorbance readings (Table 8). The use of a SO₂ pad significantly increased the total phenolics (A₂₈₀) per berry. Considered on a per gram fresh weight basis a non-significant increase was observed. No significant differences were found between total phenolics per berry at the different ethanol concentrations. However, on a weight basis the 10% ethanol treatment was significantly lower than the control, but it did not differ significantly from the other ethanol treatments. There was a quadratic trend for total phenolics per berry ($R^2 = 0.67$, $p < 0.05$) and per gram weight ($R^2 = 0.59$, $p < 0.05$) with an increasing amount of ethanol. The minimum total phenolics per berry were found at 33.3% ethanol and on a weight basis the minimum was found at 37.5%. A significant SO₂ x [E] interaction was observed for the total flavonols (A₃₆₅) per berry and per berry weight (data not shown) in 2004/05. The A₃₂₀ reading will not be discussed since only two cinnamic acids at very low concentrations were detected by HPLC. No relationship was found between sensory data and spectrophotometric analysis for both seasons.

4.3.2.3 HPLC-DAD analysis

Flavanols: Thirteen flavanols were detected by HPLC. Only the three most abundant flavanols will be discussed. The polymeric fraction (includes trimers, oligomers and polymers) was the main phenolic component identified in 'Regal Seedless'. For both seasons the two SO₂ pad treatments were significantly different in terms of the polymeric fraction when expressed on a per berry basis. When a SO₂ pad was used the

polymeric fraction was significantly higher (Table 9 and 10). On a per gram weight basis the SO₂ pad also increased the polymeric concentration, but not significantly. A possible reason is that the SO₂ pad protects the phenolics against degradation or oxidation. In 2003/04 the polymeric fraction exhibited significant differences between ethanol treatments on a per berry and weight basis. All the [E] resulted in polymer concentrations that were significantly lower than the control, but they did not differ from each other. A linear decrease ($R^2 = 0.60$, $p < 0.005$ (per berry); $R^2 = 0.65$, $p < 0.001$ (per berry weight)) was found with an increase in ethanol concentration (data not shown). Tanaka et al. (1994) has shown via HPLC that the acetaldehyde that forms from the ethanol plays an important role in the polymerisation of water-soluble proanthocyanidins, forming insoluble proanthocyanidins, which lower or remove astringency. During 2004/05 no significant differences or trends were found for the polymeric fraction between the ethanol treatments on a per gram weight basis (Table 10). On a per berry basis, no treatment differed from the control.

On a berry basis, the gallodimer1 concentration significantly increased with the use of SO₂ in 2003/04 (Table 9). The same was not observed on a weight basis. There were no significant differences between SO₂ pad treatments in terms of gallodimer1 concentration in 2004/05 (Table 10) for both units. The means of the ethanol concentrations for gallodimer1 were significantly different per berry (according to the t-test) in 2003/04, but none of the ethanol treatments were lower than the control. The ethanol treatments had no significant effect on the gallodimer1 concentration on a per berry weight basis in 2003/04 or in 2004/05.

The use of a SO₂ pad significantly increased procyanidin B2 per berry in both seasons (Table 9 and 10). On a per berry basis the use of 40 and 80% ethanol resulted in significantly higher amounts of procyanidin B2 than the control in 2003/04. A significant SO₂ x [E] interaction ($p < 0.005$) was found for procyanidin B2 on a weight basis in 2003/2004 (data not shown). The control treatment without SO₂ resulted in significantly lower concentrations of procyanidin B2 than 0%, 20%, 40%, and 80% ethanol with SO₂. In 2004/05 (Table 10), none of the ethanol treatments differed from the control.

The use of SO₂ increased the concentration of total flavanols (Fig. 4a) significantly in 2003/04 (berry: $P = 0.0084$) and in 2004/05 (berry: $P = 0.0121$; weight: $P = 0.0307$). SO₂ acts as an anti-oxidation and anti-microbial agent and thus stabilises the phenolics. There was also a non-significant increase in 2003/04 on a weight basis. All the ethanol concentrations caused a decrease in total flavanols per berry (Fig. 4b) in the first

season. The 10%, 20% and 80% ethanol treatments were significantly lower than the control. The total flavanols per gram weight of the control treatment were significantly higher than that of the ethanol treatments in 2003/04, but the ethanol concentrations from 10 to 80% did not differ significantly from each other (data not shown). A linear decrease was observed per berry ($R^2 = 0.55$, $p < 0.005$) and per berry weight ($R^2 = 0.64$, $p < 0.001$) in 2003/04 (data not shown). The ethanol treatments had no significant effect on the total flavanol concentration in 2004/05. This may be partly due to the fact that the grapes contained already low concentrations of flavanols in the second season. Significant correlations were found between astringency and total flavanols (per berry: $r = 0.42$, $p < 0.01$; per berry weight: $r = 0.45$, $p < 0.01$) in 2003/04 but the same was not found in 2004/05.

Flavonols: On a weight basis, quercetin-3-glucoside, quercetin-3-galactoside, quercetin-3-rhamnoside and kaempferol-glucoside/glucuronide, decreased significantly (according to the t-test) with the increase in ethanol concentration in 2003/04 (Table 11). These are the most abundant flavonols present within the grape and all of them followed decreasing linear trends (Table 12). The other identified flavonols, quercetin-3-rutinoside, myricetin-3-glucoside and kaempferol-3-galactoside, were only present in trace amounts. An ethanol dip of 40% and 80% resulted in concentrations that were significantly lower than the control in the case of quercetin-3-glucoside, -galactoside and total flavonols. The same was observed on a berry basis (data not shown). The SO₂ pad had no significant influence on the concentration of any of the flavonols, whether expressed per berry or per gram berry weight. In 2004/05 a significant SO₂ x [E] interaction was observed for quercetin-3-glucoside, quercetin-3-galactoside, kaempferol-glucoside/glucuronide and the total flavonols (data not shown) and no meaningful patterns were found for the compounds.

Non-flavonoids: Only one unknown benzoic acid and two unknown cinnamic acids were detected by HPLC. Their concentrations were very low and will not be discussed.

Total phenolics: When a SO₂ pad was placed in the boxes, significantly higher concentrations of total phenolics were observed for both seasons on a per berry basis (2003/04: $P = 0.0150$; 2004/05: $P = 0.0327$). When measured on a per berry weight basis the same was not found. During 2003/04 the total phenol content per berry decreased linearly ($R^2 = 0.67$; $p < 0.005$) with increasing concentration of ethanol,

where all the ethanol treatments were significant lower than the control. However, in 2004/05 no differences or trends were found between the treatments. This is not in agreement with the spectrophotometric analysis where a minimum point of total phenolics per berry could be determined. Significant correlations were found between astringency and total phenolics (per berry: $r = 0.44$, $p < 0.01$; per berry weight: $r = 0.47$, $p < 0.005$) in 2003/04 and A_{280} per berry and total phenolics per berry (2003/04: $r = 0.37$, $p < 0.05$; 2004/05: $r = 0.82$, $p < 0.0001$). It is important to note the seasonal variation. In 2004/05 the total phenolic concentration was substantially lower than in 2003/04, which could have influenced the effectiveness of ethanol dipping. The total phenolic content with an ethanol dip of 80% in 2003/04 was still higher than the control in 2004/05 (Fig. 5).

The exogenous application of ethanol is successful in removing astringency from persimmons (Yamada et al., 2002; Ortiz et al., 2005) by inducing anaerobic respiration. Ethanol is a precursor of acetaldehyde. It was found that the acetaldehyde binds to the soluble tannins to form larger insoluble tannins and thus removing the astringent taste (Taira et al., 1997). Ethanol (vapours or dipping) has been used on table grapes to prevent decay while maintaining or improving quality during cold storage (Lichter et al., 2003; Chervin et al., 2003; Karabulut et al., 2003; Pinto et al., 2006). This is very important. Alternative techniques are needed to replace the use of SO_2 pads, since SO_2 is corrosive to metals, dangerous to people allergic to sulfites and causes injury to the rachis and berries when used in high concentrations. No previous research to our knowledge has focused on the effect of ethanol on the phenolic content (neither spectrophotometric nor HPLC) of table grapes.

4.3.3 COLD STORAGE DURATION

There were speculations in the South African table grape industry that the astringency of 'Regal Seedless' increases with prolonged cold storage. This trial was conducted to investigate the influence of cold storage duration at $-0.5^{\circ}C$ in combination with two shelflife periods on astringency and phenolic content of 'Regal Seedless'.

4.3.3.1 Sensory evaluation

Sensory evaluation data are presented in Table 13. In 2002/03 and 2003/04 the cold storage and shelf life periods did not have any significant influence on the astringent taste. The astringency score tended to increase when the cold storage period was

extended in 2002/03. The eating quality (EQ) decreased significantly during cold storage as the astringency score increased. This confirms the reliability of the tasting panel. In 2003/04 the highest astringency score was observed directly after four weeks of cold storage where the EQ was the lowest, although differences between storage periods were non-significant for both variables. The lowest astringency and highest EQ were found at W7. When an additional week at 15°C was added to the cold storage period, W4 resulted in the lowest astringency for both seasons. The evaluation for skin tenacity was not consistent over the seasons. The skin tenacity score was always lower at W4, whether the extra week at 15°C was added or not. The effect of the week of shelf life at 15°C on the sensory evaluation is shown in Table 14. A negative value indicates a lower astringency, skin tenacity and eating quality after the shelf life period. A positive value illustrates a higher astringency, skin tenacity and eating quality after the extra one week at 15°C. See section 4.2.9 for explanation of calculations. No significant differences were observed for any of the taste properties at the prolonged storage at -0.5°C in both seasons, except for EQ in 2002/03. In 2002/03 grapes held at 15°C for one week following seven weeks of cold storage (W7) improved significantly in EQ compared to grapes without prior cold storage (W0) (according to the t-test).

4.3.3.2 Spectrophotometric analysis

In 2002/03 the total phenolics (A_{280}) per berry weight were significantly lower (according to the t-test) at zero weeks of cold storage when compared with four weeks of cold storage (Fig. 6). In cherries cold storage increased or decreased the total phenol levels, depending on the cultivar (Goncalves et al., 2004). In 2003/04 the total phenolics were significantly lower in the grapes with no cold storage when compared to the other cold storage treatments after shelf life (Fig. 7). The same results were found on a per berry basis (data not shown). The two seasons were not comparable, as results were inconsistent between the seasons.

The effect of the week of shelf life at 15°C is shown in Table 15. A negative value indicates lower total phenolic, flavanol and cinnamic acid content after the shelf life period. A positive value illustrates higher total phenolic, flavanol and cinnamic acid content after the extra one week at 15°C. In 2002/03 higher total phenolics were recorded at W0 and W7 than W4 after shelf life. The lower phenolics at W4 compare well with the sensory data. In 2003/04 W4 resulted in significant higher total phenolics after shelf life than W0 (according to the t-test). Results are thus inconsistent, no trends

are visible and no predictions can be made. In 2002/03 and 2003/04 the total flavanols (A_{365}) per berry weight after shelf life was the lowest at W4 and W0, respectively.

4.3.3.3 HPLC-DAD analysis

Flavanols: Eighteen and twenty-one flavanols were detected with HPLC in 2002/03 and 2003/04, respectively. A large group of these flavanols was unidentified. The total flavanols were calculated by combining the concentrations of the identified and unidentified flavanols. Gallodimer1, dimermonogallate2 and the polymeric fraction (includes trimers, oligomers and polymers) were the flavanols that were present in the highest concentration in the grapes. The results will only be discussed on a per berry weight basis since the same was found on a per berry basis.

Cold storage duration and the shelf life period did not have a significant effect on the gallodimer1 concentration, while significant differences were found for the dimermonogallate2 and the polymeric fraction (Table 16). Significantly higher concentrations of the dimermonogallate2 and polymeric fraction were found directly after four weeks of cold storage when compared to the other cold storage periods in both seasons. After a week at 15°C in 2003/04 significantly lower amounts were found at W4. In 2002/03 however, the different cold storage durations were no longer significantly different after one week of shelf life. In both seasons the concentration of total flavanols (per berry weight) followed exactly the same pattern as described for the dimermonogallate2 and polymeric fraction due to the fact that these two compounds make up a large percentage of the total flavanols (Fig. 8 and 9).

When considering the effect of the shelf period, significant differences were found for the dimermonogallate2, polymeric fraction and total flavanols in both seasons (Table 17). After a cold storage period of four weeks and a week at 15°C, the concentration of the dimermonogallate2, polymeric fraction and total flavanols were significantly lower, compared to the other cold storage durations. This coincides with the lower astringent taste and higher EQ observed in both seasons.

Flavanols: The three main flavanols present in the 'Regal Seedless' samples were quercetin-3-galactoside, quercetin-3-glucoside and quercetin-3-rhamnoside. Other flavanols were also detected, but their concentrations were very low. The total flavanols were determined as the sum of the concentrations of all the flavanols that were detected with HPLC. Dadic & Belleau (1973) found that quercetin appeared to elicit a bitter taste with weak astringency in alcohol solutions and in beer. It is possible that the flavanols

can develop a synergistic effect between themselves or the other compounds within the grape and contribute to the astringent taste even if they are separately below the detection limit.

Significant differences in flavonols were found between treatments in both seasons, except for the no shelf period in 2003/04 (Table 16). In 2002/03, after four weeks of cold storage with no extra shelf life, significantly higher concentrations were found for all three flavonols compared to the other cold storage durations. After one week at 15°C, W7 resulted in the highest (not always significant) concentrations of all three flavonols in both seasons. With no shelf life, the highest concentration of total flavonols (per berry weight) was recorded at W4 in both seasons (Fig. 10 and 11). After seven weeks of cold storage and one week of shelf life, the concentration of total flavonols was higher in both seasons compared to W7 without shelf life. The flavonol concentration was the lowest after the four week cold storage period (Table 18).

Non Flavonoids: Only one unknown benzoic acid and two unknown cinnamic acids were detected with HPLC. They were only present in trace amounts, thus they will not be discussed.

4.4 CONCLUSIONS

Zero-oxygen: The use of nitrogen had a tendency to decrease the astringent taste and the total phenolics (A_{280}), even though this decrease was only significant in 2002/03 when the total phenolic concentration was relatively low. With regards to the HPLC analysis, nitrogen was effective in lowering the concentration of flavonols, but non-significantly in both seasons. Nitrogen application increased the concentration of individual and total flavanols, but it was not significantly higher than the air treatment. Generally the best results were obtained with nitrogen exposure for four hours. A control (zero exposure time) must be added in further trials. In future treatments can be performed at higher temperatures since it has been shown that higher temperatures do not negatively affect quality parameters of table grapes (Ahumada et al., 1996). The use of CO₂ instead of N₂ can also be considered since it was found that CO₂ is more effective in removing astringency from persimmons by producing more acetaldehyde that can bind to the soluble tannins and form insoluble tannins (Zavrtanik et al. 1999).

Ethanol: During 2004/05 the total phenolic concentrations quantified by HPLC were substantially lower than in 2003/04. Thus, the concentrations were already low when the trial was conducted in 2004/05. This is a possible explanation why we did not see any effect of the ethanol dipping on the phenolic content or sensory evaluation in this season. According to the HPLC phenolic analysis in 2003/04, the 40 and 80% ethanol treatment did not only reduce the phenolic content significantly when compared to the control, but also decreased the astringency significantly. These concentrations were also effective in the control of *Botrytis cinerea* and *Alternaria* (data not shown). High ethanol concentrations did not impair the appearance of the bunches (data not shown). This is consistent with the findings of Lichter et al. (2002). An additional factor that could have contributed to the differences observed between the two seasons is the fact that in 2003/04 the trial was conducted after the bunches were harvested (postharvest) while in 2004/05 the grapes were dipped on the vines (preharvest).

In future studies, the ethanol residues on the grapes should also be determined as well as the acetaldehyde, ethanol vapour and ethylene within the boxes of grapes. It has been shown by Gabler et al. (2005) that the use of hot water ethanol treatments increases the amount of ethanol that penetrates the berry. This might improve the efficacy of the ethanol to lower phenolic concentrations. These trials should be repeated at commercial maturity for 'Regal Seedless' (18°Brix).

Further work should also be conducted to evaluate an ethanol dip followed by storage in modified atmospheres (N₂ or CO₂) on the phenolic content of grapes. It has been found that these two treatments are effective in prolonging quality during storage (Lichter et al., 2005).

Cold storage: This trial partially confirms the speculations by the table grape industry. Currently, four weeks storage at -0.5°C and one week at 15°C, is the protocol used in cold storage trials. These temperatures simulate the shipping period overseas and the shelf life period in the supermarket. Higher concentrations of total phenolics, flavanols and flavonols were found directly after the cold storage period of four weeks in comparison to the other two cold storage durations. However, by adding one week of shelf life at 15°C to the four week cold storage period, the concentrations of total phenolics, individual and total flavanols and flavonols as well as the astringent taste decreased to levels that were lower than that of the other treatments after shelf life. Thus to achieve acceptable levels of astringency and low levels of phenolic compounds, we recommend four weeks at -0.5°C and one week at 15°C.

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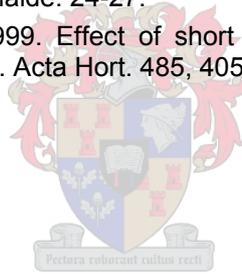


Table 1 The effect of air or nitrogen and exposure time on the sensory evaluation of 'Regal Seedless' grapes for 2002/03 and 2003/04.

Season	Treatment ^y	Astringency ^z	Skin tenacity ^z	Eating quality ^z
2002/03	Air	4.58 a	4.52 a	4.59 a
	Nitrogen	4.25 a	4.31 a	4.48 a
	LSD ^x	0.97	0.89	0.52
	P ^w (p ≤ 0.05)	0.4987	0.6400	0.6689
	T4	3.98 a	4.01 a	5.01 a
	T8	4.43 a	4.58 a	4.49 ab
	T16	4.35 a	4.54 a	4.48 ab
	T32	5.24 a	4.55 a	3.73 b
	LSD	1.41	1.30	0.77
	P (p ≤ 0.05)	0.2880	0.6962	0.0339
2003/04	Air	2.96 a	4.95 a	6.59 b
	Nitrogen	2.13 b	4.60 a	7.16 a
	LSD	0.72	0.88	0.54
	P (p ≤ 0.05)	0.0262	0.4200	0.0383
	T4	2.59 b	4.79 b	7.20 a
	T8	3.78 a	6.64 a	6.53 a
	T16	2.35 b	3.71 b	7.15 a
	T32	2.41 b	4.48 b	6.81 a
	T64	1.62 b	4.25 b	6.70 a
	LSD	1.14	1.39	0.85
P (p ≤ 0.05)	0.0107	0.0018	0.4389	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z Scores per variable – scale 0-10, 0 – no astringency, soft skin, not favourable to eat; 10 – high astringency and skin tenacity, very favourable to eat.

^y T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

Table 2 The effect of air or nitrogen and exposure time on the phenolic content (AU per berry) of 'Regal Seedless' in 2003/04.

Treatment ^z	A280 ^y	A365 ^y	A320 ^y
Air	0.0776 a	0.2260 a	0.2646 a
Nitrogen	0.0757 a	0.2065 a	0.2496 a
LSD^x	0.0041	0.0195	0.0198
P^w (p ≤ 0.05)	0.3569	0.0504	0.1361
T4	0.0665 b	0.1833 c	0.2173 c
T8	0.0820 a	0.2022 bc	0.2701 ab
T16	0.0716 b	0.2150 b	0.2449 bc
T32	0.0837 a	0.2544 a	0.2914 a
T64	0.0801 a	0.2312 ab	0.2648 ab
LSD	0.0066	0.031	0.0313
P (p ≤ 0.05)	<0.0001	0.0005	0.0004

Within treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^y A280 = total phenol content; A365 = flavanols; A320 = cinnamic acids.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

Table 3 The effect of air or nitrogen and exposure time on the phenolic content (AU per berry weight) of 'Regal Seedless' in 2002/03.

Treatment ^z	A280 ^y	A365 ^y	A320 ^y
Air	0.0122 a	0.0278 a	0.0375 a
Nitrogen	0.0117 b	0.0253 b	0.0357 a
LSD^x	0.0004	0.0024	0.0023
P^w (p ≤ 0.05)	0.0098	0.0449	0.1253
T4	0.0118 b	0.0251 b	0.0359 b
T8	0.0119 b	0.0253 b	0.0352 b
T16	0.0118 b	0.0266 b	0.0366 b
T32	0.0131 a	0.0335 a	0.0425 a
LSD	0.0006	0.0038	0.0035
P (p ≤ 0.05)	0.0001	0.0002	0.0011

Within treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours).

^y A280 = total phenol content; A365 = flavanols; A320 = cinnamic acids.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

Table 4 The effect of air or nitrogen and exposure time on the flavanol content (μg per gram berry weight), as measured by HPLC, of 'Regal Seedless' in 2002/03.

Treatment ^z	gallodimer1	procyanidin B2	di-mo-gal2 ^y	polymer	total
Air	77.66 a	5.07 a	12.61 a	577.61 a	700.82 a
Nitrogen	79.87 a	5.53 a	11.45 a	593.42 a	714.15 a
LSD^x	6.04	0.66	1.29	69.20	73.86
P^w ($p \leq 0.05$)	0.4612	0.1585	0.077	0.6453	0.7156
T4	78.26 a	5.50 a	9.98 b	521.63 b	637.42 b
T8	81.38 a	5.42 a	12.28 a	591.31 ab	717.35 ab
T16	77.99 a	5.10 a	13.36 a	602.90 ab	727.35 ab
T32	76.29 a	5.17 a	12.43 a	692.98 a	813.58 a
LSD	9.09	0.99	1.93	104.15	111.16
P ($p \leq 0.05$)	0.6061	0.6492	0.0028	0.0368	0.0408

Within treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours).

^y di-mo-gal2 = dimermonogallate2.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

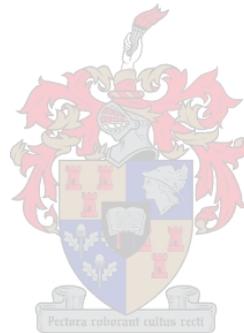


Table 5 The effect of air or nitrogen and exposure time on the concentration of flavonols (μg per gram berry weight) in 'Regal Seedless' in 2002/03 and 2003/04.

Season	Treatment ^z	q-3-gal ^y	q-3-glc ^y	k-glc/gluc ^y	q-3-rham ^y
2002/03	Air	11.14 a	20.64 a	4.28 a	9.18 a
	Nitrogen	9.61 a	17.67 a	3.42 a	8.17 a
	LSD ^x	2.81	3.10	1.06	1.02
	P ^w ($p \leq 0.05$)	0.2735	0.0596	0.1043	0.0515
	T4	9.14 b	16.68 b	3.78 ab	7.80 b
	T8	9.31 b	18.43 b	3.22 b	8.42 ab
	T16	10.60 b	20.52 ab	3.94 ab	9.25 ab
	T32	14.83 a	23.19 a	5.13 a	9.93 a
	LSD	4.19	4.62	1.58	1.52
	P ($p \leq 0.05$)	0.0219	0.0124	0.0437	0.0106
2003/04	Air	24.45 a	26.80 a	9.25 a	11.82 a
	Nitrogen	20.29 a	22.31 b	8.17 a	10.71a
	LSD	4.46	3.81	1.82	1.38
	P ($p \leq 0.05$)	0.0668	0.0219	0.2402	0.1141
	T4	18.48 bc	21.73 bc	8.02 bc	10.94 ab
	T8	16.30 c	19.86 c	5.36 c	8.99 b
	T16	28.13 a	28.59 a	11.72 a	13.01 a
	T32	24.28 ab	26.23 ab	8.82 b	11.54 a
	T64	23.15 abc	25.15 abc	8.98 ab	11.39 a
	LSD	7.06	6.04	2.89	2.19
P ($p \leq 0.05$)	0.0147	0.0487	0.0024	0.0170	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^y q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-glc/gluc = kaempferol-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

Table 6 The effect of different ethanol concentrations on the sensory evaluation of 'Regal Seedless' grapes for 2003/04 and 2004/05.

Season	Ethanol treatment (%)	Astringency ^z	Skin tenacity ^z	Eating quality ^z
2003/04	0	5.16 a	4.63 a	5.14 b
	10	3.56 ab	5.56 a	5.73 ab
	20	2.83 b	5.35 a	6.32 ab
	40	2.62 b	4.93 a	6.70 a
	80	2.20 b	5.34 a	6.48 a
LSD^y		1.60	2.27	1.24
P^x (p ≤ 0.05)		0.0055	0.9247	0.0973
2004/05	0	2.67 a	2.29 a	6.64 a
	10	2.06 a	2.10 a	7.24 a
	20	3.38 a	2.93 a	5.68 a
	40	2.49 a	2.41 a	5.45 a
	80	2.77 a	2.46 a	5.65 a
LSD		1.88	1.92	2.21
P (p ≤ 0.05)		0.7173	0.9320	0.4164

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z Scores per variable – scale 0-10, 0 – no astringency, soft skin, not favourable to eat; 10 – high astringency and skin tenacity, very favourable to eat.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 7 The effect of different ethanol concentrations on the phenolic content of 'Regal Seedless' grapes in 2003/04.

Ethanol treatment (%)	A280 ^z	A365 ^z	A320 ^z	A280 ^z	A365 ^z	A320 ^z
	AU per berry			AU per gram berry weight		
0	0.0692 a	0.2269 a	0.2509 a	0.0133 a	0.0436 a	0.0483 a
10	0.0647 a	0.2083 a	0.2355 a	0.0133 a	0.0429 a	0.0483 a
20	0.0678 a	0.2204 a	0.2438 a	0.0133 a	0.0430 a	0.0477 a
40	0.0700 a	0.2017 a	0.2349 a	0.0130 a	0.0376 a	0.0437 a
80	0.0711 a	0.2084 a	0.2380 a	0.0134 a	0.0394 a	0.0449 a
LSD^y	0.0070	0.4040	0.0366	0.0007	0.0070	0.0058
P^x (p ≤ 0.05)	0.4044	0.7117	0.8871	0.7885	0.3344	0.3650

Within columns, values with different letters are significantly different at a 5% significance level.

^z A280 = total phenol content; A365 = flavanols; A320 = cinnamic acids.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

Table 8 The effect of a SO₂ pad and different ethanol concentrations on the phenolic content of 'Regal Seedless' grapes in 2004/05.

Treatment	A280 ^z	A365 ^z	A320 ^z	A280 ^z	A365 ^z	A320 ^z
	AU per berry			AU per gram berry weight		
SO ₂₍₀₎ ^x	0.0696 b	0.2130	0.2321 a	0.0134 a	0.0410	0.0446
SO ₂₍₁₎	0.0740 a	0.2201	0.2522 a	0.0136 a	0.0404	0.0462
LSD	0.0043	- ^y	0.0241	0.0005	-	-
P (p ≤ 0.05)	0.0413	-	0.0890	0.3200	-	-
E[0] ^w	0.0749 a	0.2048	0.2306 a	0.0141 a	0.0384	0.04325
E[10]	0.0688 a	0.2043	0.2312 a	0.0131 b	0.0388	0.04394
E[20]	0.0686 a	0.2123	0.2338 a	0.0133 ab	0.0413	0.04547
E[40]	0.0711 a	0.2247	0.2492 a	0.0133 ab	0.0420	0.04632
E[80]	0.0754 a	0.2356	0.2659 a	0.0136 ab	0.0425	0.04800
LSD ^v	0.0069	-	0.0382	0.0009	-	-
P (p ≤ 0.05) ^u	0.1450	-	0.2735	0.2201	-	-

Within columns and treatments, values with different letters are significantly different at a 5% significance level.

^z A280 = total phenol content; A365 = flavanols; A320 = cinnamic acids.

^y -: data and significance level not shown due to interaction between SO₂ and [E].

^x SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^w E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^v LSD = Least significant difference.

^u P = Probability of F-ratio test.



Table 9 The effect of a SO₂ pad and different ethanol concentrations on the flavanol content of 'Regal Seedless' grapes in 2003/04.

Treatment	gallodimer1	procyanidin B2	polymer	gallodimer1	procyanidin B2	polymer
	µg/berry			µg/g berry weight		
SO ₂₍₀₎ ^y	343.98 b	60.83 b	1764.3 b	69.28 a	12.11	354.64 a
SO ₂₍₁₎	390.49 a	69.09 a	2200.0 a	73.18 a	12.94	410.81 a
LSD ^w	34.83	6.82	412.79	6.23	- ^z	74.89
P ^v (p ≤ 0.05)	0.0134	0.0241	0.0173	0.2604	-	0.0655
E[0] ^x	332.13 b	57.11 b	2717.6 a	65.07 a	11.08	527.69 a
E[10]	353.86 ab	57.70 b	1938.0 b	71.50 a	11.62	382.69 b
E[20]	368.63 ab	63.83 ab	1803.1 b	72.51 a	12.51	350.88 b
E[40]	399.91 a	74.37 a	2053.5 b	74.06 a	13.74	382.64 b
E[80]	380.11 ab	71.20 a	1445.1 b	72.69 a	13.56	278.30 b
LSD	55.17	10.80	653.83	9.87	-	118.63
P (p ≤ 0.05)	0.1506	0.0073	0.0102	0.3998	-	0.0052

Within columns and treatments, values with different letters are significantly different at a 5% significance level.

^z '-' = data and significance level not shown due to interaction between SO₂ and [E].

^y SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^x E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^w LSD = Least significant difference.

^v P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 10 The effect of a SO₂ pad and different ethanol concentrations on the flavanol content of 'Regal Seedless' grapes in 2004/05.

Treatment	gallodimer1	procyanidin B2	polymer	gallodimer1	procyanidin B2	polymer
	µg/berry			µg/g berry weight		
SO ₂₍₀₎ ^z	448.66 a	54.895 b	847.97 b	86.14 a	10.47 a	162.81 a
SO ₂₍₁₎	467.50 a	63.885 a	1019.27 a	85.95 a	11.62 a	185.69 a
LSD ^x	41.19	8.78	150.19	7.08	1.25	25.51
P ^w (p ≤ 0.05)	0.4125	0.0841	0.0223	0.9933	0.1318	0.0516
E[0] ^y	443.00 a	57.85 ab	873.60 ab	83.44 a	10.82 ab	163.99 a
E[10]	461.18 a	59.05 ab	858.00 b	88.12 a	11.26 a	163.13 a
E[20]	443.34 a	46.53 b	915.20 ab	87.68 a	9.21 b	180.78 a
E[40]	447.56 a	60.33 ab	878.30 ab	83.69 a	11.05 ab	162.18 a
E[80]	487.84 a	66.16 a	1105.30 a	88.19 a	11.91 a	199.36 a
LSD	66.12	14.39	241.07	11.36	2.05	40.94
P (p ≤ 0.05)	0.5616	0.1910	0.1754	0.7922	0.2134	0.2403

Within columns and treatments, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^x LSD = Least significant difference.

^w P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 11 The effect of a SO₂ pad and different ethanol concentrations on the flavonol content (µg/g berry weight) of 'Regal Seedless' grapes in 2003/04.

	q-3-glc ^x	q-3-gal ^x	k-glc/gluc ^x	q-3-rham ^x	total flavonols
SO ₂₍₀₎ ^z	28.75 a	25.68 a	9.87 a	13.66 a	85.91 a
SO ₂₍₁₎	27.53 a	24.62 a	9.57 a	12.80 a	82.18 a
LSD ^w	4.81	5.77	2.23	1.62	15.4
P ^v (p ≤ 0.05)	0.7819	0.8658	0.8728	0.3729	0.7728
E[0] ^y	31.62 a	34.56 a	11.15 a	14.94 a	101.81 a
E[10]	24.84 ab	27.51 ab	9.99 ab	13.04 ab	83.58 ab
E[20]	28.31 ab	28.55 ab	11.61 a	13.76 ab	90.69 ab
E[40]	20.05 b	25.53 b	7.40 b	12.00 b	71.34 b
E[80]	21.12 b	24.78 b	8.48 ab	12.44 ab	73.40 b
LSD	9.14	7.63	3.53	2.56	24.36
P (p ≤ 0.05)	0.0765	0.1091	0.0921	0.1740	0.0913

Within columns and treatments, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^x q-3-glc = quercetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-rham = quercetin-3-rhamnoside; k-glc/gluc = kaempferol-glucoside/glucuronide.

^w LSD = Least significant difference.

^v P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 12 Linear regressions found for the flavonols in 2003/04.

Flavonols	R ²	P-value	R ²	P-value
	per berry		per berry weight	
quercetin-3-glucoside	0.50	0.0202	0.60	0.0163
quercetin-3-galactoside	0.56	0.0196	0.59	0.0101
quercetin-3-rhamnoside	-	-	0.51	0.0242
kaempferol-glucoside/glucuronide	0.45	0.0446	0.46	0.0312

Table 13 The effect of cold storage duration and shelf life period on the sensory evaluation of 'Regal Seedless' in 2002/03 and 2003/04.

Season	Treatment ^y	Astringency ^z	Skin tenacity ^z	Eating quality ^z
2002/03	W0+S0	3.54 a	5.74 a	6.19 a
	W4+S0	4.03 a	4.90 a	4.66 b
	W7+S0	5.21 a	5.11 a	4.51 b
	LSD ^x	3.25	2.18	1.23
	P ^w (p ≤ 0.05)	0.5445	0.7003	0.0185
	W0+S1	3.91 a	4.62 ab	5.30 a
	W4+S1	3.59 a	3.62 b	5.30 a
	W7+S1	4.66 a	5.92 a	5.50 a
	LSD	2.73	2.08	1.58
	P (p ≤ 0.05)	0.6966	0.0926	0.9528
2003/04	W0+S0	4.80 a	4.92 a	4.56 a
	W4+S0	5.02 a	4.32 a	4.28 a
	W7+S0	3.67 a	5.29 a	5.43 a
	LSD	2.83	2.30	2.21
	P (p ≤ 0.05)	0.5506	0.6603	0.5136
	W0+S1	5.52 a	4.85 a	4.19 a
	W4+S1	4.78 a	4.71 a	4.58 a
	W7+S1	5.08 a	5.41 a	4.57 a
	LSD	3.38	2.69	2.42
	P (p ≤ 0.05)	0.8920	0.8355	0.9238

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z Scores per variable – scale 0-10, 0 – no astringency, soft skin, not favourable to eat; 10 – high astringency and skin tenacity, very favourable to eat.

^y W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 14 The effect of the shelf life period at 15°C on the sensory evaluation of 'Regal Seedless' in 2002/03 and 2003/04.

Season	Treatment ^z	Astringency	Skin tenacity	Eating quality
2002/03	W0	0.38 a	-1.13 a	-0.89 b
	W4	-0.44 a	-1.28 a	0.64 ab
	W7	-0.54 a	0.81 a	1.00 a
	LSD ^y	3.96	2.67	1.67
	P ^x (p ≤ 0.05)	0.8648	0.2096	0.0649
2003/04	W0	0.72 a	-0.07 a	-0.36 a
	W4	-0.24 a	0.39 a	0.30 a
	W7	1.40 a	0.13 a	-0.86 a
	LSD	2.93	2.38	2.94
	P (p ≤ 0.05)	0.4888	0.9138	0.6962

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 15 The effect of the shelf life period at 15°C on the phenolic content (AU per berry weight) of 'Regal Seedless' in 2002/03 and 2003/04.

Season	Treatment ^z	A280	A365	A320
2002/03	W0	0.0007 a	0.0038 a	0.0034 a
	W4	-0.0004 a	-0.0030 b	-0.0017 a
	W7	0.0006 a	0.0028 a	0.0029 a
	LSD ^y	0.0012	0.0049	0.0054
	P ^x (p ≤ 0.05)	0.1336	0.0339	0.1610
2003/04	W0	-0.0007 b	-0.0034 b	-0.0043 b
	W4	0.0008 a	0.0021 ab	0.0038 a
	W7	0.0003 ab	0.0065 a	0.0080 a
	LSD	0.0013	0.0074	0.0071
	P (p ≤ 0.05)	0.0829	0.0450	0.0089

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 16 The effect of cold storage duration and shelf life period on the concentration of individual flavanols and flavonols (μg per gram berry weight) of 'Regal Seedless' in 2002/03 and 2003/04.

Season	Treatment ^z	Flavanols			Flavonols		
		gallodimer1	di-mo-gal2 ^y	polymer	q-3-gal ^y	q-3-glc ^y	q-3-rham ^y
2002/03	W0+S0	57.69 a	7.14 b	328.0 b	3.04 b	9.10 b	5.16 b
	W4+S0	63.56 a	14.76 a	784.3 a	6.83 a	17.95 a	8.31 a
	W7+S0	55.90 a	7.35 b	337.4 b	3.69 b	10.75 b	6.00 b
	LSD ^x	10.63	6.22	258.3	2.27	3.82	1.54
	P ^w ($p \leq 0.05$)	0.3034	0.0287	0.0024	0.0063	0.0004	0.0015
	W0+S1	56.07 a	10.92 b	569.7 a	5.90 ab	16.23 ab	7.68 ab
	W4+S1	53.82 a	12.49 ab	578.4 a	3.52 b	11.04 b	6.37 b
	W7+S1	48.70 a	16.65 a	680.3 a	8.18 a	18.11 a	8.89 a
	LSD	8.44	5.48	280.4	4.03	5.99	2.20
	P ($p \leq 0.05$)	0.1962	0.1027	0.6536	0.0774	0.0609	0.0804
2003/04	W0+S0	104.15 a	13.20 b	730.25 b	7.36 a	18.36 a	9.37 a
	W4+S0	110.84 a	22.81 a	1055.95 a	8.95 a	20.11 a	9.51 a
	W7+S0	101.48 a	12.24 b	632.55 b	6.22 a	15.44 a	7.80 a
	LSD	16.15	6.13	210.91	3.59	5.27	2.26
	P ($p \leq 0.05$)	0.4477	0.0041	0.0025	0.2907	0.2032	0.2467
	W0+S1	103.12 a	25.73 a	1049.51 b	6.85 b	18.88 b	9.31 ab
	W4+S1	101.58 a	9.50 b	559.65 c	6.81 b	15.05 b	7.84 b
	W7+S1	97.84 a	25.26 a	1272.90 a	12.07 a	24.36 a	10.13 a
	LSD	13.05	4.29	139.39	3.50	4.46	1.75
	P ($p \leq 0.05$)	0.6816	<0.0001	<0.0001	0.0081	0.0018	0.0417

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C ; S0 = 0 weeks, S1 = 1 week at 15°C .

^y di-mo-gal2 = dimermonogallate2; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; q-3-rham = quercetin-3-rhamnoside.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test; ANOVA (F-test) is a more strict test than the Students t-test (LSD).

Table 17 The effect of the shelf life period at 15°C on the concentration of individual and total flavanols (μg per gram berry weight) of 'Regal Seedless'.

Season	Treatment ^z	gallodimer1	di-mo-gal2 ^y	polymer	total flavanols
2002/03	W0	-1.62 a	2.35 ab	241.6 a	263.4 a
	W4	-9.75 a	-2.27 b	-205.9 b	-238.2 b
	W7	-7.20 a	9.30 a	342.9 a	360.6 a
	LSD ^x	12.75	8.27	418.32	444.26
	P ^w ($p \leq 0.05$)	0.4030	0.0249	0.0308	0.0252
2003/04	W0	1.71 a	13.58 a	297.41 b	348.4 b
	W4	-11.88 a	-12.99 b	-482.18 c	-531.4 c
	W7	-1.86 a	14.23 a	622.21 a	685.6 a
	LSD	22.27	10.39	218.07	234.37
	P ($p \leq 0.05$)	0.3860	0.0002	<0.0001	<0.0001

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y di-mo-gal2 = dimermonogallate2.

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

Table 18 The effect of the shelf life period at 15°C on the concentration of individual and total flavonols (μg per gram berry weight) of 'Regal Seedless'.

Season	Treatment ^z	q-3-gal ^y	q-3-glc ^y	q-3-rham ^y	total flavonols
2002/03	W0	2.86 a	7.36 a	2.53 a	15.68 a
	W4	-3.31 b	7.13 a	-1.95 b	-16.33 b
	W7	4.50 a	-6.91 b	2.89 a	17.98 a
	LSD ^x	4.29	7.56	2.80	18.74
	P ^w ($p \leq 0.05$)	0.0036	0.0013	0.0035	0.0021
2003/04	W0	-0.70 b	0.08 b	-0.12 ab	-1.26 b
	W4	-2.98 b	-6.07 b	-2.10 b	-14.24 b
	W7	7.09 a	10.80 a	3.08 a	26.26 a
	LSD	5.59	7.83	3.37	18.78
	P ($p \leq 0.05$)	0.0069	0.0024	0.0215	0.0016

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; q-3-rham = quercetin-3-rhamnoside

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

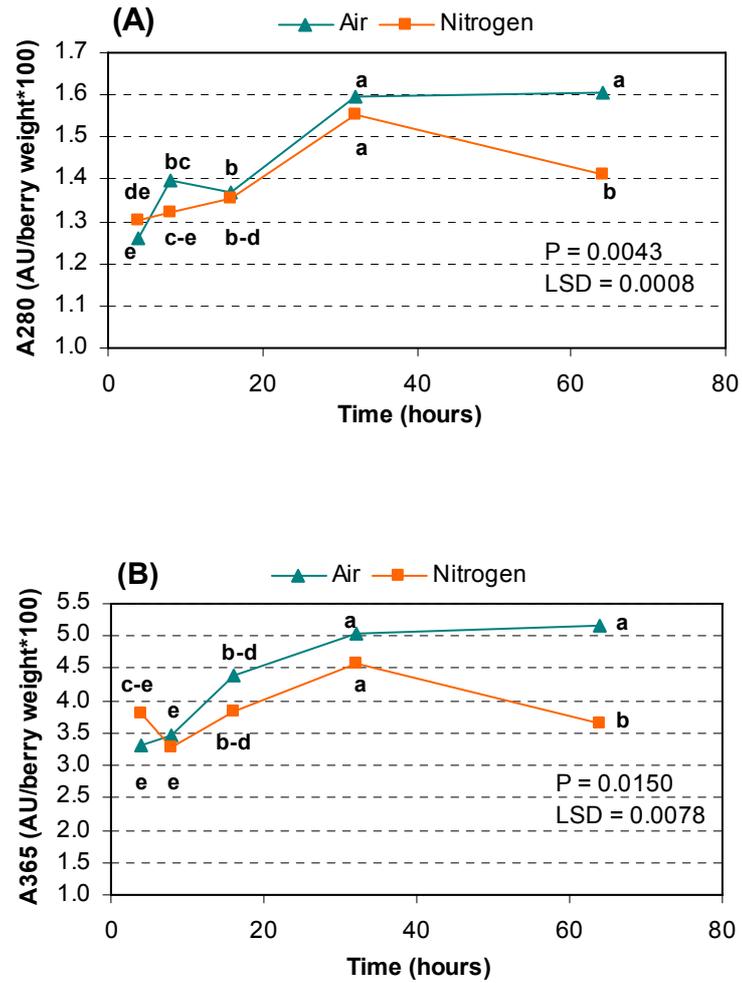


Fig. 1 The effect of air or nitrogen on the (A) total phenolics (A_{280}) and (B) flavonols (A_{365}) over treatment time on a per berry weight basis in 2003/04 as measured spectrophotometrically.

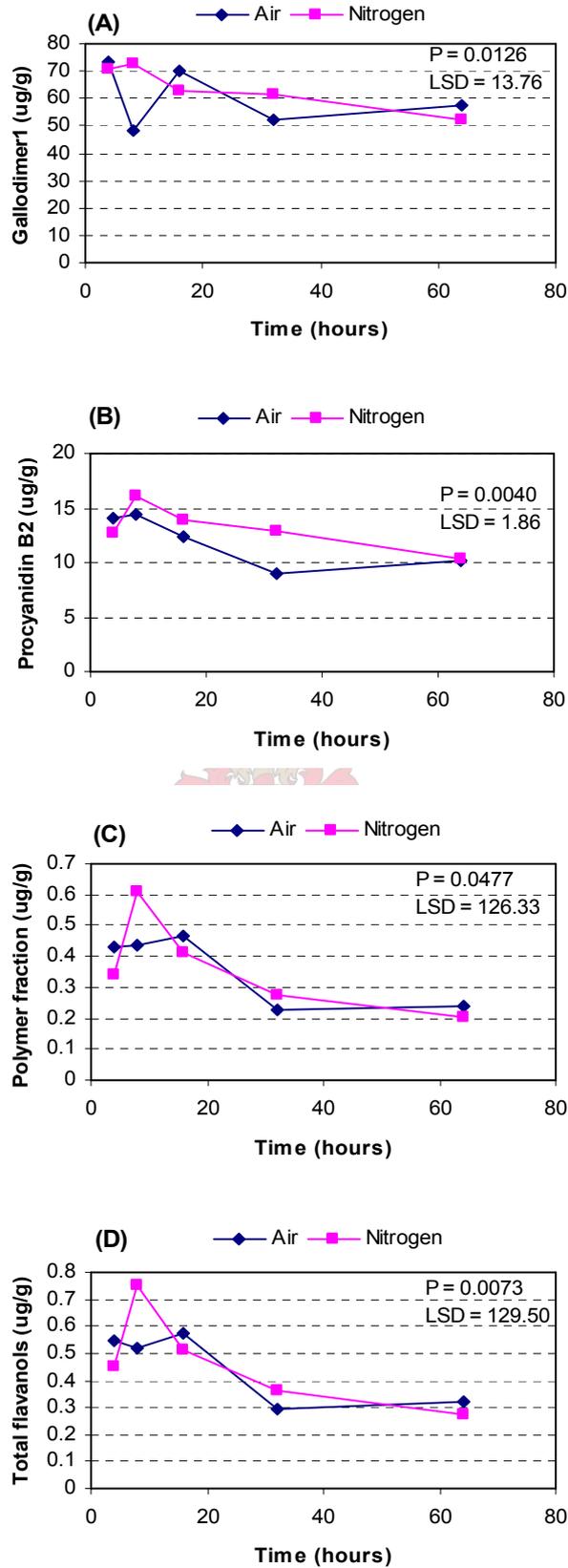


Fig. 2 The effect of air or nitrogen and exposure time on the (A) gallodimer1 (B) procyanidin B2 (C) polymeric fraction and (D) total flavanols on a per berry weight basis in 2003/04 as measured by HPLC.

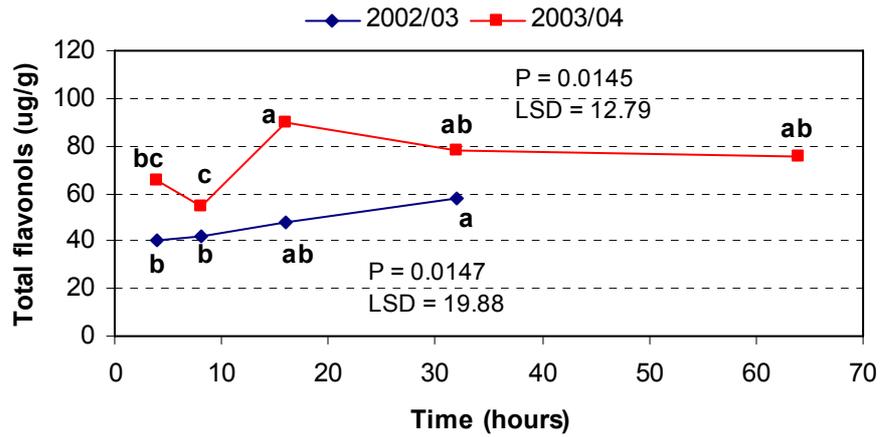


Fig. 3 The effect of gas exposure time on the total flavonol content (μg per gram berry weight) in Regal Seedless in 2002/03 and 2003/04 as measured by HPLC.

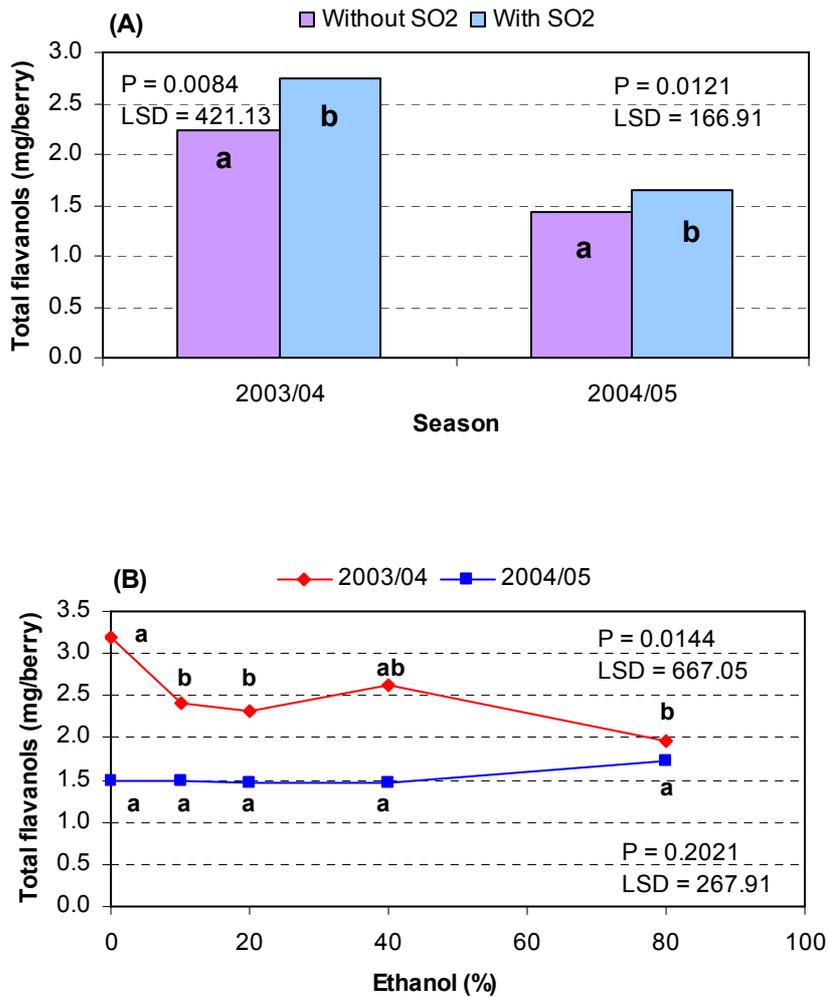


Fig. 4 The effect of a SO₂ pad (A) and different ethanol concentrations (B) on the concentration of total flavanols of Regal Seedless.

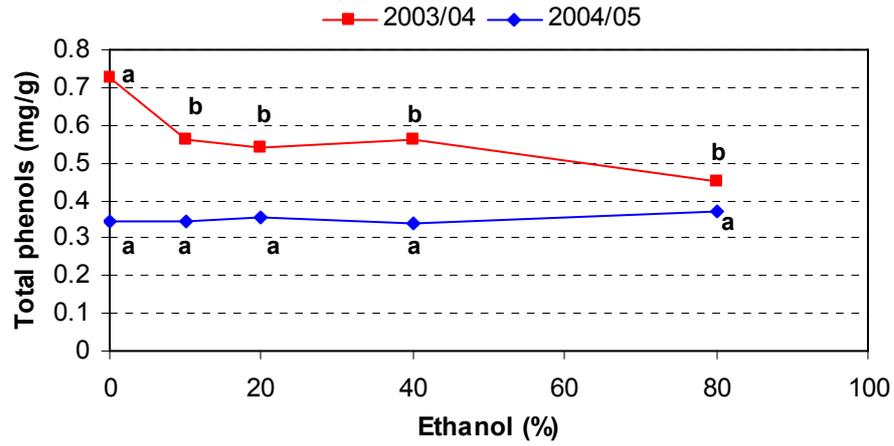


Fig. 5 The effect of different ethanol treatments on the total phenolic content (mg/g) of Regal Seedless.

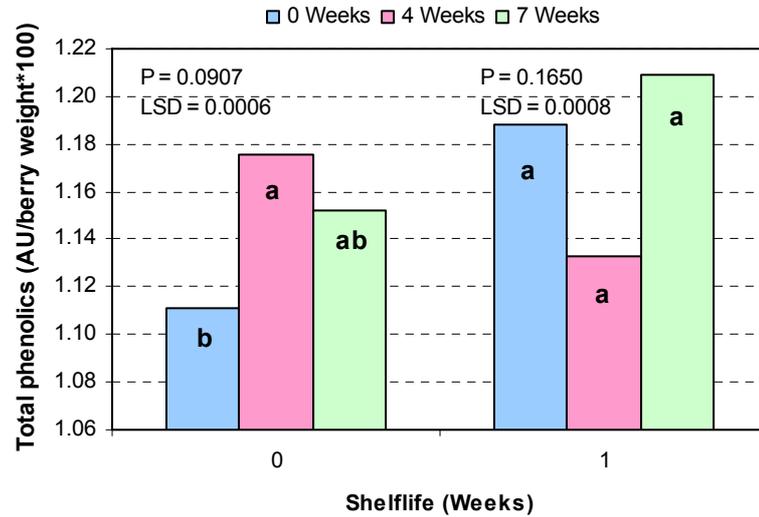


Fig. 6 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total phenolic content (AU per berry weight) of Regal Seedless in 2002/03. ANOVA (F-test) is a more strict test than the Students t-test (LSD).

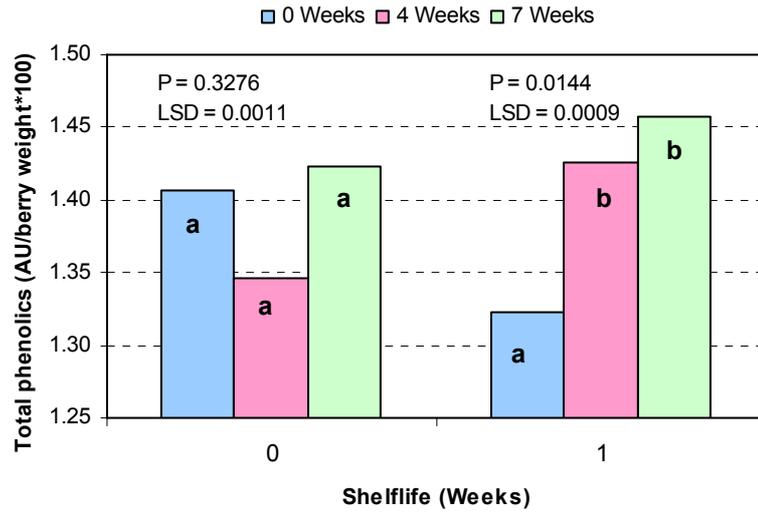


Fig. 7 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total phenolic content (AU per berry weight) of Regal Seedless in 2003/04.

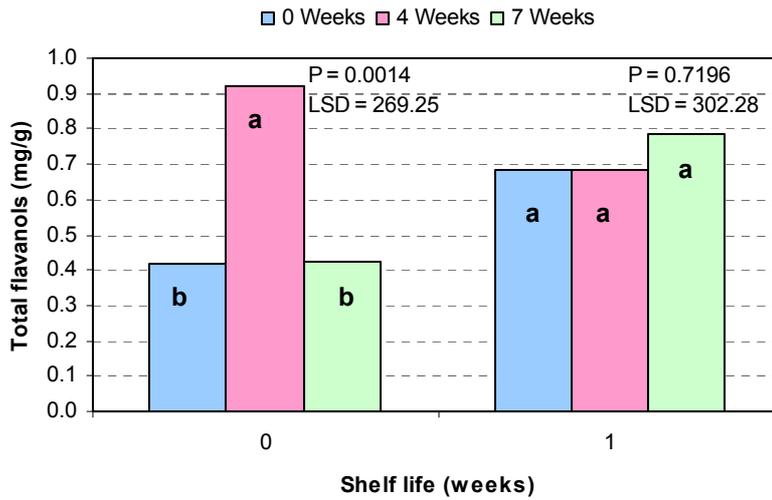


Fig. 8 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total flavanols (mg per gram berry weight) of Regal Seedless in 2002/03.

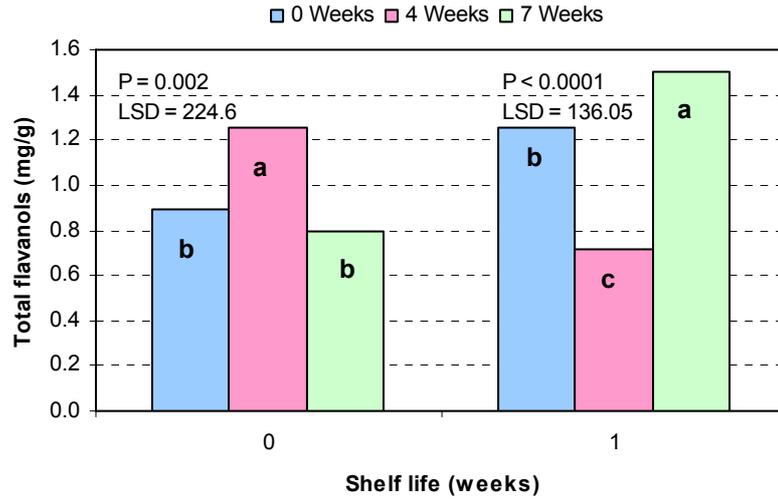


Fig. 9 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total flavanols (mg per gram berry weight) of Regal Seedless in 2003/04.

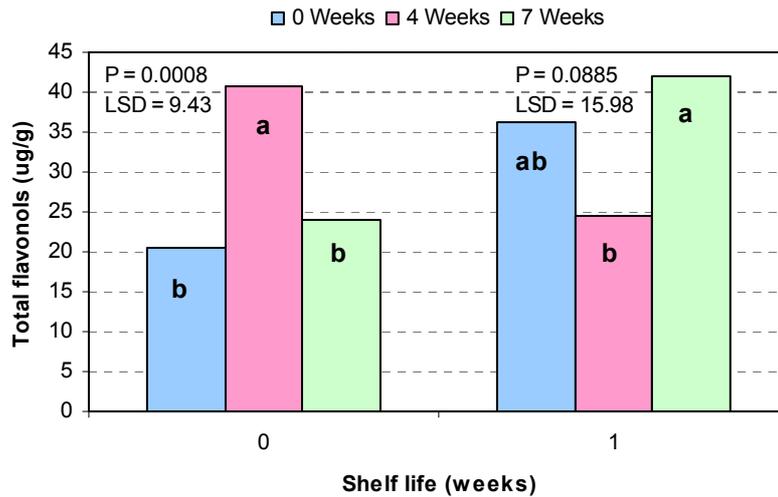


Fig. 10 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total flavonols (μg per gram berry weight) of Regal Seedless in 2002/03. ANOVA (F-test) is a more strict test than the Students t-test (LSD).

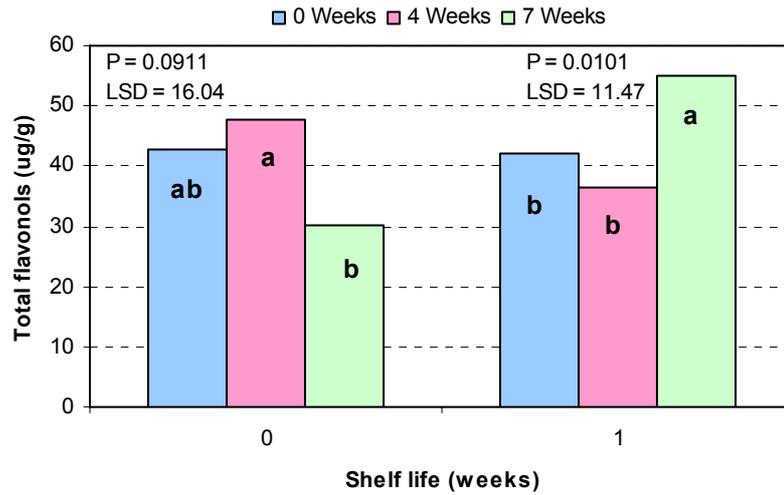
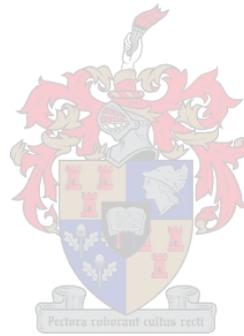


Fig. 11 The effect of cold storage duration at -0.5°C and an additional week at 15°C on the total flavonols (μg per gram berry weight) of Regal Seedless in 2003/04. ANOVA (F-test) is a more strict test than the Students t-test (LSD).



GENERAL DISCUSSION AND CONCLUSIONS



GENERAL DISCUSSION AND CONCLUSIONS

Regal Seedless is a promising South African cultivar which has quite a few positive attributes. This includes inherently large berries, very fertile vines and good cold storage ability. This grape has, however, a problem, namely the sporadic occurrence of an astringent taste. Astringency is seen as unacceptable in fresh produce. For example persimmons are well known for their astringency. This 'drying feeling' of the mouth is caused by the presence of phenolic compounds. If this problem with Regal Seedless persists, its demand by international markets will decrease and it will lead to great financial losses.

This study focused on the effect of fruit maturity and postharvest treatments (nitrogen, ethanol dip and cold storage duration) on the phenolic content and taste of Regal Seedless. The main aim was to find the treatment which resulted in the lowest phenolic concentration (specifically the concentration of flavanols) with the least astringency and acceptable eating quality. The effect on other quality parameters was also evaluated.

During the maturity trial, the total soluble solids (TSS) increased while the total titratable acidity (TTA) decreased with ripening, as expected. The measurement of TSS is the only physical parameter that the table grape producer uses to determine the harvest maturity of the grapes. Throughout this trial we looked for significant correlations between TSS and the other parameters measured to evaluate TSS not only as a maturity indicator, but also an indicator of astringency at a certain maturity. The astringent taste correlated negatively with TSS during one of the three seasons. There was a positive correlation between eating quality (EQ) and TSS in two of the seasons. One assumes that the grapes become more edible as the sugar increases, but we suspect that there is a turning point where the sugar content became too high, which is not preferred by the tasters. It is interesting to note that in all the seasons the lowest astringency score resulted in the highest eating quality rating. In the first two seasons the total phenolics per berry increased with maturity, while in the third season it increased, then decreased. If the sampling period had been extended in the first two seasons, the same pattern might have been found. The increase may indicate an increase in phenol concentration or the formation of new phenols which have higher absorbances (larger extinction coefficients). On the other hand, a decrease in total phenols per berry may indicate the breakdown of phenols later in the season or the formation of phenols with a lower 280 nm absorbance (smaller extinction coefficient)

than the original phenols. The main flavanols detected with HPLC were the polymeric fraction, a gallodimer and a dimermonogallate. In the first two seasons the flavanols decreased up to 18 and 19°Brix, respectively. In the last season the flavanols remained relatively constant and markedly lower than in previous seasons. The reason for the different pattern is that in the last season the sampling period started later and the rapid initial decrease was not observed. The total flavanols correlated negatively with TSS ($r = -0.77$, $p < 0.0001$). The eating quality (EQ) of Regal Seedless improved from 17°Brix and upwards but started to decrease from $\pm 20^\circ$ Brix. The main flavonols detected with HPLC were quercetin-galactoside, quercetin-rhamnoside and quercetin-glucoside. The concentration of total flavonols was constant in the first two seasons, but there was a substantial increase from $\pm 19^\circ$ Brix in the following season. The same increase may have been observed if the sampling period of the other years was extended. No significant correlations were found between sensory data and total phenolics (spectrophotometrically), and sensory data and HPLC data. Seasonal differences were evident in this study. The influence of weather conditions on the grape ripening process is well known and this could have added to the differences noticed between harvests. It is also responsible for fluctuations within a harvest period. The recommended maturity level for Regal Seedless to achieve the project aim is 17 - 18°Brix.

The use of a postharvest nitrogen treatment resulted in a decrease in astringency and an increase in EQ irrespective of the exposure time, but it was only significant in one season. The nitrogen treatment exhibited a tendency to decrease the total phenolics (A_{280}) per berry in both seasons, but it was not statistically significant. When the total phenolics were expressed on a per berry weight basis, nitrogen significantly decreased the total phenolics in the first season, while a significant AN (air-nitrogen) x T (time) interaction was observed in the next season where 64 hours of nitrogen application reduced the total phenolics significantly. These discrepancies between per berry and per berry weight are due to the fact that berry size is not taken into account in the former. With HPLC analysis, it was found that the use of nitrogen increased the individual and total flavanols, but not significantly when compared to the air treatment. An increase was also observed when the exposure time was extended. This increase might be because all the flavanols are quantified in catechin monomeric units while the new acetaldehyde polymers may have very different extinction coefficients, which influence the quantification. The application of nitrogen decreased the concentration of the flavonols non-significantly in both seasons. Neither air nor nitrogen significantly

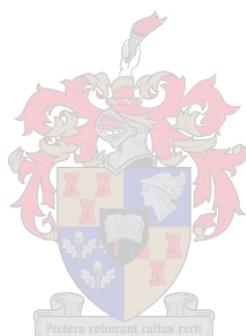
affected other quality parameters like TSS, TTA and berry shatter. The effect of zero-oxygen conditions might be more evident if the trial is conducted at the commercial maturity of Regal Seedless. The use of CO₂ can also be considered since it has been shown to be more effective than nitrogen.

During the ethanol dip trial different ethanol concentrations and two SO₂ pad treatments were tested. The SO₂ pad did not have any influence on the sensory evaluation of the grape. In the first season the astringent taste decreased and the EQ increased with an increase in ethanol concentration. The ethanol had no effect on the total phenolics (A₂₈₀) per berry or per berry weight. The use of a SO₂ pad resulted in a significantly higher concentration of individual and total flavanols on a µg per berry basis. The SO₂ pad might protect the flavanols against oxidation or breakdown reactions. In the 2003/04 season, all the ethanol concentrations tested resulted in a significant decrease in the polymeric fraction and total flavanols as well as individual and total flavonols whether expressed per berry or per berry weight. During the following season the phenolic concentrations quantified by HPLC were substantially lower than in the previous season. This could be the reason why the ethanol dip did not have any effect on the phenolic content or sensory evaluation during the second season.

The cold storage periods did not have any significant influence on the astringent taste. EQ was only significantly affected in 2002/03. When one week of shelf life at 15°C was added to four weeks of cold storage, the astringency was lower and the eating quality higher in both seasons, but the values were still not significant from the other cold storage periods. In the first season (2002/03) lower total phenolics (A₂₈₀) per berry weight were found after four weeks of cold storage and one week shelf life. This was consistent with the sensory data of 2002/03. With HPLC analysis, the concentrations of the individual flavanols and flavonols as well as the totals were the highest after four weeks of cold storage. After shelf life the concentrations of these flavanols and flavonols were lower after the four week cold storage period, when it was compared to the other cold storage periods that increased in concentrations after shelf life. To achieve low astringency and phenolic content, Regal Seedless must be stored for four weeks at -0.5°C and one week at 15°C. This is the present protocol that is used for cold storage trials.

In seasons where the phenolic content of Regal Seedless is very high and harvesting at the right maturity is not sufficient or possible, alternative methods like

zero-oxygen atmospheres (nitrogen for more than 60 h) and ethanol dips can be applied to lower the phenolic concentration and thus the astringency.



APPENDIX

APPENDIX A. ADDITIONAL DATA OF CHAPTER 3

Table 1 Post storage evaluations of Regal Seedless for three seasons.

Season	Date	Bunch weight (g)	Loose berries (%)	SO ₂ burn (%)	Botrytis (%)	Split berries (%)	Browning (%)
2002/03	20/1	1865.2 b	0.43 b	0.06 c	0.00 b	0.04 c	0.00 b
	24/1	2205.9 a	0.58 b	0.24 bc	4.71 a	0.19 bc	0.21 a
	31/1	1859.2 b	1.08 b	0.16 bc	0.37 b	0.61 ab	0.19 ab
	7/2	1730.2 b	0.71 b	3.29 a	0.06 b	0.44 bc	0.06 ab
	14/2	2096.2 a	2.86 a	0.82 ab	3.98 a	1.58 ab	0.22 a
LSD^z		220.6	0.93	0.75	1.60	0.62	0.33
P (p ≤ 0.05)^y		0.0009	<0.0001	0.0035	<0.0001	0.0106	0.1718
2003/04	20/1	1743.3 bc	1.16 ab	0.86 b	0.00 c	0.82 b	0.22 a
	27/1	1539.5 c	0.72 b	0.21 b	0.05 c	2.62 ab	0.00 a
	3/2	1982.4 a	1.49 a	0.17 b	0.81 b	0.56 b	0.40 a
	10/2	2030.9 a	0.47 b	6.46 a	0.00 c	2.95 a	0.00 a
	17/2	1919.2 ab	0.84 ab	0.41 b	2.00 a	1.04 ab	0.12 a
LSD		221.0	0.74	0.56	0.53	2.11	0.43
P (p ≤ 0.05)		0.0006	0.0741	<0.0001	<0.0001	0.0834	0.2572
2004/05	12/1	2265.9 b	1.76 a	0.53 ab	0.02 b	5.39 a	0.45 b
	19/1	2505.4 ab	0.85 ab	0.17 bc	0.00 b	3.90 a	0.62 b
	26/1	2584.3 a	0.40 b	0.04 c	0.00 b	0.91 b	0.38 b
	3/2	2374.2 ab	0.84 ab	0.36 abc	0.00 b	0.45 b	1.82 a
	9/2	2405.6 ab	1.47 a	0.94 a	1.42 a	1.07 b	1.08 ab
LSD		271.9	0.95	0.46	0.24	1.91	0.85
P (p ≤ 0.05)		0.1762	0.0496	0.0053	<0.0001	<0.0001	0.0094

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

Table 2 Subjective evaluation of stem condition and moisture in the carton of Regal Seedless for three seasons.

Season	Date	Stems ^z	Moist ^y
2002/03	20/1	3.33 b	4.83 a
	24/1	1.92 c	3.17 b
	31/1	4.75 a	2.33 b
	7/2	3.33 b	2.33 b
	14/2	1.67 c	2.33 b
LSD^x		1.08	0.88
P (p ≤ 0.05)^w		<0.0001	<0.0001
2003/04	20/1	3.50 b	3.00 a
	27/1	2.50 b	3.00 a
	3/2	2.75 b	3.00 a
	10/2	1.42 c	3.00 a
	17/2	4.83 a	3.00 a
	LSD		1.07
P (p ≤ 0.05)		<0.0001	
2004/05	12/1	3.00 a	2.25 c
	19/1	2.58 a	3.50 b
	26/1	2.58 a	2.25 c
	3/2	3.42 a	2.25 c
	9/2	3.42 a	4.75 a
LSD		1.64	0.93
P (p ≤ 0.05)		0.3711	<0.0001

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z Stem condition was ranked from 1 – 5 (where 1 is green and 5 is brown).

^y Moisture in the carton was ranked from 1 – 3 (where 1 is dry, 2 has condensation and 3 has free water).

^x LSD = Least significant difference.

^w P = Probability of F-ratio test.

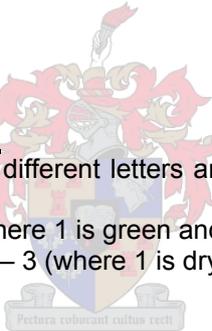


Table 3 The effect of fruit maturity on the juice analysis of Regal Seedless.

Season	Date	pH	TTA	Sugar:acid
(g/L)				
2002/03	20/1	3.58 c	7.5 a	20.34 e
	24/1	3.61 c	6.4 b	25.27 d
	31/1	3.81 b	5.6 c	29.83 c
	7/2	3.88 ab	5.6 c	33.63 b
	14/2	3.96 a	5.0 d	38.02 a
LSD^z		0.08	0.4	3.49
P (p ≤ 0.05)^y		< 0.0001	<0.0001	<0.0001
2003/04	20/1	3.40 d	8.9 a	16.40 d
	27/1	3.61 c	5.9 b	29.29 c
	3/2	3.80 b	5.4 c	34.59 b
	10/2	3.85 ab	5.3 c	37.14 b
	17/2	3.99 a	4.6 d	43.01 a
LSD		0.16	0.5	4.36
P (p ≤ 0.05)		<0.0001	<0.0001	<0.0001
2004/05	12/1	3.27 e	7.1 a	24.08 e
	19/1	3.36 d	6.4 b	29.27 d
	26/1	3.55 c	5.4 c	36.82 c
	3/2	3.68 b	4.7 d	47.46 b
	9/2	4.03 a	3.6 e	66.06 a
LSD		0.03	0.3	2.61
P (p ≤ 0.05)		<0.0001	<0.0001	<0.0001

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

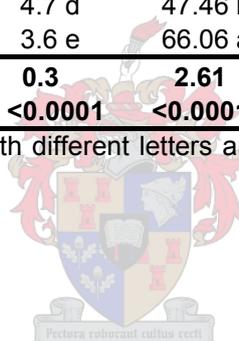


Table 4 The effect of different maturity levels on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2002/03.

Phenolic compounds	Date					LSD ^z	P ($p \leq 0.05$) ^y
	20/1	24/1	31/1	7/2	14/2		
Flavanols							
galocatehin	28.78 ab	30.88 a	24.59 b	32.83 a	27.54 ab	5.85	0.0582
gallodimer1A	304.67 ab	331.74 a	296.67 ab	271.22 b	274.87 b	50.64	0.1142
gallodimer1	333.87 ab	363.11 a	321.64 ab	304.60 b	302.85 b	51.23	0.1176
gallodimer2	34.37 ab	31.95 b	35.26 ab	37.46 a	37.71 a	3.91	0.0303
procyanidinB1	32.66 a	20.79 b	25.10 b	18.60 b	nd	6.80	0.0017
catechin	12.51	nd	nd	nd	nd		
dimer1	16.17 a	nd	14.38 a	17.62 a	16.24 a	3.71	0.2847
dimer2	nd ^w	nd	nd	nd	nd		
epicatechin	39.20 a	43.32 a	40.21 a	27.18 b	20.96 b	8.30	<0.0001
procyanidinB2	nd	nd	nd	nd	nd		
flavanol1	23.97 a	18.15 b	17.07 b	18.70 b	nd	5.02	0.0294
flavanol2	nd	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd	nd		
di-mo-gal1 ^x	16.14 a	16.48 a	nd	nd	nd	7.61	0.9076
flavanol3	15.47 a	17.20 a	20.60 a	nd	nd	9.40	0.3826
dimer3	nd	15.15 a	15.93 a	14.62 a	nd	5.25	0.8134
di-mo-gal2 ^x	83.70 ab	88.03 a	72.18 ab	64.23 b	18.25 c	20.21	<0.0001
flavanol4	nd	nd	nd	nd	nd		
epicat-gallate ^x	39.70 a	38.60 a	24.38 b	17.47 b	nd	9.73	0.0013
dimerdigallate	38.41 a	35.67 a	33.31 ab	25.12 b	nd	10.18	0.0698
trimergallate	21.63 ab	22.40 a	18.44 b	20.78 ab	nd	3.61	0.1282
flavanol5	20.46 a	19.65 a	18.41 a	16.20 a	nd	6.59	0.5722
flavanol6	nd	15.49 a	15.44 a	nd	nd		0.9708
flavanol7	19.13 a	15.35 a	18.18 a	16.90 a	nd	4.86	0.3135
poly frac ^x	4212.20 a	3897.40 ab	3211.60 c	3377.40 bc	702.80 d	643.95	<0.0001
total flavanols	4916.37 a	4589.55 ab	3816.20 c	3921.82 bc	1087.81 d	709.78	<0.0001
Flavonols							
q-3-rut ^x	5.36 a	3.46 b	4.53 ab	4.03 b	3.86 b	1.20	0.0319
m-3-glc ^x	2.78 a	nd	3.97 a	3.73 a	3.90 ab	1.49	0.2794
q-3-gal ^x	11.22 b	7.04 b	13.18 ab	18.54 a	20.06 a	7.25	0.0081
q-3-glc ^x	70.52 a	47.84 bc	57.25 abc	61.44 ab	46.07 c	14.16	0.0121
k-3-gal ^x	3.47 ab	2.85 b	4.58 a	4.60 a	4.33 a	1.26	0.0419
k-3-glc/gluc ^x	4.28 b	3.47 b	7.43 a	7.18 a	8.00 a	2.66	0.0064
q-3-rham ^x	33.81 bc	29.59 c	35.88 ab	41.63 a	28.42 c	5.99	0.0008
total flavonols	130.33 a	90.49 b	121.41 ab	141.06 a	112.22 ab	31.72	0.0272

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

^w nd = not detected.

Table 5 The effect of different maturity levels on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2002/03.

Phenolic compounds	Date					LSD ^z	P ($p \leq 0.05$) ^y
	20/1	24/1	31/1	7/2	14/2		
Flavanols							
galocatehin	5.92 a	5.69 a	4.63 b	5.67 a	5.13 ab	1.02	0.0900
gallodimer1A	62.68 a	60.98 a	55.64 ab	47.24 b	51.29 b	9.40	0.0121
gallodimer1	68.68 a	66.76 a	60.34 ab	53.01 b	56.49 b	9.46	0.0104
gallodimer2	7.06 a	5.88 b	6.62 a	6.51 ab	7.03 a	0.72	0.0164
procyanidinB1	6.71 a	3.87 bc	4.79 b	3.37 c	nd	1.33	0.0003
catechin	nd ^w	nd	nd	nd	nd		
dimer1	3.37 a	nd	2.72 a	3.05 a	2.99 a	0.66	0.2546
dimer2	nd	nd	nd	nd	nd		
epicatechin	8.06 a	7.97 a	7.57 a	4.78 b	3.91 b	1.64	<0.0001
procyanidinB2	nd	nd	nd	nd	nd		
flavanol1	4.92 a	3.30 b	3.27 b	3.34 b	nd	0.90	0.0018
flavanol2	nd	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd	nd		
di-mo-gal1 ^x	3.30 a	3.08 a	nd	nd	nd	1.57	0.7127
flavanol3	3.15 a	3.22 a	4.00 a	nd	nd	1.87	0.4624
dimer3	nd	2.76 a	2.93 a	2.64 a	nd	0.79	0.6535
di-mo-gal2 ^x	17.18 a	16.20 a	13.59 ab	11.20 b	3.42 c	3.92	<0.0001
flavanol4	nd	nd	nd	nd	nd		
epicat-gallate ^x	8.12 a	7.04 a	4.66 b	3.11 b	nd	1.77	0.0004
dimerdigallate	7.89 a	6.55 a	6.27 ab	4.40 b	nd	1.93	0.0127
trimergallate	4.45 a	4.13 ab	3.47 b	3.58 b	nd	0.80	0.0625
flavanol5	4.20 a	3.61 ab	3.48 ab	2.82 b	nd	1.16	0.1474
flavanol6	nd	2.89 a	2.92 a	nd	nd	0.78	0.9212
flavanol7	3.98 a	2.78 b	3.47 ab	3.03 b	nd	0.82	0.0423
poly frac ^x	865.40 a	715.80 b	603.90 bc	588.23 c	132.20 d	121.80	<0.0001
total flavanols	1010.13 a	843.08 b	717.64 bc	683.25 c	204.00 d	135.59	<0.0001
Flavonols							
q-3-rut ^x	1.10 a	0.64 b	0.86 ab	0.70 b	0.71 b	0.25	0.0068
m-3-glc ^x	0.58 a	nd	0.77 a	0.63 a	0.73 a	0.31	0.4894
q-3-gal ^x	2.31 bc	1.29 c	2.49 abc	3.19 ab	3.75 a	1.33	0.0130
q-3-glc ^x	14.51 a	8.83 b	10.81 b	10.69 b	8.61 b	2.94	0.0041
k-3-gal ^x	0.71 ab	0.54 b	0.87 a	0.79 a	0.80 a	0.21	0.0745
k-3-glc/gluc ^x	0.88 bc	0.64 c	1.41 a	1.27 ab	1.49 a	0.50	0.0106
q-3-rham ^x	6.96 a	5.45 b	6.74 a	7.20 a	5.30 b	1.01	0.0011
total flavonols	26.82 a	16.69 b	22.90 a	24.43 a	20.95 ab	6.10	0.0251

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

^w nd = not detected.

Table 6 The effect of different maturity levels on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Date					LSD ^z	P ($p \leq 0.05$) ^y
	20/1	27/1	3/2	10/2	17/2		
Flavanols							
gallodimer1	538.56 b	624.96 ab	548.17 b	777.65 a	722.07 a	165.84	0.0229
gallodimer2	37.99 b	40.34 ab	43.39 ab	45.83 a	41.34 ab	7.10	0.2028
procyanidinB1	83.34 a	41.78 bc	30.28 c	77.55 a	54.00 b	16.03	<0.0001
catechin	nd ^w	nd	nd	nd	nd		
dimer1	17.84 e	22.48 d	26.58 c	32.69 b	37.49 a	3.56	<0.0001
dimer2	26.00 b	26.20 b	29.07 b	33.91 a	26.59 b	3.61	0.0002
epicatechin	nd	20.10 a	24.09 a	26.11 a	21.61 a	6.35	0.1411
procyanidinB2	nd	nd	nd	25.02 a	24.33 a	6.64	0.8170
flavanol1	26.52 a	nd	nd	nd	24.20 a	15.10	0.6908
flavanol2	58.12 a	23.93 b	nd	35.88 ab	32.80 b	22.69	0.0299
trimer1	26.31 a	nd	nd	28.09 a	nd	15.10	0.7595
di-mo-gal1 ^x	46.32 a	20.45 b	24.51 b	29.58 b	26.19 b	12.65	0.0025
flavanol3	30.04	nd	nd	nd	nd		
dimer3	nd	19.73 a	24.77 a	28.28 a	30.16 a	12.68	0.3626
di-mo-gal2 ^x	153.54 a	38.89 c	39.24 c	63.16 b	54.59 bc	21.87	<0.0001
flavanol4	18.11	nd	nd	nd	nd		
epicat-gallate ^x	34.05	nd	nd	nd	nd		
dimerdigallate	41.93	nd	nd	nd	nd		
trimergallate	27.46	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	20.48	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly frac ^x	7470.80 a	3805.90 cd	2960.10 d	4703.40 b	4108.70 bc	874.87	<0.0001
total flavanols	8622.90 a	4635.60 cd	3690.40 d	5879.30 b	5175.70 bc	1006.50	<0.0001
Flavonols							
q-3-rut ^x	5.25 ab	6.37 a	4.96 ab	6.03 ab	4.76 b	1.42	0.1056
m-3-glc ^x	nd	3.91 a	4.23 a	5.77 a	8.07 a	4.64	0.2529
q-3-gal ^x	15.16 b	33.41 ab	36.25 ab	49.24 a	53.37 a	26.11	0.0533
q-3-glc ^x	97.80 a	92.09 a	73.95 a	93.97 a	91.34 a	25.56	0.4007
k-3-gal ^x	4.51 b	5.68 b	6.76 ab	8.59 a	5.25 b	2.79	0.0496
k-3-glc/gluc ^x	5.88 b	12.62 ab	19.93 a	18.57 a	11.49 ab	10.29	0.0598
q-3-rham ^x	44.05 ab	44.19 ab	34.94 b	48.17 a	43.20 ab	9.78	0.1160
total flavonols	171.75 a	195.66 a	176.13 a	226.98 a	209.29 a	72.83	0.4963
Benzoic acid							
benzoic acid1	nd	nd	nd	nd	3.98		
Cinnamic acid							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	3.22 b	2.75 bc	1.99 c	4.62 a	2.09 bc	1.14	0.0004

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

^w nd = not detected.

Table 7 The effect of different maturity levels on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Date					LSD ^z	P ($p \leq 0.05$) ^y
	20/1	27/1	3/2	10/2	17/2		
Flavanols							
gallodimer1	98.06 a	111.35 a	88.14 a	110.23 a	106.23 a	24.55	0.2881
gallodimer2	6.90 ab	7.16 a	6.94 ab	6.50 ab	6.09 b	0.94	0.1569
procyanidinB1	15.11 a	7.37 c	4.87 d	11.02 b	7.88 c	2.20	<0.0001
catechin	nd ^w	nd	nd	nd	nd		
dimer1	3.24 d	4.00 c	4.28 bc	4.64 b	5.53 a	0.53	<0.0001
dimer2	4.73 a	4.67 a	4.66 a	4.82 a	3.93 b	0.70	0.0766
epicatechin	nd	3.43 a	3.94 a	3.70 a	3.24 a	0.98	0.4453
procyanidinB2	nd	nd	nd	3.51 a	3.59 a	0.79	0.8223
flavanol1	4.77 a	nd	nd	nd	3.52 a	2.15	0.1812
flavanol2	10.49 a	4.17 b	nd	5.07 b	4.83 b	3.45	0.0024
trimer1	4.74 a	nd	nd	3.91 a	nd	1.91	0.2919
di-mo-gal1 ^x	8.35 a	3.55 b	3.85 bc	4.27 b	3.74 b	1.97	0.0002
flavanol3	5.49	nd	nd	nd	nd		
dimer3	nd	3.66 a	3.88 a	4.03 a	4.44 a	1.89	0.8314
di-mo-gal2 ^x	27.77 a	6.73 b	6.29 b	8.95 b	7.94 b	2.73	<0.0001
flavanol4	3.27	nd	nd	nd	nd		
epicat-gallate ^x	6.14	nd	nd	nd	nd		
dimerdigallate	7.62	nd	nd	nd	nd		
trimergallate	27.46	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	3.71	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly frac ^x	1352.48 a	674.14 b	474.87 c	666.58 b	603.79 b	106.08	<0.0001
total flavanols	1561.55 a	821.62 b	592.16 c	833.34 b	760.58 b	120.95	<0.0001
Flavonols							
q-3-rut ^x	0.95 ab	1.14 a	0.80 bc	0.86 bc	0.70 c	0.24	0.0084
m-3-glc ^x	nd	0.76 a	0.71 a	0.83 a	1.18 a	0.76	0.5108
q-3-gal ^x	2.73 b	6.07 ab	5.88 ab	7.00 a	7.82 a	4.10	0.1622
q-3-glc ^x	17.72 a	16.51 ab	11.92 c	13.38 bc	13.42 bc	4.22	0.0537
k-3-gal ^x	0.82 a	1.02 a	1.10 a	1.22 a	0.77 a	0.49	0.2906
k-3-glc/gluc ^x	1.06 b	2.30 ab	3.24 a	2.65 ab	1.67 ab	1.68	0.1096
q-3-rham ^x	7.99 a	7.91 ab	5.63 c	6.85 abc	6.35 bc	1.63	0.0313
total flavonols	31.11 a	35.20 a	28.48 a	32.32 a	30.70 a	11.97	0.8231
Benzoic acids							
benzoic acid1	nd	nd	nd	nd	0.60		
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	0.57 a	0.49 ab	0.32 bc	0.65 a	0.31 c	0.17	0.002

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

^w nd = not detected.

Table 8 The effect of different maturity levels on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2004/05.

Phenolic compounds	Date					LSD ^z	P (p \leq 0.05) ^y
	12/1	19/1	26/1	3/2	9/2		
Flavanols							
galocatehin	23.11 b	26.319 b	32.25 b	73.77 a	64.81 a	19.94	<0.0001
gallodimer1A	354.77 b	352.63 b	367.01 ab	377.42 ab	417.70 a	55.12	0.1218
gallodimer1	377.88 c	378.95 c	399.26 bc	451.19 ab	482.50 a	52.55	0.0007
gallodimer2	39.54 ab	36.93 b	43.48 a	40.89 ab	41.37 ab	6.25	0.3210
procyanidinB1	43.75 a	42.06 a	46.10 a	16.31 b	20.53 b	12.32	<0.0001
catechin	55.10 a	48.06 a	55.13 a	nd	20.38 b	13.15	0.0004
dimer1	18.61 bc	16.26 c	16.67 c	21.47 ab	23.07 a	3.88	0.0027
dimer2	nd ^w	nd	nd	nd	nd		
epicatechin	47.46 a	39.23 a	43.91 a	18.15 b	25.55 b	9.17	<0.0001
procyanidinB2	nd	nd	21.53 a	20.27 a	16.93 a	5.39	0.1675
flavanol1	27.96 a	20.56 a	27.90 a	20.81 a	nd	9.15	0.1417
di-mo-gal1 ^x	nd	nd	nd	nd	nd		
flavanol3	nd	nd	nd	nd	nd		
dimer3	nd	nd	nd	nd	nd		
di-mo-gal2 ^x	63.61 a	33.85 bc	40.07 b	20.76 c	23.24 bc	16.99	0.0001
flavanol4	nd	nd	nd	nd	nd		
epicat-gallate ^x	23.82	nd	nd	nd	nd		
dimerdigallate	20.95 a	17.69 a	nd	nd	nd	8.99	0.3935
trimergallate	nd	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly frac ^x	2234.80 a	2093.00 ab	2198.90 ab	1588.70 c	1764.70 bc	435.86	0.0177
total flavanols	2954.49 a	2739.01 ab	2907.46 a	2187.16 c	2402.10 bc	487.25	0.0125
Flavonols							
q-3-rut ^x	6.32 c	5.27 cd	4.54 d	10.71 a	8.81 b	1.75	<0.0001
m-3-glc ^x	9.04 c	7.34 c	10.94 bc	20.04 a	14.94 ab	5.78	0.0006
q-3-gal ^x	50.04 b	73.31 b	77.72 b	178.51 a	163.24 a	38.87	<0.0001
q-3-glc ^x	100.75 c	102.53 c	97.93 c	158.46 a	132.77 b	20.20	<0.0001
k-3-gax	7.17 b	7.55 b	7.47 b	21.12 a	20.14 a	4.27	<0.0001
k-3-glc/gluc ^x	14.00 b	21.92 b	20.40 b	68.47 a	65.79 a	13.75	<0.0001
q-3-rham ^x	50.11 c	52.38 c	51.94 c	78.79 a	63.46 b	9.70	<0.0001
total flavonols	233.81 b	270.29 b	268.75 b	536.09 a	469.14 a	87.80	<0.0001
Benzoic acids							
benzoic acid1	nd	nd	nd	3.21	nd		
benzoic acid2	nd	nd	nd	3.21	nd		
total benz. acids ^x	nd	nd	nd	6.43	nd		
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	3.06 ab	3.63 a	2.42 b	nd	nd	1.14	0.1256
caftaric acid	3.46 a	3.31 a	3.25 a	nd	nd	1.52	0.9501
total cin. acids ^x	6.52 a	6.39 a	5.18 a	nd	nd	1.36	0.1148

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside; total benz. acids = total benzoic acids; tot cin. acids = total cinnamic acids.

^w nd = not detected.

Table 9 The effect of different maturity levels on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2004/05.

Phenolic compounds	Date					LSD ^z	P ($p \leq 0.05$) ^y
	12/1	19/1	26/1	3/2	9/2		
Flavanols							
galocatehin	4.24 b	4.79 b	5.27 b	13.66 a	12.58 a	3.88	<0.0001
gallodimer1A	65.25 bc	64.13 bc	60.63 c	69.81 b	79.98 a	8.68	0.0013
gallodimer1	69.49 c	68.92 c	65.90 c	83.46 b	92.56 a	8.00	<0.0001
gallodimer2	7.26 ab	6.74 b	7.10 ab	7.59 ab	7.96 a	0.94	0.0989
procyanidinB1	8.01 a	7.61 a	7.59 a	3.03 b	3.90 b	2.04	<0.0001
catechin	10.08 a	8.62 a	9.03 a	nd	3.83 b	1.83	<0.0001
dimer1	3.38 b	2.88 b	2.87 b	3.99 a	4.44 a	0.51	<0.0001
dimer2	nd ^w	nd	nd	nd	nd		
epicatechin	8.70 a	7.09 b	7.25 ab	3.35 d	4.87 c	1.48	<0.0001
procyanidinB2	nd	nd	3.29 a	3.77 a	3.27 a	0.96	0.3292
flavanol1	5.13 a	3.73 b	4.42 ab	3.93 ab	nd	1.24	0.0653
di-mo-gal1 ^x	nd	nd	nd	nd	nd		
flavanol3	nd	nd	nd	nd	nd		
dimer3	nd	nd	nd	nd	nd		
di-mo-gal2 ^x	11.65 a	6.16 b	6.62 b	3.62 b	4.40 b	3.10	0.0001
flavanol4	nd	nd	nd	nd	nd		
epicat-gallate ^x	4.34	nd	nd	nd	nd		
dimerdigallate	3.78 a	3.17 a	nd	nd	nd	1.56	0.3585
trimergallate	nd	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly frac ^x	409.24 a	380.47 a	362.17 ab	292.18 b	339.30 ab	72.16	0.0266
total flavanols	541.45 a	497.69 ab	478.81 abc	402.96 c	461.53 bc	77.16	0.0151
Flavonols							
q-3-rut ^x	1.20 b	0.96 bc	0.74 c	1.99 a	1.70 a	0.34	<0.0001
m-3-glc ^x	1.70 b	1.33 b	1.81 b	3.70 a	2.89 a	1.07	0.0004
q-3-gal ^x	9.35 b	13.29 b	12.76 b	32.94 a	31.41 a	6.83	<0.0001
q-3-glc ^x	18.62 b	18.66 b	16.15 b	29.37 a	25.62 a	3.95	<0.0001
k-3-gal ^x	1.33 b	1.37 b	1.23 b	3.91 a	3.90 a	0.82	<0.0001
k-3-glc/gluc ^x	2.59 b	3.98 b	3.35 b	12.64 a	12.73 a	2.53	<0.0001
q-3-rham ^x	9.28 c	9.54 c	8.54 c	14.59 a	12.21 b	1.82	<0.0001
total flavonols	43.38 b	49.13 b	44.20 b	99.14 a	90.45 a	15.95	<0.0001
Benzoic acids							
benzoic acid1	nd	nd	nd	0.60	nd		
benzoic acid2	nd	nd	nd	0.60	nd		
total ben. acids ^x	nd	nd	nd	1.20	nd		
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	0.56 ab	0.67 a	0.41 b	nd	nd	0.19	0.0612
caftaric acid	0.63 a	0.61 a	0.53 a	nd	nd	0.25	0.6474
total cin. acids ^x	1.19 a	1.16 a	0.86 b	nd	nd	0.23	0.0158

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicat-gallate = epicatechingallate; poly frac = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside; total benz. acids = total benzoic acids; tot cin. acids = total cinnamic acids.

^w nd = not detected.

APPENDIX B. ADDITIONAL DATA OF CHAPTER 4

Table 10 Post storage evaluations of Regal Seedless for two seasons.

Season	Treatment ^z	Bunch weight (g)	Loose berries (%)	SO ₂ burn (%)	Botrytis (%)	Split berries (%)	Browning (%)
2002/03	Air	840.42 a	1.48 a	8.13 a	0.36 a	0.83 a	8.16 a
	Nitrogen	898.95 a	1.44 a	13.06 a	0.74 a	0.80 a	3.99 b
	LSD ^y	125.25	0.48	0.59	0.48	0.37	2.65
	P (p ≤ 0.05) ^x	0.3493	0.9086	0.2677	0.3467	0.7326	0.0029
	T4	779.57 b	1.89 a	14.24 ab	0.54 a	0.68 ab	4.71 a
	T8	1057.49 a	1.50 ab	13.15 a	0.49 a	0.36 b	8.12 a
	T16	763.69 b	0.68 b	7.07 b	0.35 a	1.17 ab	4.67 a
	T32	915.53 ab	2.05 ab	7.73 b	1.27 a	1.25 a	5.43 a
	LSD	183.35	0.70	0.86	0.70	0.55	3.88
	P (p ≤ 0.05)	0.0030	0.1219	0.0173	0.8810	0.1321	0.1173
2003/04	Air	1115.79 a	2.11 a	1.63 a	0.88 a	0.11 a	0.17 a
	Nitrogen	972.47 b	2.71 a	2.77 a	1.26 a	0.08 a	0.55 a
	LSD	92.41	0.45	0.35	0.40	0.16	0.29
	P (p ≤ 0.05)	0.0030	0.5129	0.0659	0.6528	0.9386	0.1107
	T4	1099.10 a	2.05 a	1.09 b	0.96 a	0.11 a	0.16 a
	T8	995.27 a	3.53 a	3.76 a	1.64 a	0.08 a	0.39 a
	T16	1072.72 a	1.65 a	1.56 b	0.49 a	0.16 a	0.26 a
	T32	990.38 a	3.16 a	2.72 b	1.07 a	0.05 a	0.19 a
	T64	1063.21 a	1.65 a	1.85 b	1.19 a	0.08 a	0.80 a
	LSD	146.12	0.71	0.56	0.63	0.25	0.45
P (p ≤ 0.05)	0.4736	0.5467	0.0134	0.4407	0.9442	0.5528	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

Table 11 Subjective evaluation of stem condition and moisture in the carton of Regal Seedless for two seasons.

Season	Treatment ^z	Stems ^y	Moist ^x
2002/03	Air	4.44 a	4.00 a
	Nitrogen	3.67 a	4.00 a
	LSD ^w	0.92	
	P (p ≤ 0.05) ^v	0.0938	
	T4	4.46 a	4.00 a
	T8	4.17 a	4.00 a
	T16	3.08 a	4.00 a
	T32	4.58 a	4.00 a
	LSD	1.78	
	P (p ≤ 0.05)	0.0404	
2003/04	Air	5.38 a	5.42 a
	Nitrogen	5.62 a	5.58 a
	LSD	0.96	0.32
	P (p ≤ 0.05)	0.6280	0.2969
	T4	5.00 a	5.42 a
	T8	5.21 a	5.83 a
	T16	6.00 a	5.42 a
	T32	6.08 a	5.42 a
	T64	5.21 a	5.42 a
	LSD	2.14	0.71
P (p ≤ 0.05)	0.4812	0.3617	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^y Stem condition was ranked from 1 – 5 (where 1 is green and 5 is brown).

^x Moisture in the carton was ranked from 1 – 3 (where 1 is dry, 2 has condensation and 3 has free water).

^w LSD = Least significant difference.

^v P = Probability of F-ratio test.

Table 12 The effect of air or nitrogen and exposure time on the juice analysis of Regal Seedless for two seasons.

Season	Treatment ^z	TSS	pH	TTA	Sugar:Acid
		(°Brix)		(g/L)	
2002/03	Air	20.59 a	3.99 a	3.93 b	52.63 a
	Nitrogen	20.65 a	4.04 a	4.08 a	50.69 a
	LSD ^y	0.58	0.09	0.11	2.26
	P (p ≤ 0.05) ^x	0.8524	0.2842	0.0094	0.0905
	T4	21.14 a	4.00 a	4.02 b	52.82 a
	T8	20.36 ab	4.02 a	4.02 b	50.70 a
	T16	20.27 b	4.03 a	3.90 b	52.17 a
	T32	20.77 ab	4.03 a	4.21 a	49.53 a
	LSD	0.85	0.14	0.17	3.31
	P (p ≤ 0.05)	0.1010	0.9675	0.0916	0.3903
2003/04	Air	23.51 a	4.15 a	3.60 a	66.26 a
	Nitrogen	23.69 a	4.18 a	3.56 a	67.32 a
	LSD	0.52	0.07	0.22	3.42
	P (p ≤ 0.05)	0.4797	0.3484	0.7715	0.5359
	T4	23.30 a	4.26 a	3.76 ab	63.07 bc
	T8	23.48 a	4.17 ab	3.82 a	62.03 c
	T16	23.99 a	4.18 ab	3.35 c	71.73 a
	T32	23.78 a	4.14 b	3.56 a-c	68.21 ab
	T64	23.42 a	4.09 b	3.46 bc	67.94 ab
	LSD	0.82	0.11	0.35	5.42
P (p ≤ 0.05)	0.4071	0.0403	0.0476	0.0042	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z T = exposure time to air and nitrogen atmospheres (T4 = 4 hours; T8 = 8 hours; T16 = 16 hours; T32 = 32 hours; T64 = 64 hours).

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

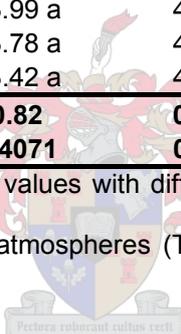


Table 13 The effect of air or nitrogen on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2002/03.

Phenolic compounds	Air	Nitrogen	LSD ^z	P ($p \leq 0.05$) ^y
Flavanols				
galocatehin	143.80 a	145.99 a	16.98	0.7946
gallodimer1A	257.02 a	272.92 a	24.27	0.1919
gallodimer1	400.82 a	418.91 a	34.68	0.2964
gallodimer2	31.69 a	30.01 a	2.44	0.1755
procyanidinB1	19.27 a	24.94 a	8.06	0.1361
catechin	nd ^x	nd		
dimer1	16.75 a	16.68 a	1.67	0.9305
dimer2	22.86 a	22.90 a	2.87	0.9751
epicatechin	14.70 a	15.08 a	3.39	0.8012
procyanidinB2	26.39 a	29.37 a	4.53	0.1889
flavanol1	nd	nd		
flavanol2	nd	nd		
trimer1	nd	nd		
dimermonogallate1	19.68 a	19.41 a	2.81	0.8403
flavanol3	nd	nd		
dimer3	20.64 a	20.28 a	2.42	0.7604
dimermonogallate2	65.05 a	60.12 a	6.77	0.1475
flavanol4	17.01 a	15.67 a	2.65	0.2948
epicatechingallate	16.90 a	21.97 a	13.62	0.3599
dimerdigallate	16.42 a	15.52 a	4.64	0.6639
trimergallate	14.26 a	15.17 a	4.29	0.6099
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	2986.90 a	3131.00 a	417.33	0.4871
total flavanols	3622.20 a	3765.30 a	452.69	0.5248
Flavonols				
quercetin-3-rutinoside	4.57 a	4.83 a	2.61	0.8348
myricetin-3-glucoside	7.24 a	6.65 a	2.23	0.5948
quercetin-3-galactoside	57.47 a	51.71 a	15.18	0.4445
quercetin-3-glucoside	105.74 a	94.41 a	14.83	0.1292
kaempferol-3-galactoside	8.65 a	7.51 a	2.01	0.2534
kaempferol-3-glucoside/glucunoride	22.11 a	18.33 a	5.66	0.1822
quercetin-3-rhamnoside	47.20 a	43.64 a	5.24	0.1754
total flavonols	251.06 a	222.47 a	44.11	0.1956
Benzoic acids				
benzoic acid1	3.27 a	3.43 a	0.37	0.3727
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	1.74 a	1.74 a	0.54	0.98

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

Table 14 The effect of air or nitrogen on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2002/03.

Phenolic compounds	Air	Nitrogen	LSD ^z	P ($p \leq 0.05$) ^y
Flavanols				
galocatehin	27.84 a	27.74 a	2.84	0.9388
gallodimer1A	49.81 a	52.14 a	4.65	0.3172
gallodimer1	77.66 a	79.87 a	6.04	0.4612
gallodimer2	6.16 a	5.73 a	0.54	0.1124
procyanidinB1	3.75 a	4.55 a	1.18	0.1471
catechin	nd ^x	nd		
dimer1	3.26 a	3.17 a	0.29	0.5224
dimer2	4.42 a	4.40 a	0.59	0.9445
epicatechin	2.85 a	2.82 a	0.47	0.8778
procyanidinB2	5.07 a	5.53 a	0.66	0.1585
flavanol1	nd	nd		
flavanol2	nd	nd		
trimer1	nd	nd		
dimermonogallate1	3.81 a	3.63 a	0.48	0.4456
flavanol3	nd	nd		
dimer3	4.01 a	3.87 a	0.46	0.5203
dimermonogallate2	12.61 a	11.45 a	1.29	0.0770
flavanol4	3.37 a	3.03 a	0.59	0.2373
epicatechingallate	3.31 a	4.02 a	2.38	0.4559
dimerdigallate	3.27 a	2.95 a	0.86	0.4130
trimergallate	2.80 a	2.63 a	0.47	0.4151
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	577.61 a	593.42 a	69.20	0.6453
total flavanols	700.82 a	714.15 a	73.86	0.7156
Flavonols				
quercetin-3-rutinoside	0.92 a	0.92 a	0.60	0.9967
myricetin-3-glucoside	1.42 a	1.23 a	0.44	0.4036
quercetin-3-galactoside	11.14 a	9.61 a	2.81	0.2735
quercetin-3-glucoside	20.64 a	17.67 a	3.10	0.0596
kaempferol-3-galactoside	1.68 a	1.40 a	0.38	0.1419
kaempferol-3-glucoside/glucunoride	4.28 a	3.41 a	1.06	0.1043
quercetin-3-rhamnoside	9.18 a	8.17 a	1.02	0.0515
total flavonols	48.88 a	41.55 a	8.57	0.0907
Benzoic acids				
benzoic acid1	0.64 a	0.65 a	0.07	0.7755
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	0.37 a	0.34 a	0.14	0.5779

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

Table 15 The effect of exposure time of air and nitrogen on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2002/03

Phenolic compounds	Hours				LSD ^z	P ($p \leq 0.05$) ^y
	4	8	16	32		
Flavanols						
galocatehin	142.62 a	154.82 a	135.74 a	147.57 a	25.56	0.3848
gallodimer1A	260.62 a	271.26 a	263.93 a	268.95 a	36.52	0.9042
gallodimer1	403.24 a	426.07 a	399.67 a	416.53 a	52.19	0.6571
gallodimer2	29.31 b	31.01 ab	30.81 ab	33.50 a	3.67	0.0954
procyanidinB1	17.34 b	21.20 ab	17.78 b	32.10 a	11.40	0.0676
catechin	nd ^x	nd	nd	nd		
dimer1	16.78 a	17.32 a	16.54 a	15.67 a	2.47	0.6307
dimer2	24.89 a	23.71 ab	20.58 b	21.11 ab	4.31	0.1011
epicatechin	16.04 a	15.30 a	14.11 a	nd	4.33	0.5340
procyanidinB2	28.48 a	28.80 a	26.55 a	28.34 a	6.81	0.8646
flavanol1	nd	nd	nd	nd		
flavanol2	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd		
dimermonogallate1	17.20 a	21.19 a	19.89 a	19.79 a	4.15	0.1836
flavanol3	nd	nd	nd	nd		
dimer3	19.02 a	21.32 a	21.42 a	19.59 a	3.63	0.3584
dimermonogallate2	51.69 b	64.17 a	68.26 a	67.80 a	10.11	0.0022
flavanol4	15.64 a	16.14 a	17.03 a	14.83 a	4.36	0.8492
epicatechingallate	nd	18.53 a	18.08 a	22.62 a	17.58	0.9290
dimerdigallate	17.31 a	13.54 a	17.65 a	15.52 a	7.09	0.4412
trimergallate	16.12 a	13.73 a	13.44 a	nd	4.55	0.2010
flavanol5	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd		
polymeric fraction	2690.20 b	3105.20 b	3097.10 b	3806.60 a	628.08	0.0283
total flavanols	3287.30 b	3764.80 b	3734.60 b	4465.90 a	681.29	0.0320
Flavonols						
quercetin-3-rutinoside	4.60 a	5.85 a	3.89 a	5.52 a	3.67	0.5557
myricetin-3-glucoside	6.16 b	6.39 b	6.71 ab	9.88 a	3.27	0.0699
quercetin-3-galactoside	48.49 b	48.43 b	54.28 b	81.62 a	22.65	0.0154
quercetin-3-glucoside	87.78 b	95.46 b	104.61 b	127.08 a	22.13	0.0029
kaempferol-3-galactoside	7.45 b	7.18 b	8.09 b	11.33 a	3.00	0.0199
k-3-glc/glu ^w	20.02 ab	16.68 b	20.18 ab	28.19 a	8.44	0.0285
quercetin-3-rhamnoside	41.08 b	43.83 b	47.34 ab	54.24 a	7.81	0.0053
total flavonols	211.10 b	218.40 b	244.32 b	315.65 a	65.81	0.0066
Benzoic acids						
benzoic acid1	3.11 a	3.44 a	3.27 a	3.62 a	0.51	0.3399
Cinnamic acids						
cinnamic acid1	nd	nd	nd	nd		
cinnamic acid2	1.67 a	1.92 a	nd	nd	0.23	0.5893

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w k-3-glc/glu = kaempferol-3-glucoside/glucuronide.

Table 16 The effect of exposure time of air and nitrogen on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2002/03

Phenolic compounds	Hours				LSD ^z	P ($p \leq 0.05$) ^y
	4	8	16	32		
Flavanols						
galocatehin	27.68 a	29.58 a	26.34 a	26.90 a	4.28	0.3501
gallodimer1A	50.58 a	51.79 a	51.65 a	49.39 a	7.00	0.7614
gallodimer1	78.26 a	81.38 a	77.99 a	76.29 a	9.09	0.6061
gallodimer2	5.68 a	5.98 a	6.01 a	6.13 a	0.81	0.4705
procyanidinB1	3.36 b	4.13 ab	3.49 b	5.60 a	1.67	0.0563
catechin	nd ^x	nd	nd	nd		
dimer1	3.26 ab	3.36 a	3.17 ab	2.87 b	0.43	0.2459
dimer2	4.86 a	4.54 ab	4.03 ab	3.88 b	0.88	0.0940
epicatechin	2.94 a	2.78 a	2.84 a	nd	0.60	0.8588
procyanidinB2	5.50 a	5.42 a	5.10 a	5.17 a	0.99	0.6492
flavanol1	nd	nd	nd	nd		
flavanol2	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd		
dimermonogallate1	3.27 b	4.04 a	3.88 ab	3.60 ab	0.70	0.0852
flavanol3	nd	nd	nd	nd		
dimer3	3.70 a	4.08 a	4.18 a	3.60 a	0.69	0.2848
dimermonogallate2	9.98 b	12.28 a	13.36 a	12.44 a	1.93	0.0028
flavanol4	3.34 a	3.05 a	3.40 a	2.70 a	0.98	0.4955
epicatechingallate	nd	3.53 a	3.54 a	4.06 a	3.07	0.9309
dimerdigallate	3.33 a	2.76 a	3.44 a	2.84 a	1.32	0.5679
trimergallate	3.05 a	2.60 a	2.63 a	nd	0.50	0.1153
flavanol5	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd		
polymeric fraction	521.63 b	591.31 ab	602.90 ab	692.98 a	104.15	0.0368
total flavanols	637.42 b	717.35 ab	727.35 ab	813.58 a	111.16	0.0408
Flavonols						
quercetin-3-rutinoside	0.87 a	1.27 a	0.78 a	1.01 a	0.85	0.5776
myricetin-3-glucoside	1.16 a	1.24 a	1.32 a	1.80 a	0.65	0.1220
quercetin-3-galactoside	9.14 b	9.31 b	10.60 b	14.83 a	4.19	0.0219
quercetin-3-glucoside	16.68 b	18.43 b	20.52 ab	23.19 a	4.62	0.0124
kaempferol-3-galactoside	1.41 b	1.39 b	1.58 ab	2.07 a	0.57	0.0349
k-3-glc/glu ^w	3.78 ab	3.22 b	3.94 ab	5.13 a	1.58	0.0437
quercetin-3-rhamnoside	7.80 b	8.42 ab	9.25 ab	9.93 a	1.52	0.0106
total flavonols	39.99 b	42.13 b	47.83 ab	57.56 a	12.79	0.0145
Benzoic acids						
benzoic acid1	0.59 a	0.68 a	0.65 a	0.63 a	0.10	0.2267
Cinnamic acids						
cinnamic acid1	nd	nd	nd	nd		
cinnamic acid2	0.35 a	0.36 a	nd	nd	0.16	0.6162

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w k-3-glc/glu = kaempferol-3-glucoside/glucuronide.

Table 17 The effect of air or nitrogen on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Air	Nitrogen	LSD ^z	P ($p \leq 0.05$) ^y
Flavanols				
gallodimer1	-	-	-	-
gallodimer2	37.99 a	38.34 a	2.66	0.7897
procyanidinB1	24.53 a	30.79 a	8.04	0.1214
catechin	nd ^x	nd		
dimer1	17.92 a	18.63 a	1.34	0.2891
dimer2	nd	nd		
epicatechin	16.99 a	17.67 a	2.57	0.5856
procyanidinB2	- ^w	-	-	-
flavanol1	16.34 b	25.59 a	8.82	0.0426
flavanol2	20.65 a	22.31 a	5.18	0.5040
trimer1	nd	nd		
dimermonogallate1	20.64 a	18.53 a	4.89	0.3789
flavanol3	nd	nd		
dimer3	19.31 a	17.45 a	4.35	0.3681
dimermonogallate2	35.19 a	39.98 a	24.57	0.6730
flavanol4	24.81 a	21.32 a	9.19	0.4073
epicatechingallate	nd	nd		
dimerdigallate	20.27 a	24.73 a	8.17	0.2045
trimergallate	20.65 a	22.21 a	6.85	0.6374
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	1932.30 a	2003.10 a	366.57	0.6994
total flavanols	-	-	-	-
Flavonols				
quercetin-3-rutinoside	7.00 a	6.28 a	1.29	0.2674
myricetin-3-glucoside	15.10 a	12.06 a	3.13	0.0566
quercetin-3-galactoside	129.27 a	108.84 a	24.02	0.0936
quercetin-3-glucoside	141.84 a	119.80 b	19.86	0.0304
kaempferol-3-galactoside	16.38 a	14.30 a	3.10	0.1837
k-3-glc/glu ^v	48.61 a	43.51 a	9.62	0.2912
quercetin-3-rhamnoside	62.76 a	57.61 a	7.47	0.1724
total flavonols	420.96 a	361.55 a	66.45	0.0785
Benzoic acids				
benzoic acid1	3.66 a	3.92 a	0.60	0.3767
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	1.43 a	1.67 a	0.43	0.2156

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v k-3-glc/gluc = kaempferol-3-glucoside/glucuronide.

Table 18 The effect of air or nitrogen on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Air	Nitrogen	LSD ^z	P ($p \leq 0.05$) ^y
Flavanols				
gallodimer1	-	-	-	-
gallodimer2	7.17 a	7.16 a	0.56	0.9793
procyanidinB1	4.44 a	5.29 a	1.18	0.1484
catechin	nd ^x	nd		
dimer1	3.37 a	3.43 a	0.21	0.5902
dimer2	nd	nd		
epicatechin	3.31 a	3.18 a	0.45	0.5446
procyanidinB2	- ^w	-	-	-
flavanol1	2.85 a	4.23 a	1.42	0.0545
flavanol2	3.69 a	3.89 a	0.72	0.5578
trimer1	nd	nd		
dimermonogallate1	3.73 a	3.34 a	0.64	0.2188
flavanol3	nd	nd		
dimer3	3.38 a	3.23 a	0.70	0.6444
dimermonogallate2	5.91 a	6.73 a	4.02	0.6614
flavanol4	3.98 a	3.57 a	1.31	0.4912
epicatechingallate	nd	nd		
dimerdigallate	3.26 a	3.97 a	1.41	0.2320
trimergallate	3.72 a	3.89 a	0.98	0.7063
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	-	-	-	-
total flavanols	-	-	-	-
Flavonols				
quercetin-3-rutinoside	1.33 a	1.18 a	0.25	0.2344
myricetin-3-glucoside	2.88 a	2.26 b	0.59	0.0421
quercetin-3-galactoside	24.45 a	20.29 a	4.46	0.0668
quercetin-3-glucoside	26.80 a	22.31 b	3.81	0.0219
kaempferol-3-galactoside	3.12 a	2.68 a	0.59	0.1428
k-3-glc/glu ^v	9.25 a	8.17 a	1.82	0.2402
quercetin-3-rhamnoside	11.82 a	10.71 a	1.38	0.1141
total flavonols	79.63 a	67.44 a	12.54	0.0565
Benzoic acids				
benzoic acid1	0.71 a	0.74 a	0.11	0.5358
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	0.28 a	0.29 a	0.05	0.5958

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v k-3-glc/glu = kaempferol-3-glucoside/glucuronide.

Table 19 The effect of exposure time of air and nitrogen on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Hours					LSD ^z	P ($p \leq 0.05$) ^y
	4	8	16	32	64		
Flavanols							
gallodimer1	-	-	-	-	-	-	-
gallodimer2	38.69 ab	42.11 a	42.04 a	35.51 bc	33.61 c	4.25	0.0003
procyanidinB1	23.91 b	38.31 a	22.22 b	nd	20.43 b	12.69	0.0174
catechin	nd ^x	nd	nd	nd	19.06		
dimer1	19.67 a	19.16 a	19.31 a	16.57 b	15.99 b	2.13	0.0026
dimer2	nd	nd	nd	nd	nd		
epicatechin	13.96 b	19.80 a	17.14 ab	16.80 ab	17.45 ab	4.18	0.1240
procyanidinB2	- ^w	-	-	-	-	-	-
flavanol1	nd	24.79 a	21.03 a	nd	nd	7.78	0.0591
flavanol2	20.91 b	29.19 a	15.67 b	nd	nd	6.20	0.0009
trimer1	nd	nd	nd	nd	nd		
dimermonogallate1	17.40 b	24.26 a	17.57 b	nd	nd	5.93	0.0357
flavanol3	nd	nd	nd	nd	nd		
dimer3	16.87 a	19.27 a	21.04 a	nd	nd	6.02	0.3276
dimermonogallate2	27.12 ab	59.82 a	23.70 ab	nd	20.25 b	37.76	0.0703
flavanol4	24.81 a	24.48 a	17.37 a	nd	nd	9.36	0.0710
epicatechingallate	nd	nd	nd	nd	nd		
dimerdigallate	20.27 a	24.73 a	nd	nd	nd	8.17	
trimergallate	19.55 b	30.66 a	16.14 b	nd	nd	8.25	0.0048
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
polymeric fraction	2050.50 b	3176.80 a	2301.50 b	1337.70 c	1183.10 c	581.03	<0.0001
total flavanols	-	-	-	-	-	-	-
Flavonols							
q-3-rut ^v	6.26 a	5.66 a	7.66 a	7.12 a	6.36 a	2.04	0.3303
m-3-glc ^v	11.31 b	10.25 b	16.77 a	14.40 ab	14.16 ab	4.97	0.0862
q-3-gal ^v	95.56 b	96.61 b	146.39 a	128.01 ab	122.16 ab	38.07	0.0515
q-3-glc ^v	111.71 b	118.00 ab	148.44 a	138.64 ab	132.67 ab	31.48	0.1536
k-3-gal ^v	13.85 b	10.77 b	20.21 a	15.23 b	15.68 ab	4.91	0.0091
k-3-glc/gluc ^v	41.06 bc	31.54 c	60.95 a	46.33 a-c	47.36 ab	15.26	0.0085
q-3-rham ^v	56.07 ab	54.06 b	67.81 a	61.05 ab	60.34 ab	11.84	0.1926
total flavonols	335.81 b	325.86 b	468.23 a	410.18 ab	398.20 ab	105.33	0.0630
Benzoic acids							
benzoic acid1	3.68 b	4.82 a	3.61 b	3.86 ab	3.53 b	1.08	0.2897
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	nd	1.88 a	1.60 ab	nd	1.46 b	0.40	0.1318

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 20 The effect of exposure time of air and nitrogen on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Hours					LSD ^z	P ($p \leq 0.05$) ^y
	4	8	16	32	64		
Flavanols							
gallodimer1	-	-	-	-	-	-	-
gallodimer2	7.57 ab	7.07 bc	8.06 a	6.75 bc	6.34 c	0.89	0.0016
procyanidinB1	4.29 b	6.29 a	4.20 b	nd	3.87 b	1.87	0.0362
catechin	nd ^x	nd	nd	nd	3.70		
dimer1	3.83 a	3.18 b	3.67 a	3.10 b	3.07 b	0.33	<0.0001
dimer2	nd	nd	nd	nd	nd		
epicatechin	2.86 a	3.30 a	3.33 a	3.22 a	3.26 a	0.73	0.8669
procyanidinB2	- ^w	-	-	-	-	-	-
flavanol1	nd	4.05 a	3.65 a	nd	nd	1.25	0.1181
flavanol2	3.75 b	4.83 a	2.97 b	nd	nd	0.87	0.0010
trimer1	nd	nd	nd	nd	nd		
dimermonogallate1	3.27 a	4.03 a	3.31 a	nd	nd	0.78	0.0736
flavanol3	nd	nd	nd	nd	nd		
dimer3	3.26 a	3.08 a	3.80 a	nd	nd	0.97	0.3729
dimermonogallate2	4.80 a	9.65 a	4.20 a	nd	3.71 a	6.19	0.1054
flavanol4	3.98 a	4.00 a	3.05 a	nd	nd	1.33	0.0862
epicatechingallate	nd	nd	nd	nd	nd		
dimerdigallate	3.26 a	3.97 a	nd	nd	nd	1.41	
trimergallate	3.53 b	5.06 a	3.09 b	nd	nd	1.17	0.0068
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
polymeric fraction	-	-	-	-	-	-	-
total flavanols	-	-	-	-	-	-	-
Flavonols							
q-3-rut ^v	1.21 ab	0.96 b	1.49 a	1.35 ab	1.22 ab	0.40	0.1299
m-3-glc ^v	2.19 bc	1.76 c	3.24 a	2.73 ab	2.69 a-c	0.94	0.0352
q-3-gal ^v	18.48 bc	16.30 c	28.13 a	24.28 ab	23.15 a-c	7.06	0.0147
q-3-glc ^v	21.73 bc	19.86 c	28.59 a	26.23 ab	25.15 a-c	6.04	0.0487
k-3-gal ^v	2.70 bc	1.83 c	3.89 a	2.89 b	2.97 ab	0.93	0.0023
k-3-glc/gluc ^v	8.02 bc	5.36 c	11.72 a	8.82 b	8.98 ab	2.89	0.0024
q-3-rham ^v	10.94 ab	8.99 b	13.01 a	11.54 a	11.39 a	2.19	0.0170
total flavonols	65.27 bc	54.89 c	90.07 a	77.73 ab	75.44 ab	19.88	0.0147
Benzoic acids							
benzoic acid1	0.73 a	0.81 a	0.70 a	0.75 a	0.68 a	0.20	0.7998
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	nd	0.31 a	0.28 a	nd	0.28 a	0.05	0.3243

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 21 The effect of SO₂ pad treatments and different ethanol treatments on the cold storage evaluations of Regal Seedless for two seasons.

Season	Treatment	Bunch weight (g)	Loose berries (%)	SO ₂ burn (%)	Botrytis (%)	Split berries (%)	Browning (%)	Alter-naria (%)
2003/04	SO ₂₍₀₎ ^z	1810.40 a	0.91 b	0	-	0	0.33 a	-
	SO ₂₍₁₎	1802.50 a	1.83 a	0	-	0	0.08 a	-
	LSD ^y	88.82	0.63		-		0.27	-
	P (p ≤ 0.05) ^x	0.8571	0.0055		-		0.3141	-
	E[0] ^w	1854.90 a	1.46 a	0	- ^v	0	0.36 a	-
	E[10]	1905.15 a	1.79 a	0	-	0	0.47 a	-
	E[20]	1980.40 a	1.71 a	0	-	0	0.11 a	-
	E[40]	1619.15 b	0.98 a	0	-	0	0.00 a	-
	E[80]	1672.65 b	0.89 a	0	-	0	0.08 a	-
	LSD	140.44	0.99		-		0.50	-
P (p ≤ 0.05)	<0.0001	0.2421		-		0.4803	-	
2004/05	SO ₂₍₀₎	2111.19 a	9.69 a	0.42 a	-	0.47 a	-	-
	SO ₂₍₁₎	2032.34 a	1.15 b	0.72 a	-	0.19 a	-	-
	LSD	171.10	0.51	0.38	-	0.26	-	-
	P (p ≤ 0.05)	0.3541	<0.0001	0.3182	-	0.032	-	-
	E[0]	2070.50 ab	9.77 a	0.24 ab	-	0.37 a	-	-
	E[10]	2208.30 a	4.81 bc	0.16 b	-	0.48 a	-	-
	E[20]	2167.40 a	8.34 ab	0.84 a	-	0.31 a	-	-
	E[40]	2026.80 ab	1.86 c	0.71 ab	-	0.16 a	-	-
	E[80]	1885.80 b	2.32 c	0.91 ab	-	0.35 a	-	-
	LSD	270.53	0.81	0.60	-	0.42	-	-
P (p ≤ 0.05)	0.1482	0.0215	0.1037	-	0.6558	-	-	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^v "-" = data not shown due to interaction.

Table 22 Subjective evaluation of stem condition and moisture in the carton of Regal Seedless for two seasons.

Season	Treatment	Stems ^z	Moist ^y
2003/04	SO ₂₍₀₎ ^x	-	5.63 a
	SO ₂₍₁₎	-	5.38 a
	LSD ^w	-	0.74
	P (p ≤ 0.05) ^v	-	0.4955
	E[0] ^u	- ^t	5.13 a
	E[10]	-	6.38 a
	E[20]	-	5.13 a
	E[40]	-	5.13 a
	E[80]	-	5.75 a
	LSD	-	1.66
	P (p ≤ 0.05)	-	0.1355
2004/05	SO ₂₍₀₎	7.55 a	5.50 a
	SO ₂₍₁₎	3.45 b	5.50 a
	LSD	0.97	
	P (p ≤ 0.05)	<0.0001	
	E[0]	4.63 b	5.50 a
	E[10]	4.94 ab	5.50 a
	E[20]	6.06 ab	5.50 a
	E[40]	5.00 ab	5.50 a
	E[80]	6.88 a	5.50 a
	LSD	2.19	
	P (p ≤ 0.05)	0.0296	

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z Stem condition was ranked from 1 – 5 (where 1 is green and 5 is brown).

^y Moisture in the carton was ranked from 1 – 3 (where 1 is dry, 2 has condensation and 3 has free water).

^x SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^w LSD = Least significant difference.

^v P = Probability of F-ratio test.

^u E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

^t “-“ = data not shown due to interaction.

Table 23 The effect of SO₂ pad treatments and different ethanol treatments on the juice analysis of Regal Seedless for two seasons.

Season	Treatment	TSS	pH	TTA	Sugar:Acid
		(°Brix)		(g/L)	
2003/04	SO ₂₍₀₎ ^z	22.40 a	4.89 a	5.23 a	43.01 a
	SO ₂₍₁₎	22.48 a	4.79 b	5.29 a	42.62 a
	LSD ^y	0.63	0.09	0.19	1.74
	P (p ≤ 0.05) ^x	0.8084	0.0341	0.5160	0.6522
	E[0] ^w	22.59 ab	4.79 a	5.43 a	41.71 a
	E[10]	21.76 b	4.83 a	5.23 a	41.78 a
	E[20]	22.53 ab	4.90 a	5.21 a	43.36 a
	E[40]	22.88 a	4.78 a	5.28 a	43.53 a
	E[80]	22.44 ab	4.88 a	5.14 a	43.70 a
	LSD	0.99	0.15	0.29	2.76
	P (p ≤ 0.05)	0.2466	0.3458	0.3749	0.3886
2004/05	SO ₂₍₀₎	20.47 b	4.00 a	2.99 a	68.83 a
	SO ₂₍₁₎	21.12 a	4.01 a	2.93 a	72.23 a
	LSD	0.61	0.07	0.13	3.55
	P (p ≤ 0.05)	0.0453	0.9824	0.3186	0.0565
	E[0]	20.69 a	4.03 a	2.93 a	70.97 a
	E[10]	21.34 a	4.04 a	3.07 a	69.54 a
	E[20]	20.63 a	3.98 a	2.99 a	69.71 a
	E[40]	20.63 a	3.99 a	2.91 a	70.94 a
	E[80]	20.79 a	4.00 a	2.91 a	71.57 a
	LSD	0.96	0.11	0.21	5.62
	P (p ≤ 0.05)	0.5493	0.7750	0.5001	0.9353

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w E[] = ethanol concentration (E[0] = 0%; E[10] = 10%; E[20] = 20%; E[40] = 40%; E[80] = 80%).

Table 24 The effect of SO₂ pad treatments on the sensory evaluation of Regal Seedless in 2003/04 and 2004/05.

Season	Treatment ^z	Astringency	Skin tenacity	Eating quality
2003/04	SO ₂₍₀₎	3.59 a	5.40 a	5.84 a
	SO ₂₍₁₎	2.95 a	4.92 a	6.31 a
	LSD ^y	1.01	1.44	0.79
	P (p ≤ 0.05) ^x	0.2088	0.5031	0.2408
2004/05	SO ₂₍₀₎	2.49 a	2.30 a	6.50 a
	SO ₂₍₁₎	2.86 a	2.57 a	5.76 a
	LSD	1.19	1.21	1.40
	P (p ≤ 0.05)	0.5324	0.6565	0.2914

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

Table 25 The effect of SO₂ pad treatments on the phenolic content of Regal Seedless in 2003/04.

Treatment	A280	A420	A365	A320
AU per berry				
SO ₂₍₀₎ ^z	0.0664 a	0.0306 a	0.2079 a	0.2335 a
SO ₂₍₁₎	0.0708 a	0.0322 a	0.2184 a	0.2478 a
LSD ^y	0.0044	0.0025	0.0255	0.0231
P (p ≤ 0.05)^x	0.0535	0.2016	0.4034	0.2175
AU per gram berry weight				
SO ₂₍₀₎	0.0133 a	0.0061 a	0.0417 a	0.0468 a
SO ₂₍₁₎	0.0132 a	0.0060 a	0.0409 a	0.0464 a
LSD	0.0004	0.0003	0.0044	0.0037
P (p ≤ 0.05)	0.8296	0.5448	0.7181	0.8026

Within columns and expressed units, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

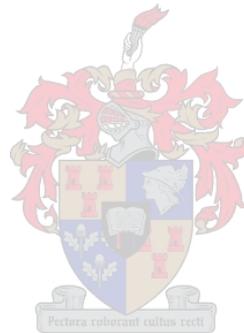


Table 26 The effect of SO₂ pad treatments on the phenolic content (µg/berry) of Regal Seedless in 2003/04.

Phenolic compounds	SO ₂₍₀₎ ^z	SO ₂₍₁₎ ^z	LSD ^y	P (p ≤ 0.05) ^x
Flavanols				
gallodimer1	343.98 b	390.49 a	34.83	0.0134
gallodimer2	- ^w	-	-	-
procyanidinB1	17.64 a	20.80 a	4.62	0.1666
catechin	nd ^v	nd		
dimer1	17.11 a	18.52 a	1.96	0.2133
dimer2	nd	nd		
epicatechin	nd	nd		
procyanidinB2	60.83 b	69.09 a	6.82	0.0241
flavanol1	nd	nd		
flavanol2	nd	23.76		
trimer1	nd	nd		
dimermonogallate1	14.34 a	15.12 a	5.60	0.9472
flavanol3	nd	nd		
dimer3	14.50 a	15.40 a	4.95	* ^u
dimermonogallate2	nd	nd		
flavanol4	nd	17.34		
epicatechingallate	nd	nd		
dimerdigallate	nd	16.58		
trimergallate	16.52 a	19.59 a	8.33	*
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	1764.30 b	2200.00 a	412.79	0.0173
total flavanols	2239.70 b	2748.90 a	421.13	0.0084
Flavonols				
quercetin-3-rutinoside	-	-	-	-
myricetin-3-glucoside	14.95 a	15.15 a	3.62	0.7211
quercetin-3-galactoside	128.03 a	131.09 a	31.98	0.6821
quercetin-3-glucoside	142.80 a	146.45 a	25.64	0.5877
kaempferol-3-galactoside	16.70 a	17.13 a	3.90	0.6968
kaempferol-3-glucoside/glucunoride	49.00 a	50.75 a	12.07	0.6610
quercetin-3-rhamnoside	67.87 a	68.00 a	8.83	0.8183
total flavonols	427.13 a	436.95 a	84.14	0.6475
Benzoic acids				
benzoic acid1	3.57 a	3.76 a	0.54	0.5191
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	1.51 a	1.54 a	0.52	*

Within rows, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w “-“ = data not shown due to interaction.

^v nd = not detected.

^u * = no P-value, degrees of freedom to low.

Table 27 The effect of SO₂ pad treatments on the phenolic content (µg/g berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	SO ₂₍₀₎ ^z	SO ₂₍₁₎ ^z	LSD ^y	P (p ≤ 0.05) ^x
Flavanols				
gallodimer1	69.28 a	73.18 a	6.23	0.2604
gallodimer2	- ^w	-	-	-
procyanidinB1	3.52 a	3.85 a	0.81	0.3768
catechin	nd ^v	nd		
dimer1	3.43 a	3.48 a	0.28	0.8434
dimer2	nd	nd		
epicatechin	nd	nd		
procyanidinB2	-	-	-	-
flavanol1	nd	nd		
flavanol2	nd	4.39		
trimer1	nd	nd		
dimermonogallate1	2.65 a	2.84 a	0.54	0.7821
flavanol3	nd	nd		
dimer3	2.99 a	2.91 a	0.76	* ^u
dimermonogallate2	nd	nd		
flavanol4	nd	3.26		
epicatechingallate	nd	nd		
dimerdigallate	nd	3.10		
trimergallate	3.04 a	3.62 a	1.25	*
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	354.64 a	410.81 a	74.89	0.0655
total flavanols	450.15 a	513.62 a	75.45	0.0450
Flavonols				
quercetin-3-rutinoside	-	-	-	-
myricetin-3-glucoside	3.01 a	2.85 a	0.67	0.7958
quercetin-3-galactoside	25.68 a	24.62 a	5.77	0.8658
quercetin-3-glucoside	28.75 a	27.53 a	4.81	0.7819
kaempferol-3-galactoside	3.36 a	3.23 a	0.72	0.8182
kaempferol-3-glucoside/glucunoride	9.87 a	9.57 a	2.23	0.8728
quercetin-3-rhamnoside	13.66 a	12.80 a	1.61	0.3729
total flavonols	85.91 a	82.18 a	15.38	0.7728
Benzoic acids				
benzoic acid1	-	-	-	-
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	0.29 a	0.29 a	0.07	*

Within rows, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w "-" = data not shown due to interaction.

^v nd = not detected.

^u * = no P-value, degrees of freedom to low.

Table 28 The effect of different ethanol treatments on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Ethanol (%)					LSD ^z	P ($p \leq 0.05$) ^y
	0	10	20	40	80		
Flavanols							
gallodimer1	332.13 b	353.86 ab	368.63 ab	399.91 a	380.11 ab	55.17	0.1506
gallodimer2	- ^x	-	-	-	-	-	-
procyanidinB1	21.10 a	15.84 a	18.77 a	20.82 a	15.64 a	8.20	0.3181
catechin	nd ^w	nd	nd	nd	nd		
dimer1	14.20 c	16.78 bc	17.56 ab	19.42 ab	19.95 a	3.13	0.0069
dimer2	nd	nd	nd	nd	nd		
epicatechin	nd	nd	nd	nd	nd		
procyanidinB2	57.11 b	57.70 b	63.83 ab	74.37 a	71.20 a	10.80	0.0073
flavanol1	nd	nd	nd	nd	nd		
flavanol2	24.29 a	nd	nd	22.96 a	nd	19.64	0.8439
trimer1	nd	nd	nd	nd	nd		
di-mo-gal1 ^v	14.55 a	16.66 a	14.25 a	nd	nd	6.26	0.5623
flavanol3	nd	nd	nd	nd	nd		
dimer3	14.50 a	15.61 a	15.18 a	nd	nd	6.11	0.8708
di-mo-gal2 ^v	nd	nd	nd	nd	nd		
flavanol4	nd	16.92 a	17.76 a	nd	nd	23.45	0.8920
epicatgallate ^v	nd	nd	nd	nd	nd		
dimerdigallate	nd	nd	nd	16.58	nd		
trimergallate	19.38 a	nd	16.52 a	19.90 a	nd	9.38	0.6221
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly fraction ^v	2717.60 a	1938.00 b	1803.10 b	2053.50 b	1445.10 b	653.83	0.0102
total flavanols	3203.90 a	2415.10 b	2312.30 b	2617.20 ab	1967.60 b	667.05	0.0144
Flavonols							
q-3-rut ^v	-	-	-	-	-	-	-
m-3-glc ^v	19.78 a	14.57 ab	16.39 ab	12.40 b	12.32 b	5.73	0.0709
q-3-gal ^v	163.49 a	122.69 ab	147.16 ab	106.99 b	108.40 b	50.65	0.1210
q-3-glc ^v	177.53 a	135.75 b	146.81 ab	136.17 b	128.01 b	40.62	0.1487
k-3-gal ^v	19.86 a	16.79 a	19.81 a	13.98 a	14.19 a	6.17	0.1455
k-3-glc/gluc ^v	57.83 ab	49.00 ab	60.09 a	39.39 b	43.24 ab	19.11	0.1338
q-3-rham ^v	76.63 a	64.25 a	70.47 a	64.09 a	64.41 a	13.98	0.2994
total flavonols	524.59 a	411.94 ab	468.23 ab	380.55 b	377.78 b	133.27	0.1482
Benzoic acids							
benzoic acid1	3.74 a	3.62 a	3.53 a	3.40 a	4.02 a	0.85	0.6514
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	1.58 a	1.49 a	nd	1.51 a	1.56 a	0.67	0.9886

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x "-" = data not shown due to interaction.

^w nd = not detected.

^v di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicatgallate = epicatechingallate; poly fraction = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 29 The effect of different ethanol treatments on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Ethanol (%)					LSD ^z	P ($p \leq 0.05$) ^y
	0	10	20	40	80		
Flavanols							
gallodimer1	65.07 a	71.50 a	72.51 a	74.06 a	72.69 a	9.87	0.3998
gallodimer2	- ^x	-	-	-	-	-	-
procyanidinB1	4.00 a	3.50 a	3.45 a	3.92 a	3.10 a	1.44	0.4562
catechin	nd ^w	nd	nd	nd	nd		
dimer1	2.80 b	3.47 a	3.48 a	3.59 a	3.81 a	0.44	0.0017
dimer2	nd	nd	nd	nd	nd		
epicatechin	nd	nd	nd	nd	nd		
procyanidinB2	-	-	-	-	-	-	-
flavanol1	nd	nd	nd	nd	nd		
flavanol2	4.44 a	nd	nd	4.32 a	nd	3.49	0.9221
trimer1	nd	nd	nd	nd	nd		
dimermonogallate1	2.70 a	3.10 a	2.68 a	nd	nd	0.60	0.2256
flavanol3	nd	nd	nd	nd	nd		
dimer3	2.99 a	2.89 a	2.92 a	nd	nd	0.94	0.9498
dimermonogallate2	nd	nd	nd	nd	nd		
flavanol4	nd	3.12 a	3.39 a	nd	nd	3.12	0.7412
epicatechingallate	nd	nd	nd	nd	nd		
dimerdigallate	nd	nd	nd	3.10	nd		
trimergallate	3.56 a	nd	3.04 a	3.73 a	nd	1.41	0.4741
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
polymeric fraction	527.69 a	382.69 b	350.88 b	382.64 b	278.30 b	118.63	0.0052
total flavanols	622.51 a	478.98 b	450.82 b	487.12 b	378.21 b	119.50	0.0070
Flavonols							
q-3-rut ^v	-	-	-	-	-	-	-
m-3-glc ^v	3.85 a	2.96 ab	3.16 ab	2.33 b	2.40 b	1.06	0.0438
q-3-gal ^v	31.62 a	24.84 ab	28.31 ab	20.05 b	21.12 b	9.14	0.0765
q-3-glc ^v	34.56 a	27.51 ab	28.55 ab	25.53 b	24.78 b	7.63	0.1091
k-3-gal ^v	3.85 a	3.42 ab	3.83 a	2.63 b	2.77 ab	1.14	0.0940
k-3-glc/gluc ^v	11.15 a	9.99 ab	11.61 a	7.40 b	8.48 ab	3.53	0.0921
q-3-rham ^v	14.94 a	13.04 ab	13.76 ab	12.00 b	12.44 ab	2.56	0.1740
total flavonols	101.81 a	83.58 ab	90.69 ab	71.34 b	73.40 b	24.36	0.0913
Benzoic acids							
benzoic acid1	-	-	-	-	-	-	-
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	0.29 a	0.28 a	nd	0.29 a	0.30 a	0.09	0.9810

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x "-" = data not shown due to interaction.

^w nd = not detected.

^v q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 30 The effect of SO₂ pad treatments on the phenolic content (µg/berry) of Regal Seedless in 2004/05.

Phenolic compounds	SO ₂₍₀₎ ^z	SO ₂₍₁₎ ^z	LSD ^y	P (p ≤ 0.05) ^x
Flavanols				
galocatehin	58.19 a	61.27 a	5.55	0.4752
gallodimer1A	390.47 a	406.24 a	38.14	0.4343
gallodimer1	448.66 a	467.50 a	41.19	0.4125
gallodimer2	35.77 a	36.94 a	1.98	0.3180
procyanidinB1	nd ^w	18.52		
catechin	nd	31.95		
dimer1	20.84 a	20.55 a	2.24	0.7470
dimer2	nd	nd		
epicatechin	17.26 b	22.43 a	3.82	0.0146
procyanidinB2	54.90 b	63.89 a	8.78	0.0841
flavanol1	nd	nd		
flavanol2	nd	nd		
trimer1	nd	nd		
dimermonogallate1	nd	nd		
flavanol3	nd	nd		
dimer3	34.10 a	36.38 a	6.04	0.3413
dimermonogallate2	nd	32.11		
flavanol4	nd	nd		
epicatechingallate	nd	nd		
dimerdigallate	nd	nd		
trimergallate	nd	nd		
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	847.97 b	1019.27 a	150.19	0.0223
total flavanols	1429.67 b	1652.96 a	166.91	0.0121
Flavonols				
quercetin-3-rutinoside	5.97 a	4.65 b	1.19	0.0290
myricetin-3-glucoside	- ^v	-	-	-
quercetin-3-galactoside	-	-	-	-
quercetin-3-glucoside	-	-	-	-
kaempferol-3-galactoside	12.49 a	12.42 a	2.91	0.8967
kaempferol-3-glucoside/glucunoride	-	-	-	-
quercetin-3-rhamnoside	-	-	-	0.6745
total flavonols	-	-	-	-
Benzoic acids				
benzoic acid1	4.08 a	3.50 a	0.63	0.1085
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	1.76 a	1.91 a	0.63	0.4834

Within rows, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w nd = not detected.

^v "-" = data not shown due to interaction.

Table 31 The effect of SO₂ pad treatments on the phenolic content (µg/g berry weight) of Regal Seedless in 2004/05.

Phenolic compounds	SO ₂₍₀₎ ^z	SO ₂₍₁₎ ^z	LSD ^y	P (p ≤ 0.05) ^x
Flavanols				
galocatehin	11.18 a	11.27 a	1.06	0.9208
gallodimer1A	74.96 a	74.69 a	6.52	0.9944
gallodimer1	86.14 a	85.95 a	7.08	0.9933
gallodimer2	6.88 a	6.80 a	0.33	0.5960
procyanidinB1	nd ^w	3.34		
catechin	nd	5.85		
dimer1	4.00 a	3.77 a	0.36	0.2428
dimer2	nd	nd		
epicatechin	3.32 b	4.11 a	0.61	0.0184
procyanidinB2	10.47 a	11.62 a	1.25	0.1318
flavanol1	nd	nd		
flavanol2	nd	nd		
trimer1	nd	nd		
dimermonogallate1	nd	nd		
flavanol3	nd	nd		
dimer3	6.64 a	6.62 a	0.86	0.8725
dimermonogallate2	nd	6.13		
flavanol4	nd	nd		
epicatechingallate	nd	nd		
dimerdigallate	nd	nd		
trimergallate	nd	nd		
flavanol5	nd	nd		
flavanol6	nd	nd		
flavanol7	nd	nd		
polymeric fraction	162.81 a	185.69 a	25.51	0.0516
total flavanols	274.45 b	302.12 a	25.95	0.0307
Flavonols				
quercetin-3-rutinoside	1.15 a	0.85 b	0.22	0.0094
myricetin-3-glucoside	- ^v	-	-	-
quercetin-3-galactoside	-	-	-	-
quercetin-3-glucoside	-	-	-	-
kaempferol-3-galactoside	-	-	-	-
kaempferol-3-glucoside/glucunoride	-	-	-	-
quercetin-3-rhamnoside				0.2501
total flavonols	-	-	-	-
Benzoic acids				
benzoic acid1	0.78 a	0.67 a	0.13	0.1154
Cinnamic acids				
cinnamic acid1	nd	nd		
cinnamic acid2	0.34 a	0.35 a	0.10	0.4869

Within rows, values with different letters are significantly different at a 5% significance level.

^z SO₂₍₀₎ = without SO₂ pad; SO₂₍₁₎ = with SO₂ pad.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w nd = not detected.

^v "-" = data not shown due to interaction.

Table 32 The effect of different ethanol treatments on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2004/05.

Phenolic compounds	Ethanol (%)					LSD ^z	P ($p \leq 0.05$) ^y
	0	10	20	40	80		
Flavanols							
galocatehin	57.27 ab	59.98 ab	52.79 b	63.41 a	62.25 a	8.91	0.1775
gallodimer1A	385.73 a	401.20 a	390.55 a	384.15 a	425.59 a	61.22	0.5716
gallodimer1	443.00 a	461.18 a	443.34 a	447.56 a	487.84 a	66.12	0.5616
gallodimer2	37.06 a	35.99 a	34.51 a	35.81 a	37.62 a	3.17	0.3766
procyanidinB1	nd ^x	18.52	nd	nd	nd		
catechin	32.18 a	31.81 a	nd	nd	nd	4.95	0.8269
dimer1	20.50 a	20.51 a	20.30 a	21.02 a	21.02 a	3.60	0.9904
dimer2	nd	nd	nd	nd	nd		
epicatechin	22.35 a	19.31 a	16.49 a	19.01 a	19.56 a	6.26	0.4554
procyanidinB2	57.85 ab	59.05 ab	46.53 b	60.33 ab	66.16 a	14.39	0.1910
flavanol1	nd	nd	nd	nd	nd		
flavanol2	nd	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd	nd		
di-mo-gal1 ^v	nd	nd	nd	nd	nd		
flavanol3	nd	nd	nd	nd	nd		
dimer3	34.98 a	34.46 a	nd	nd	nd	5.51	0.8070
di-mo-gal2 ^v	33.12 a	30.08 a	nd	nd	nd	9.25	0.4141
flavanol4	nd	nd	nd	nd	nd		
epicatgallate ^v	nd	nd	nd	nd	nd		
dimerdigallate	nd	nd	nd	nd	nd		
trimergallate	nd	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
poly fraction ^v	873.60 ab	858.00 b	915.20 ab	878.30 ab	1105.30 a	241.07	0.1754
total flavanols	1496.40 a	1496.30 a	1463.80 a	1462.00 a	1725.40 a	267.91	0.2021
Flavonols							
q-3-rut ^v	6.08 a	4.74 a	5.51 a	5.48 a	4.74 a	1.90	0.5186
m-3-glc ^v	- ^w	-	-	-	-	-	-
q-3-gal ^v	-	-	-	-	-	-	-
q-3-glc ^v	-	-	-	-	-	-	-
k-3-gal ^v	12.33 a	12.24 a	13.51 a	12.91 a	11.55 a	4.67	0.9406
k-3-glc/gluc ^v	-	-	-	-	-	-	-
q-3-rham ^v	48.90 a	48.73 a	51.41 a	52.65 a	51.22 a	10.73	0.9183
total flavonols	-	-	-	-	-	-	-
Benzoic acids							
benzoic acid1	4.18 a	3.40 a	4.26 a	3.86 a	3.78 a	0.98	0.3605
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	2.11 a	1.76 a	nd	1.89 a	1.78 a	0.67	0.6243

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v di-mo-gal1 = dimermonogallate1; di-mo-gal2 = dimermonogallate2; epicatgallate = epicatechingallate; poly fraction = polymeric fraction; q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 33 The effect of different ethanol treatments on the phenolic content ($\mu\text{g/g}$ berry weight) of Regal Seedless in 2004/05.

Phenolic compounds	Ethanol (%)					LSD ^z	P ($p \leq 0.05$) ^y
	0	10	20	40	80		
Flavanols							
galocatehin	10.75 a	11.46 a	10.47 a	11.89 a	11.29 a	1.70	0.4819
gallodimer1A	72.69 a	76.67 a	77.21 a	71.81 a	76.90 a	10.47	0.7062
gallodimer1	83.44 a	88.12 a	87.68 a	83.69 a	88.19 a	11.36	0.7922
gallodimer2	6.96 a	6.87 a	6.82 a	6.71 a	6.83 a	0.53	0.8781
procyanidinB1	nd ^x	3.34	nd	nd	nd		
catechin	5.85 a	5.85 a	nd	nd	nd	0.52	0.9990
dimer1	3.85 a	3.92 a	4.02 a	3.92 a	3.80 a	0.59	0.9631
dimer2	nd	nd	nd	nd	nd		
epicatechin	4.17 a	3.68 a	3.27 a	3.53 a	3.59 a	1.00	0.4245
procyanidinB2	10.82 ab	11.26 a	9.21 b	11.05 ab	11.91 a	2.05	0.2134
flavanol1	nd	nd	nd	nd	nd		
flavanol2	nd	nd	nd	nd	nd		
trimer1	nd	nd	nd	nd	nd		
dimermonogallate1	nd	nd	nd	nd	nd		
flavanol3	nd	nd	nd	nd	nd		
dimer3	6.59 a	6.69 a	nd	nd	nd	0.78	0.7539
dimermonogallate2	6.22 a	5.96 a	nd	nd	nd	1.47	0.6512
flavanol4	nd	nd	nd	nd	nd		
epicatechingallate	nd	nd	nd	nd	nd		
dimerdigallate	nd	nd	nd	nd	nd		
trimergallate	nd	nd	nd	nd	nd		
flavanol5	nd	nd	nd	nd	nd		
flavanol6	nd	nd	nd	nd	nd		
flavanol7	nd	nd	nd	nd	nd		
polymeric fraction	163.99 a	163.13 a	180.78 a	162.18 a	199.36 a	40.94	0.2403
total flavanols	281.09 a	285.01 a	289.28 a	271.06 a	311.48 a	41.65	0.3146
Flavonols							
q-3-rut ^v	1.14 a	0.90 a	1.09 a	1.04 a	0.85 a	0.35	0.3792
m-3-glc ^v	- ^w	-	-	-	-	-	-
q-3-gal ^v	-	-	-	-	-	-	-
q-3-glc ^v	-	-	-	-	-	-	-
k-3-gal ^v	-	-	-	-	-	-	-
k-3-glc/gluc ^v	-	-	-	-	-	-	-
q-3-rham ^v	9.17 a	9.29 a	10.16 a	9.89 a	9.25 a	1.89	0.7812
total flavonols	-	-	-	-	-	-	-
Benzoic acids							
benzoic acid1	0.80 a	0.66 a	0.83 a	0.75 a	0.71 a	0.20	0.4076
Cinnamic acids							
cinnamic acid1	nd	nd	nd	nd	nd		
cinnamic acid2	0.39 a	0.33 a	nd	0.36 a	0.30 a	0.11	0.4406

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w '-' = data not shown due to interaction.

^v q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 34 The effect of cold storage duration and shelf life period on the post storage evaluations of Regal Seedless for two seasons.

Season	Treatment ^z	Bunch weight	Loose berries	SO ₂ burn	Botrytis	Split berries	Browning
		(g)	(%)	(%)	(%)	(%)	(%)
2002/03	W0+S0	* ^w	*	*	*	*	*
	W4+S0	1817.23 a	1.78 a	4.70 a	1.14 a	0.36 a	0.00 b
	W7+S0	1801.57 a	1.17 a	5.65 a	0.50 a	1.03 a	0.51 a
	LSD ^y	59.40	0.54	1.23	1.09	0.83	0.51
	P (p ≤ 0.05) ^x	0.5698	0.2969	0.4184	0.9202	0.3224	0.0317
	W0+S1	1836.90 a	3.93 a	0.41 b	0.00 a	0.52 a	0.00 b
	W4+S1	1813.73 ab	0.38 b	8.42 a	0.27 a	0.26 a	0.55 a
	W7+S1	1708.40 b	6.56 a	3.09 a	0.18 a	0.87 a	0.28 ab
	LSD	113.29	0.64	1.11	0.51	0.85	0.51
	P (p ≤ 0.05)	0.064	<0.0001	0.0089	0.4093	0.7645	0.0597
2003/04	W0+S0	1810.73 b	2.90 a	0.00 b	0.00 a	0.00 b	0.00 b
	W4+S0	1880.20 a	0.62 a	2.75 a	0.05 a	2.15 a	0.73 a
	W7+S0	1871.50 a	0.82 a	5.68 a	0.14 a	1.17 a	0.04 b
	LSD	51.30	1.03	0.68	0.31	0.62	0.24
	P (p ≤ 0.05)	0.0200	0.1255	<0.0001	0.5087	0.0002	<0.0001
	W0+S1	1903.37 a	0.38 b	2.86 b	0.00 b	4.37 a	0.32 a
	W4+S1	1883.23 ab	1.21 ab	9.00 a	0.44 ab	2.58 a	0.95 a
	W7+S1	1865.33 b	1.43 a	10.85 a	0.502 a	1.48 a	0.08 a
	LSD	31.88	0.65	1.02	0.51	0.94	0.77
	P (p ≤ 0.05)	0.0640	<0.0001	0.0080	0.4093	0.7645	0.0597

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w "*" missing values.

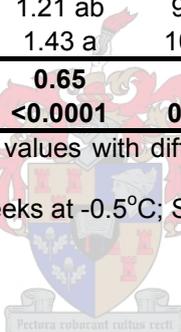


Table 35 The effect of the shelf life period at 15°C on the cold storage evaluations of Regal Seedless in 2002/03 and 2003/04.

Season	Treatment ^z	Bunch weight (g)	Loose berries (%)	SO ₂ burn (%)	Botrytis (%)	Split berries (%)	Browning (%)
2002/03	W0	*	*	*	*	*	*
	W4	-3.50 a	-1.40 b	3.73 a	-0.87 a	-0.10 a	0.55 a
	W7	-93.17 a	5.39 a	-2.56 a	-0.34 a	-0.25 a	-0.23 a
	LSD ^y	159.62	0.96	1.13	1.63	1.42	1.01
	P (p ≤ 0.05) ^x	0.2392	0.0003	0.0687	0.8259	0.6521	0.096
2003/04	W0	92.63 a	-2.52 b	2.86 a	0.00 a	4.30 a	0.32 a
	W4	3.03 b	0.59 a	6.25 a	0.39 a	0.44 b	0.22 a
	W7	3.46 b	0.19 a	3.90 a	0.41 a	0.03 b	0.05 a
	LSD	38.81	0.99	1.43	0.65	1.31	0.88
	P (p ≤ 0.05)	0.0002	0.0063	0.5151	0.2422	0.0049	0.7046

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w "*" missing values.

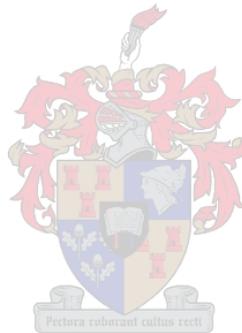


Table 36 Subjective evaluation of stem condition and moisture in the carton of Regal Seedless for two seasons.

Season	Treatment ^z	Stems ^y	Moist ^x
2002/03	W0+S0	* ^u	*
	W4+S0	1.08 b	4.67 a
	W7+S0	3.17 a	2.58 b
	LSD ^w	0.58	0.77
	P (p ≤ 0.05) ^v	<0.0001	<0.0001
	W0+S1	3.00 b	2.58 a
	W4+S1	3.00 b	2.58 a
	W7+S1	4.70 a	2.58 a
	LSD	1.34	0.31
	P (p ≤ 0.05)	0.0081	1.0000
2003/04	W0+S0	1.00 b	1.42 b
	W4+S0	2.58 a	4.33 a
	W7+S0	3.10 a	4.50 a
	LSD	0.77	0.43
	P (p ≤ 0.05)	<0.0001	<0.0001
	W0+S1	3.83 b	4.33 a
	W4+S1	5.00 a	4.33 a
	W7+S1	5.00 a	1.83 b
	LSD	1.12	0.87
	P (p ≤ 0.05)	0.0230	<0.0001

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^y Stem condition was ranked from 1 – 5 (where 1 is green and 5 is brown).

^x Moisture in the carton was ranked from 1 – 3 (where 1 is dry, 2 has condensation and 3 has free water).

^w LSD = Least significant difference.

^v P = Probability of F-ratio test.

^u “*” missing values.

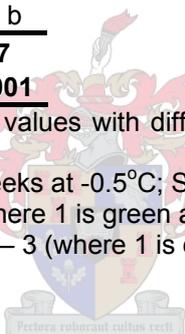


Table 37 The effect of cold storage duration and shelf life period on the juice analysis of Regal Seedless for 2002/03 and 2003/04.

Season	Treatment ^z	TSS	pH	TTA	Sugar:Acid
		(°Brix)		(g/L)	
2002/03	W0+S0	18.28 a	*W	*	*
	W4+S0	17.83 a	3.72 a	6.35 a	28.26 a
	W7+S0	18.08 a	3.75 a	6.10 a	29.82 a
	LSD ^y	1.05	0.11	0.58	4.15
	P (p ≤ 0.05) ^x	0.6630	0.5641	0.3620	0.4193
	W0+S1	18.53 a	3.93 a	5.13 b	36.42 a
	W4+S1	18.30 a	3.91 a	5.42 b	34.02 a
	W7+S1	17.83 a	3.72 b	6.42 a	27.83 b
	LSD	1.14	0.10	0.41	4.24
	P (p ≤ 0.05)	0.4329	0.0006	<0.0001	0.0018
2003/04	W0+S0	19.43 a	3.97 a	3.85 b	50.65 a
	W4+S0	18.32 a	3.68 b	5.12 a	36.30 b
	W7+S0	18.10 a	3.88 a	5.19 a	35.05 b
	LSD	1.45	0.17	0.49	5.97
	P (p ≤ 0.05)	0.1375	0.0050	<0.0001	<0.0001
	W0+S1	17.67 a	*	7.60 a	23.36 c
	W4+S1	18.30 a	3.88 a	4.77 c	38.66 a
	W7+S1	17.42 a	3.81 a	6.04 b	28.92 b
	LSD	1.12	0.10	0.51	3.82
	P (p ≤ 0.05)	0.2533	0.1586	<0.0001	<0.0001

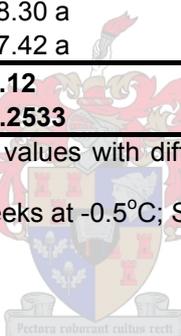
Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w “*” missing values.

**Table 38** The effect of the shelf life period at 15°C on the juice analysis of Regal Seedless in 2002/03 and 2003/04.

Season	Treatment ^z	TSS	pH	TTA	Sugar:Acid
		(°Brix)		(g/L)	
2002/03	W0	0.25 a	*W	*	*
	W4	0.47 a	0.19 a	-0.93 b	5.76 a
	W7	-0.25 a	-0.03 b	0.32 a	-2.00 b
	LSD ^y	1.75	0.15	0.68	5.50
	P (p ≤ 0.05) ^x	0.6758	0.0092	0.0021	0.0104
2003/04	W0	-1.77 a	*	3.76 a	-27.29 c
	W4	-0.02 a	0.20 a	-0.35 c	2.36 a
	W7	-0.86 a	-0.10 b	0.90 b	-6.69 b
	LSD	1.85	0.21	0.79	7.33
	P (p ≤ 0.05)	0.1479	0.0102	<0.0001	<0.0001

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

^w “*” missing values.

Table 39 The effect of cold storage duration and shelf life period on the phenolic content (AU per berry) of Regal Seedless in 2002/03 and 2003/04.

Season	Treatment ^z	A280	A420	A365	A320
2002/03	W0+S0	0.0655 ab	0.0271 a	0.1168 a	0.1773 a
	W4+S0	0.0683 a	0.0290 a	0.1331 a	0.1924 a
	W7+S0	0.0598 b	0.0266 a	0.1188 a	0.1765 a
	LSD ^y	0.0075	0.0031	0.0226	0.0250
	P (p ≤ 0.05) ^x	0.0868	0.2686	0.2943	0.3624
	W0+S1	0.0661 ab	0.0269 b	0.1300 a	0.1824 a
	W4+S1	0.0681 a	0.0307 a	0.1186 a	0.1884 a
	W7+S1	0.0606 b	0.0258 b	0.1256 a	0.1859 a
	LSD	0.0058	0.0029	0.0270	0.0253
	P (p ≤ 0.05)	0.0366	0.0067	0.6789	0.8823
2003/04	W0+S0	0.0864 a	0.0381 a	0.1832 a	0.2501 a
	W4+S0	0.0778 a	0.0316 b	0.1604 a	0.2159 a
	W7+S0	0.0823 a	0.0364 ab	0.1674 a	0.2363 a
	LSD	0.0088	0.0059	0.0325	0.0347
	P (p ≤ 0.05)	0.1383	0.0742	0.3300	0.1300
	W0+S1	0.0795 b	0.0314 c	0.1564 b	0.2156 c
	W4+S1	0.0855 ab	0.0379 b	0.1786 b	0.2474 b
	W7+S1	0.0873 a	0.0434 a	0.2094 a	0.2921 a
	LSD	0.0061	0.0038	0.0242	0.0238
	P (p ≤ 0.05)	0.0367	<0.0001	0.0011	<0.0001

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

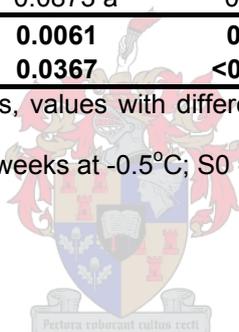


Table 40 The effect of cold storage duration and shelf life period on the phenolic content (AU per gram berry weight) of Regal Seedless in 2002/03 and 2003/04.

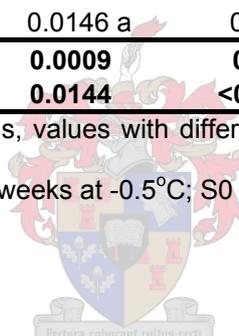
Season	Treatment ^z	A280	A420	A365	A320
2002/03	W0+S0	0.0111 b	0.0046 b	0.0198 a	0.0301 b
	W4+S0	0.0118 a	0.0050 ab	0.0228 a	0.0331 ab
	W7+S0	0.0115 ab	0.0051 a	0.0229 a	0.0340 a
	LSD ^y	0.0006	0.0005	0.0037	0.0038
	P (p ≤ 0.05) ^x	0.0907	0.0456	0.1334	0.0811
	W0+S1	0.0119 a	0.0048 a	0.0235 a	0.0329 ab
	W4+S1	0.0113 a	0.0051 a	0.0197 a	0.0313 b
	W7+S1	0.0121 a	0.0051 a	0.0251 a	0.0372 a
	LSD	0.0008	0.0004	0.0055	0.0050
	P (p ≤ 0.05)	0.1650	0.2183	0.1304	0.0584
2003/04	W0+S0	0.0141 a	0.0062 a	0.0298 a	0.0408 a
	W4+S0	0.0135 a	0.0055 a	0.0278 a	0.0374 a
	W7+S0	0.0142 a	0.0063 a	0.0290 a	0.0409 a
	LSD	0.0011	0.0009	0.0052	0.0058
	P (p ≤ 0.05)	0.3276	0.1152	0.6906	0.3401
	W0+S1	0.0132 b	0.0052 c	0.0260 b	0.0359 c
	W4+S1	0.0143 a	0.0063 b	0.0298 b	0.0412 b
	W7+S1	0.0146 a	0.0073 a	0.0350 a	0.0488 a
	LSD	0.0009	0.0007	0.0044	0.0039
	P (p ≤ 0.05)	0.0144	<0.0001	0.0021	<0.0001

Within seasons, treatments and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C; S0 = 0 weeks, S1 = 1 week at 15°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

**Table 41** The effect of the shelf life period at 15°C on the phenolic content (AU per berry) of Regal Seedless in 2002/03 and 2003/04.

Season	Treatment ^z	A280	A420	A365	A320
2003/03	W0	0.0004 a	-0.0006 a	0.0139 a	0.0088 a
	W4	-0.0037 a	0.0004 a	-0.0200 b	-0.0129 a
	W7	0.0018 a	-0.0001 a	0.0114 a	0.0107 a
	LSD ^y	0.0107	0.0042	0.0247	0.0305
	P (p ≤ 0.05) ^x	0.5642	0.8666	0.0325	0.2582
2003/04	W0	-0.0057 b	-0.0056 b	-0.0237 b	-0.0301 b
	W4	0.0077 a	0.0063 a	0.0183 ab	0.0315 a
	W7	0.0036 a	0.0068 a	0.0417 a	0.0524 a
	LSD	0.0083	0.0068	0.0437	0.0431
	P (p ≤ 0.05)	0.0110	0.0028	0.0232	0.0038

Within seasons and columns, values with different letters are significantly different at a 5% significance level.

^z W0 = 0 weeks, W4 = 4 weeks, W7 = 7 weeks at -0.5°C.

^y LSD = Least significant difference.

^x P = Probability of F-ratio test.

Table 42 The effect of cold storage duration with no shelf life on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
galocatehin	29.30 a	33.73 a	44.86 a	22.95	0.3554
gallodimer1A	309.66 ab	347.46 a	236.51 b	76.65	0.0228
gallodimer1	338.96 ab	381.19 a	281.37 b	73.50	0.0351
gallodimer2	37.46 ab	45.61 a	32.81 b	9.54	0.0358
procyanidinB1	18.42 a	24.27 a	nd	9.67	0.1894
catechin	nd ^x	28.15	nd		
dimer1	16.14 a	18.48 a	13.59 b	2.48	0.0038
dimer2	nd	nd	nd		
epicatechin	33.36 a	37.14 a	29.09 a	8.81	0.1839
procyanidinB2	25.72 b	35.90 a	22.61 b	8.53	0.0199
flavanol1	25.72 a	36.71 a	nd	17.96	0.1850
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	nd	25.05	nd		
flavanol3	nd	nd	nd		
dimer3	22.13	nd	nd		
dimermonogallate2	44.17 b	88.20 a	36.78 b	34.83	0.0121
flavanol4	nd	nd	nd		
epicatechingallate	nd	26.43 a	18.43 b	6.94	0.0303
dimerdigallate	31.91 a	37.97 a	21.51 b	8.11	0.0039
trimergallate	23.34 a	24.78 a	13.33 a	12.74	0.1369
flavanol5	26.76 a	24.81 a	nd	7.09	0.5276
flavanol6	nd	18.17	nd		
flavanol7	nd	nd	nd		
polymeric fraction	2010.50 b	4723.50 a	1684.40 b	1588.1	0.0017
total flavanols	2545.60 b	5551.60 a	2113.90 b	1700.2	0.0011
Flavonols					
q-3-rut ^w	5.02 a	8.74 a	5.42 a	3.74	0.0899
m-3-glc ^w	4.67 a	7.73 a	3.94 a	4.23	0.1110
q-3-gal ^w	18.01 b	41.41 a	18.52 b	14.09	0.0040
q-3-glc ^w	54.15 b	108.44 a	53.95 b	27.20	0.0007
k-3-gal ^w	5.67 b	8.98 a	3.78 b	2.94	0.0040
k-3-glc/gluc ^w	9.90 b	21.25 a	7.39 b	7.05	0.0016
q-3-rham ^w	30.62 b	50.02 a	30.13 b	10.73	0.0016
total flavonols	122.16 b	246.58 a	120.91 b	64.41	0.0010
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 43 The effect of cold storage duration and one week shelf life on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
galocatehin	29.49 b	25.05 b	96.63 a	12.19	<0.0001
gallodimer1A	283.02 a	252.38 a	148.19 b	50.43	0.0228
gallodimer1	312.51 a	277.43 ab	244.82 b	56.59	0.0671
gallodimer2	44.26 a	34.69 b	26.01 c	7.35	0.0004
procyanidinB1	22.97 a	18.31 ab	14.78 b	8.00	0.1002
catechin	21.56	nd ^x	nd		
dimer1	16.80 a	14.62 a	nd	4.36	0.2773
dimer2	nd	nd	nd		
epicatechin	36.50 a	30.67 a	21.81 b	8.52	0.0093
procyanidinB2	30.67 ab	36.06 a	27.20 b	8.59	0.1198
flavanol1	32.81 a	23.14 a	nd	15.14	0.1746
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	22.02 a	nd	18.43 a	4.14	0.0804
flavanol3	nd	nd	nd		
dimer3	nd	nd	18.27		
dimermonogallate2	61.51 a	63.97 a	83.28 a	29.76	0.2637
flavanol4	nd	nd	nd		
epicatechingallate	22.28 a	nd	23.87 a	8.65	0.6764
dimerdigallate	28.52 a	24.35 a	26.44 a	5.04	0.2716
trimergallate	25.93 a	21.71 ab	15.97 b	6.35	0.0155
flavanol5	22.36 a	19.34 a	nd	7.99	0.4019
flavanol6	16.15 a	16.35 a	13.32 a	3.92	0.2102
flavanol7	nd	nd	nd		
polymeric fraction	3202.50 a	2950.70 a	3409.00 a	1511.30	0.8133
total flavanols	3828.60 a	3492.80 a	3925.80 a	1640.30	0.8417
Flavonols					
q-3-rut ^w	7.95 a	3.83 b	4.36 b	3.41	0.0426
m-3-glc ^w	7.05 a	4.38 a	6.55 a	6.13	0.6665
q-3-gal ^w	32.36 ab	17.86 b	40.71 a	20.16	0.0809
q-3-glc ^w	90.00 a	56.43 b	90.52 a	30.72	0.0503
k-3-gal ^w	6.82 a	4.08 a	7.34 a	3.61	0.1517
k-3-glc/gluc ^w	14.55 a	8.50 a	16.96 a	9.94	0.2077
q-3-rham ^w	42.59 ab	32.60 b	44.50 a	11.33	0.0867
total flavonols	200.14 ab	124.75 b	209.85 a	80.45	0.0776
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 44 The effect of cold storage duration with no shelf life on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
galocatehin	4.98 a	5.61 a	8.89 a	4.31	0.1506
gallodimer1A	52.70 a	57.95 a	47.01 a	12.22	0.1958
gallodimer1	57.69 a	63.56 a	55.90 a	10.63	0.3034
gallodimer2	6.37 a	7.59 a	6.53 a	1.48	0.1959
procyanidinB1	2.92 a	4.04 a	nd	1.31	0.0818
catechin	nd ^x	4.42	nd		
dimer1	2.68 b	3.09 a	2.71 b	0.24	0.0032
dimer2	nd	nd	nd		
epicatechin	5.64 a	6.15 a	5.81 a	1.21	0.6700
procyanidinB2	4.33 b	5.96 a	4.49 b	1.20	0.0199
flavanol1	4.09 a	6.09 a	nd	2.39	0.0868
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	nd	4.16	nd		
flavanol3	nd	nd	nd		
dimer3	3.50	nd	nd		
dimermonogallate2	7.14 b	14.76 a	7.35 b	6.22	0.0287
flavanol4	nd	nd	nd		
epicatechingallate	nd	4.42 a	3.71 a	1.08	0.1611
dimerdigallate	5.05 b	6.35 a	4.29 b	1.01	0.0026
trimergallate	3.70 a	4.11 a	2.66 a	1.87	0.2115
flavanol5	4.24 a	4.14 a	nd	0.93	0.7961
flavanol6	nd	2.96	nd		
flavanol7	nd	nd	nd		
polymeric fraction	328.00 b	784.30 a	337.40 b	258.27	0.0024
total flavanols	418.10 b	922.10 a	422.80 b	269.25	0.0014
Flavonols					
q-3-rut ^w	0.85 b	1.43 a	1.08 ab	0.57	0.1122
m-3-glc ^w	0.79 a	1.27 a	0.78 a	0.65	0.1612
q-3-gal ^w	3.04 b	6.83 a	3.69 b	2.27	0.0063
q-3-glc ^w	9.10 b	17.95 a	10.75 b	3.82	0.0004
k-3-gal ^w	0.94 b	1.48 a	0.75 b	0.44	0.0053
k-3-glc/gluc ^w	1.68 b	3.50 a	1.47 b	1.09	0.0019
q-3-rham ^w	5.16 b	8.31 a	6.00 b	1.54	0.0015
total flavonols	20.55 b	40.78 a	24.08 b	9.43	0.0008
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 45 The effect of cold storage duration and one week shelf life on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
galocatehin	5.31 b	4.87 b	19.21 a	1.98	<0.0001
gallodimer1A	50.76 a	48.94 a	29.49 b	7.47	<0.0001
gallodimer1	56.07 a	53.82 a	48.70 a	8.44	0.1962
gallodimer2	8.02 a	6.75 a	5.18 b	1.45	0.0031
procyanidinB1	4.06 a	3.63 a	3.02 a	1.67	0.3631
catechin	3.95	nd ^x	nd		
dimer1	2.93 a	2.86 a	nd	0.66	0.8142
dimer2	nd	nd	nd		
epicatechin	6.54 a	5.95 a	4.36 b	1.43	0.0181
procyanidinB2	5.50 b	7.03 a	5.39 b	1.53	0.0672
flavanol1	5.83 a	4.57 a	nd	3.10	0.3679
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	3.87 a	nd	3.69 a	0.95	0.6649
flavanol3	nd	nd	nd		
dimer3	nd	nd	3.64		
dimermonogallate2	10.92 b	12.49 ab	16.65 a	5.48	0.1027
flavanol4	nd	nd	nd		
epicatechingallate	3.83 a	nd	4.78 a	1.80	0.2554
dimerdigallate	4.98 a	4.82 a	5.28 a	0.90	0.5029
trimergallate	4.59 a	4.26 ab	3.19 b	1.32	0.0815
flavanol5	3.94 a	3.81 a	nd	1.72	0.8599
flavanol6	2.78 a	3.13 a	2.76 a	0.77	0.4164
flavanol7	nd	nd	nd		
polymeric fraction	569.7 a	578.4 a	680.3 a	280.35	0.6536
total flavanols	681.50 a	683.90 a	783.40 a	302.28	0.7196
Flavonols					
q-3-rut ^w	1.45 a	0.75 b	0.88 ab	0.67	0.0941
m-3-glc ^w	1.29 a	0.88 a	1.32 a	1.19	0.7239
q-3-gal ^w	5.90 ab	3.52 b	8.18 a	4.03	0.0774
q-3-glc ^w	16.23 ab	11.04 b	18.11 a	5.99	0.0609
k-3-gal ^w	1.24 a	0.80 a	1.48 a	0.72	0.1659
k-3-glc/gluc ^w	2.65 a	1.68 a	3.42 a	2.00	0.2112
q-3-rham ^w	7.68 ab	6.37 b	8.89 a	2.20	0.0804
total flavonols	36.23 ab	24.45 b	42.06 a	15.98	0.0885
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 46 The effect of the shelf life period at 15°C on the on the phenolic content (µg/berry) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P (p ≤ 0.05) ^y
	0	4	7		
Flavanols					
galocatehin	0.19 b	-8.68 b	51.77 a	26.83	0.0005
gallodimer1A	-26.64 a	-95.08 a	-88.32 a	77.34	0.1497
gallodimer1	-26.45 a	-103.76 a	-36.56 a	78.06	0.1047
gallodimer2	6.81 a	-10.92 b	-6.80 b	12.54	0.0220
procyanidinB1	-2.44 a	-2.37 a	nd	30.68	0.9929
catechin	nd ^x	nd	nd		
dimer1	1.59 a	-4.29 b	nd	2.60	0.0015
dimer2	nd	nd	nd		
epicatechin	3.13 a	-6.47 b	-6.97 b	9.41	0.0597
procyanidinB2	4.94 a	0.16 a	4.59 a	11.61	0.6288
flavanol1	-3.72 a	-14.40 a	nd	47.79	0.5686
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	nd	nd	nd		
flavanol3	nd	nd	nd		
dimer3	nd	nd	nd		
dimermonogallate2	9.95 ab	-24.24 b	46.50 a	45.90	0.0144
flavanol4	nd	nd	nd		
epicatechingallate	nd	nd	-1.13		
dimerdigallate	3.19 a	-12.02 b	4.77 a	12.22	0.0162
trimergallate	-1.94 a	-4.18 a	1.56 a	24.86	0.7616
flavanol5	-7.06 a	-5.61 a	nd	26.79	0.8881
flavanol6	nd	-1.61	nd		
flavanol7	nd	nd	nd		
polymeric fraction	1192.00 a	-1773.00 b	1725.00 a	2405.5	0.0155
total flavanols	1283.00 a	-2059.00 b	1812.00 a	2584.8	0.0122
Flavonols					
q-3-rut ^w	2.93 a	-4.92 b	-0.73 ab	5.34	0.0193
m-3-glc ^w	2.38 a	-0.97 a	3.35 a	5.39	0.1871
q-3-gal ^w	14.35 a	-23.56 b	22.18 a	24.06	0.0023
q-3-glc ^w	35.85 a	-52.01 b	36.57 a	45.05	0.0009
k-3-gal ^w	0.03 a	-4.91 b	3.57 a	4.68	0.0035
k-3-glc/gluc ^w	5.14 a	-12.75 b	9.58 a	13.62	0.0067
q-3-rham ^w	11.97 a	-17.42 b	14.38 a	16.89	0.0017
total flavonols	77.98 a	-121.83 b	88.95 a	109.74	0.0013
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 47 The effect of the shelf life period at 15°C on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2002/03.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
galocatehin	0.32 b	-0.74 b	10.32 a	5.13	0.0006
gallodimer1A	-1.94 a	-9.01 ab	-17.52 b	13.11	0.0687
gallodimer1	-1.62 a	-9.75 a	-7.20 a	12.75	0.4030
gallodimer2	1.65 a	-0.84 b	-1.35 b	2.14	0.0206
procyanidinB1	-0.31 a	0.05 a	nd	5.49	0.8053
catechin	nd ^x	nd	nd		
dimer1	0.29 a	-0.28 a	nd	0.70	0.0929
dimer2	nd	nd	nd		
epicatechin	0.89 a	-0.19 ab	-1.43 b	1.36	0.0101
procyanidinB2	1.16 a	1.06 a	0.91 a	1.87	0.9574
flavanol1	-0.48 a	-1.61 a	nd	7.80	0.7067
flavanol2	nd	nd	nd		
trimer1	nd	nd	nd		
dimermonogallate1	nd	nd	nd		
flavanol3	nd	nd	nd		
dimer3	nd	nd	nd		
dimermonogallate2	2.35 ab	-2.27 b	9.30 a	8.27	0.0249
flavanol4	nd	nd	nd		
epicatechingallate	nd	nd	-0.23		
dimerdigallate	0.70 ab	-1.41 b	1.00 a	2.16	0.0338
trimergallate	-0.20 a	-0.01 a	0.31 a	4.25	0.9549
flavanol5	-1.04 a	-0.32 a	nd	4.78	0.6973
flavanol6	nd	0.29	nd		
flavanol7	nd	nd	nd		
polymeric fraction	241.6 a	-205.9 b	342.9 a	418.32	0.0308
total flavanols	263.4 a	-238.2 b	360.6 a	444.26	0.0252
Flavonols					
q-3-rut ^w	0.60 a	-0.69 b	-0.14 ab	0.90	0.0224
m-3-glc ^w	0.54 a	-0.05 a	0.68 a	0.99	0.2239
q-3-gal ^w	2.86 a	-3.31 b	4.50 a	4.29	0.0036
q-3-glc ^w	7.36 a	7.13 a	-6.91 b	7.56	0.0013
k-3-gal ^w	0.11 ab	-0.68 b	0.73 a	0.82	0.0053
k-3-glc/gluc ^w	1.12 a	-1.82 b	1.95 a	2.46	0.0110
q-3-rham ^w	2.53 a	-1.95 b	2.89 a	2.80	0.0035
total flavonols	15.68 a	-16.33 b	17.98 a	18.74	0.0021
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	nd	nd	nd		

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 48 The effect of cold storage duration with no shelf life on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	636.83 a	632.61 ab	573.67 b	59.91	0.0841
gallodimer2	44.66 a	41.41 a	44.65 a	7.61	0.5598
procyanidinB1	57.96 a	65.36 a	53.73 a	17.18	0.3594
catechin	nd ^x	nd	nd		
dimer1	24.64 a	23.59 a	27.02 a	4.78	0.3255
dimer2	19.62 a	21.03 a	22.92 a	7.42	0.6412
epicatechin	nd	17.79 a	17.23 a	6.75	0.7556
procyanidinB2	45.89 a	43.49 a	53.55 a	12.78	0.2579
flavanol1	nd	22.49 a	17.08 a	7.11	0.1021
flavanol2	27.90 a	36.34 a	23.02 a	15.63	0.2141
trimer1	nd	16.02	nd		
dimermonogallate1	36.28 a	36.09 a	27.96 a	9.99	0.1772
flavanol3	nd	20.60	nd		
dimer3	19.94 a	nd	17.29 b	0.94	0.0068
dimermonogallate2	82.00 b	130.01 a	69.78 b	37.97	0.0087
flavanol4	nd	nd	nd		
epicatechingallate	32.90 a	31.59 a	20.81 a	21.71	0.4231
dimerdigallate	23.92 ab	31.84 a	18.65 b	10.01	0.0407
trimergallate	nd	22.79	nd		
flavanol5	nd	nd	nd		
flavanol6	nd	15.93	nd		
flavanol7	nd	nd	nd		
polymeric fraction	4519.80 b	6030.20 a	3604.90 b	1412.50	0.0101
total flavanols	5503.00 b	7191.80 a	4551.4 b	1507.20	0.0086
Flavonols					
q-3-rut ^w	9.00 a	6.92 a	7.59 a	2.81	0.2679
m-3-glc ^w	7.91 a	6.56 a	4.96 a	3.04	0.1628
q-3-gal ^w	45.25 a	51.54 a	35.24 a	22.05	0.3162
q-3-glc ^w	112.98 a	115.34 a	87.82 a	34.42	0.2158
k-3-gal ^w	9.39 a	10.19 a	6.58 a	4.36	0.2256
k-3-glc/gluc ^w	21.59 ab	28.09 a	14.51 b	13.53	0.1410
q-3-rham ^w	57.68 a	54.58 a	44.43 a	15.26	0.1998
total flavonols	262.23 ab	273.21 a	172.94 b	97.83	0.0878
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	3.76 a	2.12 b	1.58 b	1.04	0.0018

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 49 The effect of cold storage duration and one week shelf life on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	619.97 a	608.49 a	586.32 a	82.96	0.6862
gallodimer2	42.33 a	43.05 a	44.73 a	6.47	0.7248
procyanidinB1	73.60 b	40.89 c	87.71 a	11.39	<0.0001
catechin	nd ^x	nd	nd		
dimer1	23.42 b	26.54 a	26.19 ab	3.03	0.0875
dimer2	29.83 a	28.41 a	25.11 a	10.36	0.5939
epicatechin	22.03 a	17.94 a	19.17 a	4.62	0.2152
procyanidinB2	47.63 b	55.25 ab	62.62 a	9.73	0.0172
flavanol1	22.47 b	18.36 c	27.41 a	3.93	0.0012
flavanol2	37.61 b	22.95 c	61.22 a	6.83	<0.0001
trimer1	19.64 a	nd	21.34 a	5.28	0.4610
dimermonogallate1	46.18 a	24.71 b	51.44 a	11.72	0.0007
flavanol3	21.95 b	nd	33.59 a	5.37	0.0007
dimer3	nd	16.22	nd		
dimermonogallate2	154.65 a	56.96 b	150.71 a	23.77	<0.0001
flavanol4	21.51 a	nd	20.47 a	3.46	0.5002
epicatechingallate	37.26 a	nd	48.07 a	18.78	0.2285
dimerdigallate	37.06 a	16.69 b	45.57 a	10.83	0.0005
trimergallate	24.75 b	17.38 b	34.69 a	9.90	0.0083
flavanol5	nd	nd	nd		
flavanol6	17.66 a	nd	21.90 a	6.25	0.1480
flavanol7	nd	nd	nd		
polymeric fraction	6293.80 b	3367.30 c	7627.50 a	901.88	<0.0001
total flavanols	7542.60 b	4317.20 c	8980.30 a	897.23	<0.0001
Flavonols					
q-3-rut ^w	8.09 a	6.93 a	8.04 a	1.79	0.3226
m-3-glc ^w	5.73 a	5.06 a	7.61 a	2.63	0.1325
q-3-gal ^w	41.18 b	40.51 b	72.29 a	20.93	0.0078
q-3-glc ^w	113.44 b	89.98 b	145.10 a	23.60	0.0006
k-3-gal ^w	8.74 ab	7.75 b	10.64 a	2.76	0.1104
k-3-glc/gluc ^w	20.86 a	20.72 a	24.99 a	9.14	0.5412
q-3-rham ^w	55.91 ab	46.94 b	60.42 a	9.65	0.0278
total flavonols	253.94 b	217.04 b	329.07 a	65.08	0.0071
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	3.37 a	1.86 b	4.04 a	0.95	0.0009

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 50 The effect of cold storage duration with no shelf life on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	104.15 a	110.84 a	101.48 a	16.15	0.4477
gallodimer2	7.27 a	7.22 a	7.88 a	1.08	0.3792
procyanidinB1	9.44 a	11.44 a	9.41 a	2.79	<0.0001
catechin	nd ^x	nd	nd		
dimer1	4.02 b	4.06 ab	4.78 a	0.74	0.0835
dimer2	3.23 a	3.70 a	4.05 a	1.46	0.4971
epicatechin	nd	3.29 a	3.05 a	0.87	0.3595
procyanidinB2	7.49 b	7.51 b	9.44 a	1.85	0.0750
flavanol1	nd	3.92 a	3.00 a	1.68	0.2045
flavanol2	4.51 a	6.34 a	4.04 a	2.38	0.1219
trimer1	nd	2.80	nd		
dimermonogallate1	5.94 a	6.28 a	4.89 a	1.63	0.2105
flavanol3	nd	3.52	nd		
dimer3	3.41 a	nd	2.92 b	0.45	0.0420
dimermonogallate2	13.20 b	22.81 a	12.24 b	6.13	0.0041
flavanol4	nd	nd	nd		
epicatechingallate	5.12 a	5.57 a	3.68 a	3.86	0.4854
dimerdigallate	3.86 b	5.60 a	3.23 b	1.74	0.0274
trimergallate	nd	4.00	nd		
flavanol5	nd	nd	nd		
flavanol6	nd	2.90	nd		
flavanol7	nd	nd	nd		
polymeric fraction	730.25 b	1055.95 a	632.55 b	210.91	0.0025
total flavanols	890.70 b	1259.40 a	799.50 b	224.60	0.0020
Flavonols					
q-3-rut ^w	1.47 a	1.21 a	1.33 a	0.43	0.4091
m-3-glc ^w	1.29 a	1.13 a	0.87 a	0.46	0.1926
q-3-gal ^w	7.36 a	8.95 a	6.22 a	3.59	0.2907
q-3-glc ^w	18.36 a	20.11 a	15.44 a	5.27	0.2032
k-3-gal ^w	1.53 a	1.77 a	1.16 a	0.70	0.2102
k-3-glc/glc ^w	3.50 ab	4.90 a	2.56 b	2.28	0.1243
q-3-rham ^w	9.37 a	9.51 a	7.80 a	2.26	0.2467
total flavonols	42.62 ab	47.57 a	30.23 b	16.04	0.0911
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	0.62 a	0.37 b	0.28 b	0.19	0.0100

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/glc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 51 The effect of cold storage duration and one week shelf life on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	103.12 a	101.58 a	97.84 a	13.05	0.6816
gallodimer2	7.04 a	7.16 a	7.50 a	1.11	0.6660
procyanidinB1	12.29 b	6.80 c	14.65 a	1.96	<0.0001
catechin	nd ^x	nd	nd		
dimer1	3.91 b	4.42 a	4.36 a	0.43	0.0440
dimer2	4.91 a	4.73 a	4.19 a	1.65	0.6050
epicatechin	3.62 a	2.99 a	3.20 a	0.70	0.2119
procyanidinB2	7.90 b	9.22 ab	10.48 a	1.59	0.0125
flavanol1	3.74 b	2.99 c	4.59 a	0.69	0.0011
flavanol2	6.28 b	3.79 c	10.24 a	1.17	<0.0001
trimer1	3.33 a	nd	3.57 a	0.96	0.5543
dimermonogallate1	7.67 a	4.16 b	8.63 a	2.01	0.0009
flavanol3	3.65 b	nd	5.62 a	0.90	0.0006
dimer3	nd	2.70	nd		
dimermonogallate2	25.73 a	9.50 b	25.26 a	4.29	<0.0001
flavanol4	3.56 a	nd	3.39 a	0.60	0.5056
epicatechingallate	6.23 a	nd	7.95 a	2.90	0.2161
dimerdigallate	6.17 a	2.77 b	7.57 a	1.53	0.0001
trimergallate	4.13 ab	2.90 b	5.81 a	1.72	0.0101
flavanol5	nd	nd	nd		
flavanol6	2.84 a	nd	3.65 a	0.97	0.0851
flavanol7	nd	nd	nd		
polymeric fraction	1049.51 b	559.65 c	1272.90 a	139.39	<0.0001
total flavanols	1257.27 b	718.05 c	1498.89 a	136.05	<0.0001
Flavonols					
q-3-rut ^w	1.34 a	1.16 a	1.35 a	0.32	0.3651
m-3-glc ^w	0.95 a	0.85 a	1.27 a	0.45	0.1388
q-3-gal ^w	6.85 b	6.81 b	12.07 a	3.50	0.0081
q-3-glc ^w	18.88 b	15.05 b	24.36 a	4.46	0.0018
k-3-gal ^w	1.45 ab	1.30 b	1.77 a	0.46	0.1181
k-3-glc/gluc ^w	3.47 a	3.50 a	4.16 a	1.53	0.5687
q-3-rham ^w	9.31 ab	7.84 b	10.13 a	1.75	0.0417
total flavonols	42.26 b	36.37 b	55.10 a	11.47	0.0101
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	0.56 a	0.31 b	0.67 a	0.15	0.0004

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 52 The effect of the shelf life period at 15°C on the phenolic content ($\mu\text{g}/\text{berry}$) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	1.84 a	-39.85 a	19.98 a	118.93	0.5435
gallodimer2	-1.67 a	2.37 a	-0.13 a	13.65	0.7961
procyanidinB1	14.62 b	-25.71 c	32.75 a	17.84	<0.0001
catechin	nd ^x	nd	nd		
dimer1	-1.53 a	3.05 a	-0.70 a	6.55	0.2988
dimer2	11.85 a	5.68 a	1.90 a	15.19	0.4203
epicatechin	nd	-1.78 a	3.31 a	41.40	0.3627
procyanidinB2	4.49 a	11.91 a	6.13 a	17.74	0.6195
flavanol1	nd	-4.84 a	7.58 a	17.93	0.0966
flavanol2	8.16 b	-12.58 c	37.33 a	18.47	0.0004
trimer1	nd	nd	nd		
dimermonogallate1	12.67 a	-13.17 b	23.99 a	20.44	0.0081
flavanol3	nd	nd	nd		
dimer3	nd	nd	nd		
dimermonogallate2	80.30 a	-71.14 b	86.98 a	56.00	0.0001
flavanol4	nd	nd	nd		
epicatechingallate	-0.59 a	nd	15.97 a	85.43	0.4920
dimerdigallate	11.52 b	-11.85 c	27.38 a	14.38	0.0024
trimergallate	nd	-6.50	nd		
flavanol5	nd	nd	nd		
flavanol6	nd	nd	nd		
flavanol7	nd	nd	nd		
polymeric fraction	1668.50 b	-2575.70 c	3854.90 a	1263.40	<0.0001
total flavanols	1960.70 b	-2812.50 c	4277.80 a	1350.70	<0.0001
Flavonols					
q-3-rut ^w	-0.96 a	-0.24 a	0.90 a	4.57	0.6860
m-3-glc ^w	-2.48 b	-3.10 b	3.24 a	4.39	0.0179
q-3-gal ^w	-5.03 b	-16.14 b	43.92 a	33.59	0.0067
q-3-glc ^w	-1.73 b	-31.44 b	67.46 a	46.55	0.0025
k-3-gal ^w	-0.76 b	-3.23 b	4.67 a	5.19	0.0217
k-3-glc/gluc ^w	-1.47 ab	-10.07 b	11.70 a	16.41	0.0462
q-3-rham ^w	-1.85 b	-10.26 b	20.10 a	20.66	0.0252
total flavonols	-12.49 b	-74.53 b	160.43 a	111.45	0.0016
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	-0.19 b	-0.08 b	2.58 a	1.76	0.0201

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.

Table 53 The effect of the shelf life period at 15°C on the phenolic content ($\mu\text{g}/\text{gram}$ berry weight) of Regal Seedless in 2003/04.

Phenolic compounds	Week			LSD ^z	P ($p \leq 0.05$) ^y
	0	4	7		
Flavanols					
gallodimer1	1.71 a	-11.88 a	-1.86 a	22.27	0.3860
gallodimer2	-0.14 a	0.05 a	-0.35 a	2.01	0.9096
procyanidinB1	2.64 a	-4.84 b	5.19 a	3.22	<0.0001
catechin	nd ^x	nd	nd		
dimer1	-0.18 a	0.45 a	-0.38 a	1.10	0.2774
dimer2	1.80 a	0.74 a	0.12 a	2.52	0.4079
epicatechin	nd	-0.66 a	0.48 a	8.70	0.3451
procyanidinB2	0.84 a	1.74 a	0.62 a	2.72	0.6381
flavanol1	nd	-1.04 a	1.05 a	4.11	0.1609
flavanol2	1.49 b	-2.42 c	6.12 a	3.00	0.0003
trimer1	nd	nd	nd		
dimermonogallate1	2.17 a	-2.38 b	3.90 a	3.29	0.0056
flavanol3	nd	nd	nd		
dimer3	nd	nd	nd		
dimermonogallate2	13.58 a	-12.99 b	14.23 a	10.39	0.0002
flavanol4	nd	nd	nd		
epicatechingallate	0.26 a	nd	2.61 a	13.77	0.5389
dimerdigallate	2.09 b	-2.22 c	4.47 a	2.36	0.0020
trimergallate	nd	-1.53	nd		
flavanol5	nd	nd	nd		
flavanol6	nd	nd	nd		
flavanol7	nd	nd	nd		
polymeric fraction	297.41 b	-482.18 c	622.21 a	218.07	<0.0001
total flavanols	348.40 b	-531.40 c	685.60 a	234.37	<0.0001
Flavonols					
q-3-rut ^w	-0.14 a	-0.09 a	0.11 a	0.74	0.7615
m-3-glc ^w	-0.40 b	-0.54 b	0.51 a	0.70	0.0154
q-3-gal ^w	-0.70 b	-2.98 b	7.09 a	5.59	0.0069
q-3-glc ^w	0.08 b	-6.07 b	10.80 a	7.83	0.0024
k-3-gal ^w	-0.10 ab	-0.60 b	0.73 a	0.87	0.0224
k-3-glc/gluc ^w	-0.18 ab	-1.84 b	1.83 a	2.84	0.0530
q-3-rham ^w	-0.12 ab	-2.10 b	3.08 a	3.37	0.0215
total flavonols	-1.26 b	-14.24 b	26.26 a	18.78	0.0160
Benzoic acids					
benzoic acid1	nd	nd	nd		
Cinnamic acids					
cinnamic acid1	nd	nd	nd		
cinnamic acid2	-0.02 b	-0.02 b	0.41 a	0.29	0.0228

Within rows, values with different letters are significantly different at a 5% significance level.

^z LSD = Least significant difference.

^y P = Probability of F-ratio test.

^x nd = not detected.

^w q-3-rut = quercetin-3-rutinoside; m-3-glc = myricetin-3-glucoside; q-3-gal = quercetin-3-galactoside; q-3-glc = quercetin-3-glucoside; k-3-gal = kaempferol-3-galactoside; k-3-glc/gluc = kaempferol-3-glucoside/glucuronide; q-3-rham = quercetin-3-rhamnoside.