PRE- AND POST HARVEST FACTORS INFLUENCING THE EATING QUALITY OF SELECTED NECTARINE (Prunus persica (L.) Batsch) CULTIVARS

By

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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.
Pectura roburant cultus recti

Date

Signature

SUMMARY

Fruit quality, and especially eating quality, of nectarines has become very important to markets and consumers in recent years. Pre- and post harvest factors that influence the eating quality of nectarines were studied to optimise fruit quality at harvest and to maintain this quality during export. This will ensure good returns for a producer and will maximise his profit.

The influence of the variables canopy position, initial fruit size and bearing position was studied to determine the variation in fruit quality within a nectarine tree. 'Red Jewel' and 'Ruby Diamond' fruit from the upper part of the tree canopy had significantly higher total soluble solids (TSS). Fruit position on the shoot does not seem to play a significant role in fruit quality for 'Red Jewel' nectarines, which will allow producers to leave more than one fruit per bearer if necessary. Fruit thinning is an important means to improve fruit size and quality in 'Red Jewel', but poor thinning can cause extreme variability in size and quality. Fruit that were small at thinning remained significantly smaller, weighed less, had lower sugars and higher acids at harvest. If it is possible to reduce the variation in size at thinning, fruit will be much more homogenous at harvest.

The effect of pre-conditioning (PC) prior to storage and controlled atmosphere (CA) storage was evaluated on 'Red Jewel' and 'Spring Bright' nectarines. Free juice percentage was determined at the end of a simulated export protocol. The severity of woolliness differed between the two seasons for both nectarine cultivars. PC, to a firmness of 6 kg, followed by regular atmosphere (RA) storage increased percentage free juice significantly in 'Spring Bright' and 'Red Jewel' nectarines. However, a PC protocol for each cultivar and each producer must be determined beforehand to ensure fruit quality. CA storage is another technique that can be used to prevent the development of chilling injury (CI) symptoms. Both 'Spring Bright' and 'Red Jewel' showed an increase in percentage free juice with the use of CA storage during both seasons.

The eating quality of nectarines depends on the composition of the individual sugars and organic acids and the ratio between them. Sucrose, fructose, glucose and sorbitol were found to be the major sugars in all evaluated nectarine cultivars. Sucrose was the dominant sugar in all cultivars at optimum maturity. The three main organic acids in nectarine cultivars were malic, citric and quinic acid, with malic acid being dominant at optimum maturity. Small amounts of shikimic, fumaric and succinic acid were also observed. It was evident that cultivars differ in the composition of sugar and organic acids at optimum maturity, especially the standard acid cultivars and the new low-acid cultivars. Individual sugars and organic acids in cultivars also differ in how they react during storage.



OPSOMMING

VOOR- EN NAOES FAKTORE WAT DIE EETKWALITEIT VAN GESELEKTEERDE NEKTARIEN (*Prunus persica* (L.) Batsch) CULTIVARS BEÏVLOED

Vrugkwaliteit, en in besonder eetkwaliteit, van nektariens het baie belangrik geword vir die hedendaagse mark en verbruiker. Voor- en naoes faktore wat die eetkwaliteit van nektariens kan beïnvloed, is ondersoek om die vrugkwaliteit by oes te optimaliseer en om hierdie kwaliteit te handhaaf tydens die uitvoerproses. Dit sal bydrae daartoe dat die produsent 'n goeie opbrengs verdien en maksimum wins genereer.

The invloed van die veranderlikes nl. aanvanklike vruggrootte, posisie in die boom en draposisie is ondersoek om die variasie in vrugkwaliteit te bepaal. 'Red Jewel' en 'Ruby Diamond' nektatriens van die boomste deel van die boom het hoër suikervlakke gehad as vrugte onder in die boom. Draposisie het nie 'n betekenisvolle invloed op vrugkwaliteit van 'Red Jewel' gehad nie, wat produsente sal toelaat om meer as een vrug per draeenheid te los, indien nodig. Vruguitdunning is 'n belangrike manier om vrugkwaliteit en –grootte van 'Red Jewel' nektariens te verbeter, maar swak uitdunning kan lei tot groot variasie in vrugkwaliteit en –grootte. Vrugte wat klein was tydens handuitdunning, het klein gebly, minder geweeg en laer suikers met hoër sure gehad tydens oes. As dit moontlik is om die variasie in vruggrootte te verminder tydens handuitdunning, sal vrugte baie meer homogeen wees tydens oes.

Die effek van hoë temperatuur kondisionering (HTK) voor opberging en beheerde atmosfeer (BA) opberging is geëvalueer op 'Red Jewel' en 'Spring Bright' nektariens. Vrysap persentasie is bepaal aan die einde van 'n gesimuleerde uitvoer protokol. The graad van voosheid het verskil vir die twee seisoene vir beide cultivars. HTK tot 'n fermheid van 6 kg, gevolg deur gewone atmosfeer (GA) opberging, het persentasie

vrysap betekenisvol vir 'Spring Bright' en 'Red Jewel' vermeerder. Nietemin, a HTK protokol vir elke cultivar en produsent moet vooraf bepaal word om vrugkwaliteit te verseker. BA opberging is 'n alternatiewe metode om die ontwikkeling van simptome van koueskade (KS) te voorkom. In beide seisoene het 'Spring Bright' en 'Red Jewel' nektariens 'n toename in persentasie vrysap getoon met die gebruik van BA opberging.

Die eetkwaliteit van nektariens word ook beïnvloed deur die samestelling en verhouding van die individuele suikers en organiese sure. Sukrose, fruktose, glukose en sorbitol is die vier hoof suikers in die geëvalueerde cultivars. Sukrose is die dominante suiker in alle cultivars tydens optimum plukrypheid. Die drie hoof organiese sure in nektariens is malaat-, sitraat-, en quinaatsuur, met malaatsuur as die dominante suur tydens optimum plukrypheid. Baie klein hoeveelhede van shikimaat-, fumaraat-, en suksinaatsuur is ook in alle nektarienmonsters waargeneem. Dit was ooglopend dat cultivars verskil in die samestelling en ontwikkeling van individuele suikers en organiese sure, veral die normale hoë-suur cultivars en die nuwe lae-suur cultivars. Die suikers en organiese sure van cultivars verander ook verskillend tydens gesimuleerde uitvoer.

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INTRODUCTION

The increase in hectares of nectarines in South Africa in recent years includes new cultivars with different growth and fruiting characteristics, more full red fruit colour and better eating quality than the existing older cultivars. Imported cultivars are also often not as well adapted to the South African climate, soil and water conditions as locally bred cultivars. Nectarines grown in South Africa are mostly earmarked for the export market and thus necessitate strict adherence to export standards. To ensure superior fruit quality in overseas markets, special attention must be given to all aspects of production, picking, packing and shipping of these nectarine cultivars.

The aim of the first paper was to determine what causes the variation in fruit quality within 'Red Jewel' and 'Ruby Diamond' nectarine trees and to aid growers in some production aspects and picking. The effect of canopy position, initial fruit size and bearing position on fruit quality characteristics were tested. Dann and Jerie (1988), Forlani et al. (2002) and Luchsinger et al. (2002) reported that fruit from the top of the tree canopy was bigger and had higher TSS levels than fruit from the bottom of the canopy. Fruit thinning can cause extreme variability in fruit size and quality (Giacalone et al., 2002). Corelli-Grappadelli and Coston (1991) and Marini and Sowers (1994) suggested that fruit size, fruit mass and TSS are influenced by crop density and length of the bearing shoot, but not position on the shoot.

The second paper was aimed at determining if post harvest conditioning treatments or controlled atmosphere (CA) storage or the combination of these, can alleviate and prevent the symptoms of chilling injury (CI) of 'Red Jewel' and 'Spring Bright' nectarines. These symptoms are a result of low temperature storage (-0.5°C) for extended periods, but the symptoms only appear once the fruit is ripened after storage (Crisosto et al., 1999). The CI is then manifested as a lack of free juice also known as mealiness or woolliness (Von Mollendorf et al., 1992). Various authors (Truter et al., 1993; Combrink and Visagie, 1997; Zhou et al., 2000; Choi and Lee, 2001) have shown

that pre-conditioning (PC) can alleviate the onset of woolliness in nectarines. CA storage, with elevated CO_2 and reduced O_2 concentrations, is also used with great success in various parts of the world to prevent the development of CI symptoms (Lurie, 1992; Truter and Combrink, 1992; Zhou et al., 2000; Choi and Lee, 2001).

The aim of the third paper was to identify and quantify the individual sugar and organic acids in nectarine cultivars during maturation and storage. The composition of sugars and organic acids has a direct effect on the eating quality of nectarines (Esti et al., 1997). The dominant sugars in nectarines and peaches are sucrose, glucose, fructose and sorbitol (Robertson et al., 1990; Génard et al., 1999; Wu et al., 2005). The three major non-volatile organic acids are malic, citric and quinic acid (Génard et al., 1999; Wu et al., 2002). The difference between normal high-acid cultivars and a new low-acid cultivar was also evaluated.

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Literature review: composition of sugars and organic acids in peach and nectarine fruit

1.1 INTRODUCTION

Fruit producers around the world are constantly trying to satisfy markets and consumers by producing outstanding fruit quality. In doing so, the producer ensures a maximum return for his product and eventually earns good profits. While trying to achieve this, they need to know exactly what the specific markets demand in terms of fruit quality specifications. Appearance and eating quality are two of the most important factors influencing consumer acceptance of a product. Bruhn (1995) concluded that with peaches and nectarines, consumers prefer full red coloured fruit. Fruit must be appealing to the eye, but the taste must also encourage a consumer to come back and buy more.

In stone fruit, where the turnover of new cultivars are very rapid, a producer needs to have access to the latest and most sought after cultivars. Consistent high yield, good fruit size, sufficient colour development, firmness and resistance to the most common pathogens are only some of the characteristics of new nectarine cultivars (Dirlewanger et al., 1999). A producer can distinguish himself from the competition with new and superior cultivars in an industry where there is an over production of stone fruit around the world. In the past few years the eating quality of fruit, and especially stone fruit, has become a very important quality trait (Crisosto et al., 2002). Demand is for juicy, melting fruit, but the flavour and taste components are also very important.

Organic compounds, i.e. acids, sugar and pectins, and the composition of these have become an important quality trait (Selli and Sansavini, 1995). According to Chapman and Horvat (1990), fruit taste and equally important, flavour properties are largely defined by the composition of these compounds as well as the sugar: acid ratio. In order to improve the organoleptic quality of peach and nectarine cultivars according to consumer acceptance, breeding programmes started to focus on producing genotypes with excellent taste, high sugar levels, and balanced sugar: acid ratios (Esti et al., 1997).

1.2 SUGARS

After water (85 – 90%), sugars are the next most abundant constituent of peaches and nectarines (Wills et al., 1983). Sugar content of peaches and nectarines may vary considerably with cultivar, maturity and environmental conditions (Crisosto et al., 1997). According to Génard and Souty (1996) the eating quality of peaches and nectarines to a great extent depends on the sweetness, which is related to total sugar content. Crisosto et al. (2002) concluded during an 'in-store' consumer acceptance trial for 'Elegant Lady' peach and 'Spring Bright' nectarine, that consumer acceptance is significantly affected by ripe soluble solids content (RSSC). Later studies by Crisosto and Crisosto (2005) indicate that consumer acceptance reaches a plateau for the two acidic cultivars 'Elegant Lady' and 'Spring Bright' at a RSSC level of 11 – 12 %, but for the low-acid cultivars 'Ivory Princess' and 'Honey Kist' consumer acceptance increases as RSSC increases without reaching a plateau. These low-acid peach ('Ivory Princess') and nectarine ('Honey Kist') cultivars achieved 100 % acceptance with RSSC of 16 and 15 %, respectively.

Soluble solids content is directly influenced by the composition of the individual sugars. In most rosaceous plants the main sugars are sucrose, fructose, glucose, and sorbitol (Brady, 1993). In peaches and nectarines sucrose is dominant at maturity, followed by the reducing sugars (glucose and fructose) and then sorbitol (Deshpande and Salunke, 1964; Sweeney et al., 1970; Chapman and Horvat, 1990; Moriguchi et al., 1990; Robertson et al., 1990; Génard et al., 1999; Wu et al., 2005). Very small amounts of maltose, galactose, and xylose have also been reported by Wrolstad and Shallenberger (1981) and Chapman and Horvat (1990).

1.2.1 Sucrose

Sucrose is, at maturity, by far the prevailing soluble sugar in peaches and nectarines (Deshpande and Salunkhe, 1964; Moriguchi et al., 1990; Esti et al., 1997). Sucrose content in immature peach fruit is very low and then it rapidly increases to account for up to 40 to 85% of soluble carbohydrates at maturity (Bassi and Selli, 1990; Liverani and Cangini 1991; Vizzotto et al., 1996; Dirlewanger et al., 1999).

Selli and Sansavini (1995) found that fruit are rich in soluble solids by the end of pit hardening, and thereafter sucrose steadily rises in the last 20 days prior to harvest. Chapman and Horvat (1990) reported a sigmoidal increase in sucrose from 95 to 109 DAFB (days after full bloom), followed by little change between 109 and 123 DAFB. A second sigmoidal increase occurs 123 to 137 DAFB. This pattern in sucrose levels during maturation closely resembles the double-sigmoidal fruit growth curves observed in most stone fruit (Romani and Jennings, 1971).

Moriguchi et al. (1990) found that sucrose levels in 'Hakuto' peach remain constantly low during the immature stage of development. As fruit matures, sucrose rises rapidly and becomes the major component (70%) of total accumulated sugars in ripe fruit. The significant increase in sucrose levels is accompanied by a rapid increase in sucrose synthase (SS) activity. Hubbard et al. (1991) reported a 5-fold increase in SS activity and a slight increase in sucrose phosphate synthase (SPS), with the increase in sucrose levels near maturity. SPS was also detected in peach flesh extracts by Morighuchi et al. (1990), but the activities were low throughout development. They concluded that the accumulation and biosynthesis of sucrose in peach fruit depends on SS.

Vizzotto et al. (1996) also reported a rapid increase in sucrose levels in stage III of fruit growth, but found no significant increase in SS activity and the SPS activity remained lower than those reported by Moriguchi et al. (1990) and Hubbard et al. (1991). These results of Vizzotto et al. (1996) suggest that the increase of sucrose in the

mesocarp of developing peach fruit might be as a result of the disappearance of hydrolytic enzyme activities.

Génard and Souty (1996) reported that the phloem sap of peach trees contains sucrose and sorbitol in almost equal quantities as the only sugars in the phloem. Translocated sucrose and sorbitol could be taken up by the mesocarp tissue by an apoplastic and/or symplastic route of phloem unloading, due to the presence of plasmodesmata (Masia et al., 1992). Sucrose can arrive via the phloem into the fruit, be hydrolyzed by acid invertase into glucose and fructose, or be synthesised by sucrose phosphate synthase from glucose and fructose. Sucrose synthase is a reversible enzyme, because it is involved in synthesis and hydrolysis. Hubbard et al. (1991) found that the balance between sucrose hydrolysis and synthesis in the fruit is probably in the favour of hydrolysis, because glucose and fructose are absent from the phloem sap. As the enzymes involved in sucrose hydrolysis and synthesis fluctuates during fruit growth, the balance between sucrose and reducing sugars shifts towards sucrose (Génard and Souty, 1996). These results are in agreement with those of Vizzotto et al. (1996), who showed that sucrose hydrolysing enzymes decline rapidly with accumulation of sucrose, without a rise of enzymes involved in synthetic activities.

Génard et al. (1999) investigated the correlation between peach fruit growth and the sugar content. They divided fruit growth into two phases: pit growth as the first stage and flesh growth as the second. Fruit growing intensively during the second phase had the highest sucrose and the lowest reducing sugars (glucose and fructose) concentration at harvest. They concluded that fruit growth curves are an indicator of fruit composition at harvest and that the accumulation of sucrose in the fruit is proportional to the assimilate supply to the fruit.

Sucrose plays an important role in the taste of peach and nectarine fruit. Pangborn (1963) investigated the relative taste intensities of different sugars in comparison to sucrose. In terms of sweetness, if sucrose is rated 1, then fructose is rated 1.75 and

glucose 0.75. Because sucrose is the main sugar at harvest and fructose the sweetest, the composition of these two sugars will dominate the taste of nectarine and peach fruit.

Fig. 1: The structure of a sucrose molecule (Drawn from Matthews and Van Holde, 1990)

1.2.2 Reducing sugars - glucose and fructose

Of the reducing sugars in peaches and nectarines, glucose is usually the most abundant at maturity (Wrolstad and Shallenberger, 1981; Meredith et al., 1989; Bassi and Selli, 1990, Selli and Sansavini, 1995). However, some researchers have reported glucose and fructose in comparable amounts (Chapman and Horvat, 1990; Wu et al., 2005), and sometimes higher fructose levels (Robertson et al., 1990; Byrne et al., 1991; Esti et al., 1997; Versari et al., 2002; Wu et al., 2003).

In immature fruit, glucose and fructose are normally the dominant sugars (Moriguchi et al., 1990; Hubbard et al., 1991; Liverani and Cangini, 1991; Vizzotto et al., 1996), but Brooks et al. (1993) reported that sucrose is the major sugar in peaches and nectarines at all stages of maturity.

Selli and Sansavini (1995) found that the initial high glucose and fructose levels start to decline over the last 20 days prior to harvest. This decrease in reducing sugars seems to correlate with the biosynthesis of sucrose as the fruit matures. Glucose and fructose drop to less than 1.5% of fruit fresh weight. Liverani and Cangini (1991) found that the

reducing sugars in four Italian grown peaches followed the same trend during development. Their concentration increased up to 87 – 94 DAFB and then decreased rapidly until harvest. No significant fluctuations in glucose and fructose concentrations throughout ripening were observed by Moriguchi et al. (1990), Hubbard et al. (1991) and Brooks et al. (1993).

Moriguchi et al. (1990) concluded that 'Hakuto' peach fruit contained a substantial amount of sorbitol oxidase activity. This implies that transported sorbitol from the phloem is usually converted to glucose in the fruit. They also found that sorbitol oxidase activity is two to three times higher in peach than in pear fruit. Thus, sorbitol that was transported by the phloem may be metabolised differently in peach than in pear.

Contrary to this, Génard and Souty (1996) found that most of the sorbitol in peach fruit is converted to fructose, rather than glucose. This conversion is made possible by various sorbitol dehydrogenases enzymes. Sucrose that arrives via the phloem into the fruit can also be hydrolysed into fructose and glucose by acid invertase or neutral invertase. Vizzotto et al. (1996) observed high levels of insoluble acid-, soluble acid- and neutral invertase in immature fruit. During maturation, enzyme levels drop to about one-tenth of the initial level, being almost undetectable at maturity. Moriguchi et al. (1990) found similar acid invertase activities in young and developing fruit, but found an increase in the latter part of fruit development.

Génard et al. (1999) correlated poor fruit growth in the last phase before harvest, with high reducing sugar concentrations at harvest. Since glucose and fructose are substrates for growth and respiration, the negative correlation with rapid fruit growth is apparent. They also found no significant changes in reducing sugar percentage throughout fruit ripening. This finding supports the results of Moriguchi et al. (1990).

Of the reducing sugars, fructose plays the most important role in taste perception (Pangborn, 1963). The higher the fructose content of the fruit, the sweeter it will taste. Wu et al. (2003) reported that genotypes with low fructose content should not be

considered for breeding purposes. It was also found that glucose could cause a slight bitterness at high concentrations. Robertson and Meredith (1988) reported that "high quality" peaches contain higher levels of fructose and lower levels of glucose and sorbitol than "low quality" fruit.

Fig. 2: The structure of a glucose molecule (Matthews and Van Holde, 1990).

Fig. 3: The structure of a fructose molecule (Matthews and Van Holde, 1990).

1.2.3 Sorbitol

The levels of sorbitol, a poly-alcohol sugar, are usually very low and relatively variable, in peaches and nectarines, ranging from 0.1 % to 5.5 % of total sugars (Wrolstad and Shallenberger, 1981; Bassi and Selli, 1990; Robertson et al., 1990;

Liverani and Cangini, 1991; Robertson et al., 1992; Brooks et al., 1993; Moing et al., 1998b; Génard et al., 1999; Versari et al., 2002; Wu et al., 2003).

Throughout fruit development sorbitol levels never exceed 6 % of total sugars (Liverani and Cangini, 1991) and at about 94 DAFB sorbitol levels reach this maximum and then decrease towards maturity. This reduction of sorbitol was closely correlated with the beginning of fruit ripening. Chapman and Hovat (1990) found the same tendency in the sorbitol levels throughout development, but found the maximum level at about 109 DAFB. Brooks et al. (1993) also reported an initial increase in sorbitol levels until pit hardening, when sorbitol levels reached 9.1 %. When the ripening processes started, sorbitol content dropped significantly to below 6 % of total sugars. Selli and Sansavini (1995) reported no fluctuations in sorbitol levels during development, but found a slight increase in sorbitol near maturity.

Moing et al. (1992) reported that the phloem sap of peach trees contains equal amounts of sorbitol and sucrose and these are the only sugars in the phloem. These can be taken up by the mesocarp tissue by an apoplastic and/or symplastic route of phloem unloading, due to the presence of plasmodesmata (Masia et al., 1992). The fact that sorbitol content is always low in peach and nectarine fruit suggests that sorbitol is metabolised into reducing sugars (Moriguchi et al., 1990). They also demonstrated that sorbitol oxidase, which catalyses the conversion of sorbitol into glucose, is an essential enzyme of sorbitol metabolism in 'Hakuto' peaches. Yamaki and Ishikawa (1986) found that the various sorbitol dehydrogenases that convert sorbitol into fructose are also important in other cultivars. Thus, transported sorbitol may be metabolised differently in different cultivars.

The findings of Robertson and Meredith (1988) suggest that 'low quality' peaches contain a higher percentage of sorbitol and glucose and lower percentage fructose than 'high quality' peaches. Because sorbitol is usually not present in very high concentrations at harvest, it will not play a critical role in the taste of peaches and

nectarines. However, fruit that are picked immature and still contain high sorbitol levels, taste less sweet than fruit that are picked at optimum maturity.

Fig. 4: The structure of a sorbitol molecule (Matthews and Van Holde, 1990)

1.3 ORGANIC ACIDS

Organic acids are minor components in peach and nectarine fruit, but they make an important contribution to organoleptic quality, in combination with sugars and aromatic compounds (Wang et al., 1993). Sweeney et al. (1970), Esti et al. (1997) and Crisosto et al. (2002) showed that consumers are more sensitive to the RSSC (Ripe Soluble Solid Content): RTA (Ripe Titratable Acidity) ratio than to fruit acidity on its own. Crisosto and Crisosto (2005) concluded that consumer acceptance of two high-acid and two low-acid peach and nectarine cultivars was associated with RSSC, regardless of RTA. Fruit acidity is influenced by many factors such as cultivar, environmental conditions, canopy position, crop load, ripening, fruit maturity and rootstock (Crisosto et al., 1997).

As with sweetness, the total acidity of peach and nectarine fruit is directly influenced by the composition of the different organic acids (Esti et al., 1997; Moing et al., 1998b). The three major non-volatile organic acids in peaches and nectarines are malic-, citric-, and quinic acid (Wills et al., 1983; Meredith et al., 1989; Chapman and Horvat, 1990; Robertson et al., 1990; Byrne et al., 1991; Wang et al., 1993; Moing et al., 1998a; Génard et al., 1999; Wu et al., 2002). Very small amounts of succinic, shikimic, ascorbic and oxalic acid were also reported by Sweeney et al. (1970), Chapman and Horvat (1990), Liverani and Cangini (1991), Selli and Sansavini (1995) and Wu et al. (2005).

Significant correlations have been reported for citric and malic acid and their contribution to the perception of sourness (Esti et al., 1997). Some authors suggest that the malic to citric acid ratio could be used as an index of maturity (Meredith et al., 1989; Chapman and Horvat, 1990). European and American cultivars are more acidic than the low- and sub-acid peach and nectarine cultivars that were introduced from China (Moing et al., 1998b). Esti et al. (1997) found that the high level of sweetness of these low-acid cultivars is not correlated to high sucrose levels, but to low malic and citric acid content.

1.3.1 Malic acid

In fruit pulp cells, malate and citrate are biosynthesised in the cytoplasm and the mitochondrion respectively, and stored in the vacuole. Although malate and citrate have a close metabolic connection (Kreb cycle / Tricarboxylic acid cycle), they differ greatly with fruit development (Wu et al., 2002). Malic is, in general, the main acid at maturity (50 – 60 % of total organic acids) (Meredith et al., 1989; Liverani and Cangini, 1991; Wang et al., 1993; Moing et al., 1998b; Dirlewanger et al., 1999; Versari et al., 2002; Wu et al., 2005) and in immature peaches malic acid is usually found in lower concentrations.

Chapman and Horvat (1990) monitored the changes in nonvolatile acids during the maturation of 'Monroe' peaches. They found that malic acid remained fairly constant until 123 DAFB, then increased rapidly for the next 7 days (130 DAFB), and then declined slowly towards maturity (144 DAFB). At 123 DAFB malic acid became the main acid and remained so until harvest. Meredith et al. (1989) examined the malic acid levels of 'Harvester' peaches picked at different maturities. As maturity (measured with colour chips) increased, malic acid increased accordingly. Selli and Sansavini (1995) however found no increase in malic acid from 63 DAFB until maturity in Italian grown peaches and nectarines.

Wu et al. (2002) found the seasonal development of malic acid to be more influenced by and sensitive to environmental conditions. They found a decrease in malate concentration at the beginning of fruit growth and an increase towards maturity over a few seasons. They concluded that the level of malate is only marginally influenced by PEPC (phosphoenolpyruvate carboxylase) and malic enzyme, so it is apparent that it is mainly controlled by vacuolar storage. Malate concentration in the vacuole is controlled by the proton pump. Assimilate supply favours the arrival of more cations, mainly potassium, into the vacuole through phloem flow. A negative and positive correlation could be found between assimilate supply and malate concentration at the beginning of fruit growth and at maturity, respectively.

Liverani and Cangini (1991), Moing et al. (1998b) and Wu et al. (2005) found the same trend in the malic acid concentration throughout fruit development of peach and nectarine cultivars. An initial reduction in malic acid concentration was observed up to about 94 DAFB, thereafter a sharp increase in concentration was seen towards maturity. This work was done on standard acid cultivars. Moing et al. (1998a) reported that the malic acid levels during development in a low-acid cultivar 'Jalousia', is characterised by the absence of malate accumulation near maturity. At maturity, 'Fantasia' fruit (standard acid cultivar) had 3 times higher malic acid content compared to 'Jalousia' fruit. Other studies (Byrne et al., 1991) on low-acid cultivars found the same lack of accumulation of malic acid at maturity. 'Sam Houston', a low-acid peach, has approximately twice as little malic acid levels than the high acid cultivars. Picha et al. (1989) concluded that low-acid cultivars contain less malic acid than normal cultivars at any stage during development.

According to Moing et al. (1998b) there are three hypotheses to explain these results. These concern malate synthesis, catabolism and compartmentation, which are unrelated hypotheses. Concerning malate synthesis, PEPC (phosphoenolpyruvate carboxylase) is regulated by phosphorylation by a PEPC kinase. Therefore differences in malate build-up by peach genotypes could result from differences in PEPC kinases content and/or activity. As previously mentioned, malate is stored in the vacuole to accumulate in mesocarp cells. Malate and citrate cross the tonoplast by means of the same carrier. Vacuolar malate uptake is driven by H⁺-ATPase or H⁺-pyrophosphatase. Therefore, an

inactive proton pump or malate transporter is a relevant hypothesis. Hawker (1969) and Guttiérrez-Granda and Morrison (1992) suggested that intracellular compartmentation rather than enzyme availability regulates malic acid metabolism during development of grape berries. Moing et al. (1998b) stated that if malate storage in the vacuole compartment is impeded in low acid cultivars, malate in the cytosol could be catabolised by malic enzyme and malic dehydrogenases before maturity.

Génard et al. (1999) studied the stability of the relationship between organic acids and fruit growth. They divided fruit growth in two phases: pit growth with the first phase of fruit growth, and a second stage fruit growth towards maturity. They found that fruit growing intensively during the second phase had the highest malic acid and sucrose concentration. Fruits growing weakly during the second phase probably reach a low level of maturity and consequently had low levels of malic acid and sucrose. They concluded that malic acid probably has a high metabolic priority, explaining the positive relationship with growth.

Although malic acid is only a minor component of peaches and nectarines, it makes an important taste contribution to the sensory perception of sourness (Esti et al., 1997). Crisosto et al. (2002) concluded that consumer acceptance for some cultivars is related to RTA (Ripe Titratable Acidity). Because malic acid is dominant at maturity it plays a critical role in the perception of acidity. It was reported by Pangborn (1963) that the taste of malic acid is not as strong as citric acid, but it persists for longer. Consumers vary in their appreciation of acidity level, but in general a high sugar level and, to a lesser extent, high acid level seem to be favourable to most consumers (Wu et al., 2003). Selli and Sansavini (1995) reported that Italian consumers prefer a lower sugar-acid (6.5:1) ratio rather than a very high ratio (13.3:1).

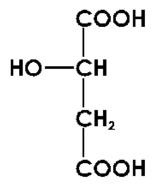


Fig. 5: The structure of a malic acid molecule (Matthews and Van Holde, 1990)

1.3.2 Citric acid

Citric acid is the second most abundant organic acid in most peach and nectarine cultivars (Sweeney et al., 1970; Wills et al., 1983; Bassi and Selli, 1990; Byrne et al., 1991; Liverani and Cangini, 1991; Vizzotto et al., 1996; Versari et al., 2002; Wu et al., 2005). Li and Woodroof (1968) and Esti et al. (1997), however, reported that citric acid is the dominant acid in some peach cultivars, followed by malic acid. Citric acid levels are reported to be 20 - 25 % of total acids at maturity (Byrne et al., 1991; Wang et al., 1993; Dirlewanger et al., 1999; Wu et al., 2003).

Citrate is synthesised in the mitochondrion and stored in the vacuole of peach and nectarines (Wu et al., 2002). Changes in citric acid levels differ greatly from malic acid during fruit development. Wu et al. (2002) stated that, of the di- and tricarboxylic acids in the mitochondrion, citrate is the main substrate for growth and respiration. They presented a model that predicts that the rate of citrate synthesis or degradation depends strongly on the mesocarp weight. Increasing initial fruit weight promotes citrate concentration, whereas at maturity, increasing fruit weight reduces citrate concentration. This could be explained by a relative reduction in the 'mitochondrial equipment', which diminishes the potential for citrate synthesis.

Meredith et al. (1989), Chapman and Horvat (1990), Liverani and Cangini (1991) and Wu et al. (2002) concluded that citrate levels increase to become the dominant acid in immature fruit and then decrease as maturity progressed. Wu et al. (2002) found that assimilate supply at the beginning of fruit growth increased citrate accumulation, while near maturity more assimilate supply decreased citrate levels. Génard et al. (1999) reported that peaches contained very high citric acid levels at the time of thinning, but fruit growing strongly during the last phase of fruit growth had the lowest citric acid levels at maturity. They concluded that there is an analogy in the sucrose-reducing sugars and malic acid-citric acid pairs, since sucrose and malic acid are storage components, whereas reducing sugars and citric acid are used as substrates for growth and respiration.

Moing et al. (1998a) compared the acid metabolism of a standard acid cultivar ('Fantasia') and a low-acid cultivar ('Jalousia'). Citric acid levels were similar in both cultivars until 80 DAFB when levels in 'Fantasia' started to increase rapidly and no accumulation occurred in 'Jalousia'. At maturity citric acid levels for 'Fantasia' were 6 times higher, compared to 'Jalousia'.

As one of the major acids in peaches and nectarines citric acid plays an important role in the sensory perception of acidity (Esti et al. 1997). Pangborn (1963) reported that the taste of citric acid appears before that of malic acid and is perceived as more acidic, but it can not persist as long as malic acid.

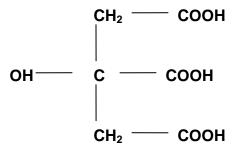


Fig. 6: The structure of a citric acid molecule (Matthews and Van Holde, 1990)

1.3.3 Quinic acid

Quinic acid is the third most important acid in peaches and nectarines in terms of abundance (Sweeney et al., 1970; Wills et al., 1983; Chapman and Horvat, 1990; Byrne et al., 1991; Wang et al. 1993; Moing et al., 1998b; Dirlewanger et al., 1999; Versari et al., 2002; Wu et al., 2002; Wu et al., 2003). These authors reported quinic acid levels that range from 1-5 % of total acids at maturity. Chapman and Horvat (1990) and Wu et al. (2002) reported that quinic acid is one of the major acids at the beginning of fruit growth.

As fruit matures, the quinic acid concentration decreases rapidly from about 80 DAFB to very low levels at optimum maturity (Chapman and Horvat, 1990; Chapman et al., 1991; Wang et al., 1993; Wu et al., 2002). Wu et al. (2005) stated that the lowest quinic acid concentration corresponded with physiological maturity. Wu et al. (2002) reported that high assimilate supply accelerates the decrease in quinic acid. Quinic and shikimic acid are both important intermediary metabolites connecting carbohydrate metabolism and aromatic biosynthesis (Jensen, 1985). He emphasised the importance of quinic acid in the biosynthesis of aromatic compounds, therefore the decreasing quinic acid levels may be indicative of the accumulation of aromatic compounds (Wu et al., 2002).

Wu et al. (2003) reported that quinic acid imparts a slightly sour and bitter taste and has important antibacterial properties beneficial to health.

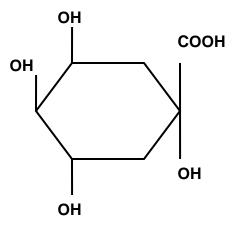


Fig. 6: The structure of a quinic acid molecule (Matthews and Van Holde, 1990)

1.3.4 Other acids

Low levels of succinic acid have been reported by Sweeney et al. (1970) Bassi and Selli (1990), Chapman and Horvat (1990), Selli and Sansavini (1995), Versari et al. (2002) and Chinnici et al. (2005). Bassi and Selli (1990) and Selli and Sansavini (1995) reported succinic acid levels as high as 19 % of total acids in peaches grown in Italy. Succinic acid levels decreased significantly as maturity progressed (Selli and Sansavini, 1995).

The presence of shikimic acid in peaches and nectarines has also been reported by Wang et al. (1990) and Wu et al. (2002), but in very low quantities (<1 % of total acids). Shikimic acid is an important metabolite connecting the carbohydrate metabolism and aromatic biosynthesis (Jensen, 1985). As maturity progressed, shikimic acid levels decreased, indicating the accumulation of aromatic compounds (Wu et al., 2002).

Fumaric (Wang et al., 1993; Chinnici et al., 2005), oxalic and ascorbic acid (Liverani and Cangini, 1991; Selli and Sansavini, 1995) have also been reported in peach and nectarine fruit, but all at very low levels (<1 % of total acids).

1.4 FACTORS AFFECTING SUGAR AND ACID CONTENT

1.4.1 Maturity

The effect of maturity has been discussed in previous sections.

1.4.2 Assimilate and water supply

Wu et al. (2002) concluded that irrigation might cause a decrease in acid concentrations by decreasing the levels of carbohydrates, reducing acid transport to the fruit, or causing the dilution of acids in the fruit.

The effect on assimilate supply on sugar and organic acid concentration has also been discussed in previous sections.

1.4.3 Cultivar

Brooks et al. (1993) evaluated the variation in sugar content for 54 different peach selections. Sucrose content varied between 3.7 - 1.1 % of fresh weight, while reducing sugars varied between 2.6 - 0.6 % of fresh weight and sorbitol levels were between 1.5 - 0.24 %. The highest total sugars percentage for a cultivar was 8.3 %, while the lowest was 3.9 % of fresh weight. This variation can be used to select and breed peach and nectarine cultivars with higher sugar content.

Esti et al. (1997) compared the sugar and acid composition of 21 commercial nectarine cultivars. Substantial variation between cultivars was found for sucrose, glucose and fructose levels. The low-acid cultivar 'Douceur' had sucrose levels of $9.8~\rm g$ / $100~\rm g$ fresh weight, compared to $4.3~\rm g$ / $100~\rm g$ fresh weight for the high-acid cultivar 'Iris

Rosso'. Major differences in the non-volatile acid levels were also found. Malic acid was not found in greater quantities than citric acid in all cultivars. 'Iris Rosso', 'Maria Aurelia', 'Argento di Roma' and 'Morciani 51' had higher citric acid levels at maturity, which will have a significant influence on the eating quality of these fruit (Pangborn 1963). Versari et al. (2002) compared the composition of three commercial peach cultivars grown in Italy. They found that 'Suncrest' peach had significantly higher glucose, fructose, sorbitol and malic acid levels, than 'Redhaven' and 'Maria Marta'.

Robertson et al. (1990) compared the quality characteristics of six yellow- and five white-fleshed peach cultivars. Although the sugar contents of the yellow- and white-fleshed cultivars were not significantly different, sucrose, glucose and fructose as well as soluble solids tended to be higher for white-fleshed cultivars. These data support the findings of Picha et al. (1989). Liverani and Cangini (1991) concluded that the white-fleshed cultivar 'Triestina' contained higher sucrose, fructose and malic acid levels, than yellow-fleshed peaches.

Byrne et al. (1991) studied the variability of sugar and acids in 12 peach genotypes, including one low-acid cultivar. 'Sam Houston', the low-acid cultivar, had lower malic, citric and quinic acid levels than the other cultivars. This cultivar also had higher levels of sucrose, lower levels of glucose and fructose, but the same relative sweetness as the high-acid cultivars. Moing et al. (1998b) compared the sugar and acid composition of a normal acid ('Fantasia') and low-acid ('Jalousia') nectarine. At maturity 'Fantasia' contained two and five times more malic and citric acid, respectively. Sucrose, reducing sugar and sorbitol concentrations were the same for both cultivars at maturity.

1.4.4 Canopy position

Génard and Bruchou (1992) sampled peach fruit from different canopy positions to determine the variation in quality within a tree. Fruit from the upper canopy had higher sucrose content and lower citric acid content than fruit from the lower part of the canopy.

Fruit exposed to sunlight in the afternoon had higher citric acid content and lower sucrose and malic acid content than fruit exposed to sunlight in the morning. They also found that fruit borne by thick shoots had higher malic acid levels than fruit on thinner bearing units.

1.5 CORRELATIONS BETWEEN CERTAIN SUGARS AND ORGANIC ACIDS

Sucrose content is usually positively correlated with malic acid, as both of their levels increase as maturity progresses (Wu et al., 2003). Chapman and Horvat (1990) proposed that the maximum levels of sucrose and malic acid could be used as a reliable index for physiological maturity of peaches. The reducing sugars and citric acid decrease with advancing maturity and also show a positive correlation with each other (Génard et al., 1999). Glucose and fructose contents are also always closely correlated (Chapman and Horvat, 1990; Wu et al., 2003). As previously mentioned, there is an analogy in the sucrose-reducing sugars and malic acid-citric acid pairs.

Wu et al. (2002) reported a positive correlation for quinic acid and shikimic acid as they both decrease with advancing maturity. This decrease in concentration for both acids is fundamental in the formation of aromatic compounds (Jensen, 1985).

1.6 CONCLUSION

The organoleptic quality and sensory acceptability of peaches and nectarines are largely defined by their composition of individual soluble sugars and organic acids. Consumer reaction towards peaches and nectarines has been shown to be associated with total soluble solids content and the soluble solids: titratable acidity ratio.

Sucrose, fructose, glucose and sorbitol have been identified as the major sugars, and malic, citric and quinic acid as the major organic acids, in peaches and nectarines. There

is still much contradictory literature in how they are metabolised during ripening on the tree, but the majority of research focussed on this aspect. Very little information is available on how sugars and organic acids change during storage.

The composition of individual sugars and organic acids is important information because it can be an indicator of fruit maturity and quality. It can also be used to develop peach and nectarine cultivars which will satisfy market and consumer demands.

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PAPER 1. THE EFFECT OF CANOPY POSITION, INITIAL FRUIT SIZE AND BEARING POSITION ON FRUIT QUALITY OF 'RED JEWEL' AND 'RUBY DAIMOND' NECTARINES (*Prunus persic a* (L.) Batsch).

ABSTRACT

The influence of the variables canopy position, initial fruit size and bearing position was studied to determine the variation in fruit quality within a nectarine tree. 'Red Jewel' and 'Ruby Diamond' fruit from the upper part of the tree canopy had significantly higher TSS. 'Red Jewel' nectarine matured from the top of the trees, so indicating that these fruit should be picked prior to the rest of the tree. As these differences are, however, not visible at harvest due to the full red colour development of these cultivars, producers will have to sample fruit from different positions in trees to determine where to harvest when. Fruit position on the shoot does not seem to play a significant role in fruit quality for 'Red Jewel' nectarines, which will allow producers to leave more than one fruit per bearer if necessary. Fruit thinning is an important means to improve fruit size and quality in 'Red Jewel', but poor thinning can cause extreme variability in size and quality. The variation in fruit size following hand thinning remains the same until harvest. Fruit that were small at thinning remained significantly smaller, weighed less, had lower sugars and higher acids at harvest. If it is possible to reduce the variation in size at thinning, fruit will be much more homogenous at harvest.

KEYWORDS: Canopy position, initial fruit size, bearing position, fruit quality, 'Red Jewel', 'Ruby Diamond'.

1. Introduction

The increase in hectares of nectarines in recent years includes new cultivars with different growth and fruiting habits, more full red fruit colour and better eating quality than the existing older cultivars. Imported cultivars are also often not as well adapted to the South African climate, soil and water conditions as locally bred cultivars. In South

Africa these cultivars are ear-marked for the export market and thus necessitate strict adherence to export standards.

According to Luchsinger and Reginato (2001), although maturity is only one feature of fruit quality, it has a great influence on post harvest behaviour during marketing, as well as on the ultimate organoleptic quality of the fruit. Fruit maturity at harvest will determine the fruits' susceptibility to mechanical bruising, post harvest performance and potential storage life (Crisosto et al., 1997). Over mature fruit will not be able to withstand the rigours of post harvest handling and may have increased susceptibility to fruit rotting organisms. Immature fruit are incapable of ripening to their full potential, will lose water more readily and may be more susceptible to physiological disorders like woolliness (van Mollendorf, 1987). Characteristics that change with advancing maturity like ground colour, total soluble solids (TSS), titratable acidity (TA) (Lill et al., 1989) and firmness (Brovelli et al., 1998) are valuable harvesting indicators in assessing maturity. Crisosto (1994) concluded that a combination of ground colour and firmness may be a better index to assay nectarine maturity. Existing cultivars can mostly be picked on ground colour, which correlates well with firmness (National Department of Harvesting the newer cultivars, however, is a Agriculture specifications, 2004). challenge as they develop a full red over colour, masking the ground colour, before fruit firmness is within the quality specification. Brovelli et al. (1998) investigated a range of peach and nectarine cultivars, and found that for each cultivar a different maturity indice gave the best guideline to optimum picking maturity.

Various factors influence fruit quality, and more specifically eating quality, of fruit at harvest e.g. sunlight (Kappel et al., 1983; Erez and Flore, 1986), leaf: fruit ratio (Marini et al., 1991), canopy position (Saenz, 1991; Forlani et al., 2002; Luchsinger et al., 2002), number of fruit per tree (Marini and Sowers, 1994; Johnson and Handley, 1989; Giacalone et al., 2002), and the immediate microclimate of an individual fruit (Mancinelli, 1984; Marini et al., 1991; Salvador and Lizana, 1998). Producers have to manage these factors to ensure well coloured, mature fruit with high sugars and also a high TSS: TA ratio.

The aim of this study was to determine the ripening pattern and the variation in fruit quality within the tree in order to facilitate harvesting of full red cultivars.

2. Materials and methods

The trials were conducted on two farms (Verdun Estates and Lushof Farms) in the Warm Bokkeveld area near Ceres (33° 13'S, 19° 20'E, 503 m.a.s.l.) in South Africa during the 2003/2004 and 2004/2005 seasons. Two nectarine cultivars were used, 'Ruby Diamond' at Verdun Estates and 'Red Jewel' at Lushof Farms. Both cultivars on SAPO 778 clonal rootstocks were planted in 2000 at 4.5 m x 1.5 m spacing and trained to a central leader system. The row direction for both cultivars is north-south. Average production per hectare for both 'Red Jewel' and 'Ruby Diamond' was ±20 tons in their fourth leaf and the average fruit size was between 62 – 68 mm. Standard commercial orchard management practices were followed. ReTain® was applied at 830g/1000*l* on 'Red Jewel' at Lushof about ten days before harvest in the 2004/2005 season.

2.1 Trial 1: Effect of canopy position

The effect of canopy position on fruit quality at harvest was evaluated on both cultivars to determine whether this could be used to assist at harvest to reduce variability in maturity. Ten trees (2003/04) and 15 trees (2004/05) were chosen randomly in each orchard and tagged during dormancy. The trees were closely monitored during blossoming to see if there were any differences in full bloom date in any part of the tree. Trees were divided into three sections: bottom, middle and top (in the first season only two sections, viz., bottom and top). Following fruit set, 5 fruit / position / tree were tagged. Fruit were tagged on the western side of the trees and the bearing units (one-year-old shoots) were all of the same approximate length and diameter. Fruit size was measured weekly with a Mitutoyo CD-6"C digital calliper to determine the fruit growth curve of each cultivar and position.

2.2 Trial 2: Effect of bearing position

This trial was only conducted in the 2004/2005 season. Twenty trees each of 'Ruby Diamond' and 'Red Jewel' were chosen randomly in the two orchards. The trees were closely monitored during blossoming to see if there were any differences in full bloom date on a one-year-old shoot between the terminal and distal flower buds. Following fruit set (20 - 30 days after full bloom) the trees were thinned by hand according to the six different treatments / combinations illustrated in Fig. 1. The aim was to evaluate the influence of bearing position *per se* and to evaluate the effect of neighbouring fruit in different positions on the same bearing unit on the variability in harvest maturity. The one-year-old shoots were all approximately of the same length and diameter and in the middle section on the outside of the trees.

2.3 Trial 3: Effect of initial fruit size

Twenty trees each of 'Ruby Diamond' and 'Red Jewel' were chosen randomly in the two orchards. After the producer completed commercial hand thinning of fruit (40 DAFB), the variation in fruit size was determined with a calliper. The fruit was then divided into three groups, viz. small (<22 mm), medium (22 mm – 26 mm) and large (>26 mm). All the bearing positions were positioned in the bottom section of the trees and on the same quality bearing wood. Bearing positions were all lateral in the middle of the one-year-old shoots.

2.4 Data recorded:

In both seasons fruit were harvested during the optimum harvest window (firmness \pm 9.0 kg). In the first season, following harvest, fruit were taken to the ARC Infruitec-Nietvoorbij laboratories for maturity indexing. The following data were recorded: Fruit firmness (kg) was determined using a G üss Fruit Texture Analyser 20 by inserting the 11.2 mm probe into the fruit flesh after (\pm 2 mm) skin was removed from opposite sides

of the fruit cheek. The fruit of each replication were then put into an AEG ESF103 Juice extractor to isolate the juice. The juice was used to determine total soluble solids (TSS) (in °Brix) with an Atago PR32 digital refractometer (0 - 32 °Brix range and temperature calibrated). Fruit size (mm) was measured with a Mitutoyo CD-6"C digital calliper.

In the second season, fruit were taken to our laboratories after harvest. Maturity indexing was done as follows: Fruit firmness (kg) was determined using a G üss FTA (Fruit Texture Analyser) 20 by inserting the 11.2 mm probe into the fruit flesh after (±2 mm) skin was removed from opposite sides of the fruit. Fruit size (mm) and fruit mass (g) were determined with an EFM (Electronic Fruit Measurement). The fruit of each replication were then put into an AEG ESF103 Juice Extractor to isolate the juice. The juice was used to determine total soluble solids (TSS) (in °Brix) with an Atago PR32 digital refractometer (0 - 32 °Brix range and temperature calibrated) and titratable acidity (TA) (in % malic acid) with a Metrohm AG 719 S Titrino which titrated with 0.1 M NaOH to a pH of 8.2.

2.5 Trial lay-out and data analysis:

A complete randomised design was used. Data were analysed using the GLM (general linear models) procedure in the Statistical Analysis Systems (SAS), Enterprise Guide 3.0.

3. Results and discussion

3.1 Effect of canopy position

'Red Jewel' nectarine did not show any differences in full bloom dates for different parts of the trees in both seasons. The fruit growth curves of 'Red Jewel' (Figure 2a) in the first season were very similar for different canopy positions. The normal double sigmoidal pattern of growth for stone fruit as described by Brady (1993) was not observed because fruit growth measurements only started 45 days after full bloom

(DAFB). At harvest there were no significant differences between the size and firmness of the two canopy positions (Table 1). TSS for fruit from the top part of the trees was significantly higher than of fruit from the bottom, confirming the results of Dann and Jerie (1988) and Crisosto et al. (1997).

In the second season the fruit growth curve of 'Red Jewel' (Figure 3) showed the normal double sigmoidal pattern of growth as described by Brady (1993). Again, fruit from the different parts of the canopy had very similar growth curves. At harvest there were no significant differences in size and mass for fruit from different canopy positions (Table 1). The firmness of the fruit in the bottom of the trees was significantly higher than those in the middle and the tops. These fruit were, therefore, less mature and had significantly lower sugars and higher acids than fruit from the rest of the tree (Table 1). Fruit from the top part of the trees had significantly higher sugars than the rest of the canopy, confirming the results of Marini (1985), Dann and Jerie (1988) and Crisosto et al. (1997). The lower canopy positions can produce good fruit quality if it is allowed to mature (Forlani et al., 2002). Fruit that is shaded during the final stage of fruit growth (three weeks before harvest) was found to have lower TSS values (Marini et al., 1991). Picking 'Red Jewel' on size will result in variation in firmness and TSS. It is recommended that the top part of the trees be picked first and fruit from the bottom part of the canopy can be picked when more mature.

During the first season 'Ruby Diamond' nectarine also did not show the double sigmoidal growth pattern, but there was a difference in how fruit from the bottom and top of the canopy developed (Figure 2b). Fruit from the top part of the canopy were significantly bigger and sweeter than fruit from the bottom part at the optimum harvest date (Table 2), confirming the results of Marini (1985), Dan and Jerie (1988) and Crisosto et al. (1997). There was, however, no significant difference in firmness between these canopy positions. Marini et al. (1991) concluded that these differences in fruit size and TSS are because of shading in the bottom canopy of the tree during final fruit swell. The effects of shading can be negated by summer pruning from three weeks before harvest.

These results suggest that 'Ruby Diamond' nectarine should be picked when fruit firmness is within the quality specification to minimise the variation in maturity, but the variation in size and TSS within the batch of fruit will be significant.

There were no data for 'Ruby Diamond' in the second season due to a hailstorm destroying the whole crop one week before harvest.

3.2 Effect of bearing position

The effect of bearing position was only investigated in the 2004/2005 season. 'Red Jewel' nectarine blossomed very uniformly on the one-year-old shoots and there was no visible difference between full bloom date of terminal and distal buds (data not shown). There were no significant differences between treatments for fruit size, fruit mass, firmness, total soluble solids (TSS) and titratable acidity (TA) at harvest (Table 3), indicating that a terminal fruit did not influence the maturity or size of basal or middle fruit and vice versa. These results confirm the results of Corelli-Grappadelli and Coston (1991) and Marini and Sowers (1994) who suggested that fruit size, fruit mass and TSS are influenced by crop density and length of the bearing shoot, but not position on the shoot. These authors hypothesised that differences in fruit size resulted from altered carbohydrate partitioning between plant parts. Actively growing shoots may be strong sinks for carbohydrates, and young fruit developing near the terminal bud may be at a more competitive disadvantage than fruit farther down the shoot. In contrast, Spencer and Couvillon (1975) reported that peaches developing near the terminal bud of the fruiting shoot were larger than fruit at the base of the shoot. We, however, could not confirm this.

These results suggest that tree training and pruning for 'Red Jewel' can be done to reduce the number of bearing units, leaving more fruit per one-year-old shoot and therefore allowing selection of superior quality shoots as bearers.

3.3 Effect of initial fruit size

No differences in full bloom date between different positions in the canopy were observed for 'Red Jewel' (data not shown). Fruit size following commercial hand thinning was between 21 mm and 28 mm. Fruit that were large at thinning (>26 mm) were significantly bigger, weighed more and had higher sugars than medium and small fruit at harvest (Table 4). Large fruit were also significantly softer and had lower acids than medium and small fruit on the same harvest date. This indicates that large fruit at the time of hand thinning were probably more mature than smaller fruit at that time and remained more advanced in maturity throughout their development. Giacalone et al. (2002) concluded that incorrect thinning can cause extreme variability in fruit size and quality. Spencer and Couvillon (1975) found that flower buds at terminal nodes bloomed earlier than buds at basal positions and that these terminal fruit were also larger at harvest.

In an attempt to reduce this variation at harvest, producers must thin on size, rather than position on the shoot.

4. Conclusion

Different factors within the tree can influence the fruit quality of nectarines considerably. Fruit position in the tree canopy significantly influenced fruit quality for 'Red Jewel' and 'Ruby Diamond' nectarines. Fruit from the upper part of the tree canopy had significantly higher TSS in both cultivars. 'Red Jewel' nectarine trees seem to mature from the top of the trees, so these fruit can be picked prior to the rest of the tree. As these differences are, however, not visible at harvest due to the full red colour development of these cultivars, producers will have to sample fruit from different positions in trees to determine where to harvest when. Fruit position on the shoot does not seem to play a significant role in fruit quality for 'Red Jewel' nectarines, which will allow producers to leave more than one fruit per bearer if necessary. Leaving fruit in the terminal position, however, still has disadvantages due to the movement of the bearer and

therefore fruit finish might be sacrificed. Fruit thinning is an important means to improve fruit size and quality in 'Red Jewel', but poor thinning can cause extreme variability in size and quality. The variation in fruit size following hand thinning remains the same until harvest. Fruit that were small at thinning remained significantly smaller, weighed less, had lower sugars and higher acids at harvest. If it is possible to reduce the variation in size at thinning, fruit will be much more homogenous at harvest. This will also reduce problems with post harvest handling of 'Red Jewel'.

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<u>Table 1.</u>
Means of the fruit size, mass, firmness, total soluble solids (TSS) and titratable acidity (TA) in relation to canopy position of 'Red Jewel' nectarines from Lushof for the 2003/2004 and 2004/2005 seasons.

Position	Size (mm)	Mass (g)	Firmness (kg)	TSS (°Brix)	TA (%Malic)
2003/2004					
Bottom	58.99 a		6.54 a	14.03 b	
Тор	58.47 a		7.73 a	15.82 a	
LSD (5%)	2.52		2.35	1.44	
Pr>f	0.6780		0.3120	0.0150	
2004/2005					
Bottom	58.16 a	117.35 a	9.63 a	15.4 c	1.44 a
Middle	58.90 a	123.01 a	6.54 b	17.4 b	1.29 b
Тор	58.98 a	124.90 a	5.13 b	19.6 a	1.25 b
LSD (5%)	1.45	9.18	1.58	0.5	0.07
Pr>f	0.470	0.241	<0.0001	< 0.0001	< 0.0001

<u>Table 2.</u>
Means of the size, firmness, total soluble solids (TSS) and titratable acidity (TA) of 'Ruby Diamond' nectarines from Verdun Estates at harvest for the 2003/2004 season.

Position	Size (mm)	Firmness (kg)	TSS (°Brix)
2003/2004			
Bottom	59.25 b	7.81 a	10.1 b
Тор	63.37 a	8.98 a	13.1 a
LSD (5%)	2.85	1.95	1.3
<i>Pr>f</i>	0.0070	0.2250	< 0.0001



<u>Table 3.</u>
Means of the size, mass, firmness, total soluble solids (TSS) and titratable acidity (TA) of 'Red Jewel' nectarines relative to the bearing position in the 2004/2005 season.

Treatment	Size (mm)	Mass (g)	Firmness (kg)	TSS (°Brix)	TA (%Malic)
T	59.66 ns	115.06 ns	7.11 ns	14.0 ns	1.32 a
M	59.70	113.83	7.90	14.3	1.27 ab
В	60.40	118.00	7.42	14.7	1.17 b
T + M	58.32	105.74	6.61	13.6	1.16 b
$\mathbf{M} + \mathbf{T}$	59.08	110.78	8.26	13.6	1.27 ab
T + B	57.74	103.29	7.94	14.0	1.24 ab
$\mathbf{B} + \mathbf{T}$	57.91	104.52	7.39	14.1	1.23 ab
M + B	59.35	113.45	6.79	14.3	1.26 ab
$\mathbf{B} + \mathbf{M}$	59.81	115.20	7.98	14.2	1.23 ab
LSD (5%)	ns	ns	ns	ns	0.12
Pr>f	0.3485	0.2667	0.7693	0.1012	0.0528

(T = Terminal fruit; M = Middle of bearing unit; B = Basal fruit)

Data in rows represent bearing position first mentioned.

<u>Table 4.</u>
Means of the size, mass, firmness, total soluble solids (TSS) and titratable acidity (TA) at harvest of 'Red Jewel' nectarines as determined by initial fruit size in the 2004/2005 season.

Initial fruit size	Size (mm)	Mass (g)	Firmness (kg)	TSS (°Brix)	TA (%Malic)
Small	53.95 с	88.67 c	10.41 a	13.8 b	1.37 a
Medium	55.95 b	104.95 b	9.34 b	13.5 с	1.22 b
Large	59.16 a	123.64 a	6.96 c	14.82 a	1.16 c
LSD (5%)	1.07	6.33	0.82	0.1	0.044
Pr>f	< 0.0001	<0.0001	< 0.0001	< 0.0001	< 0.0001



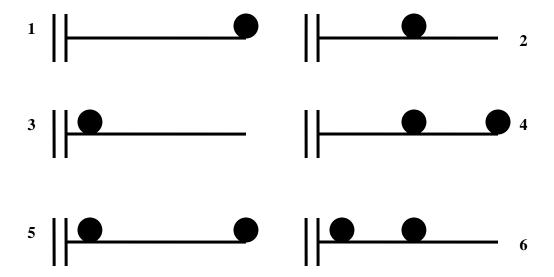
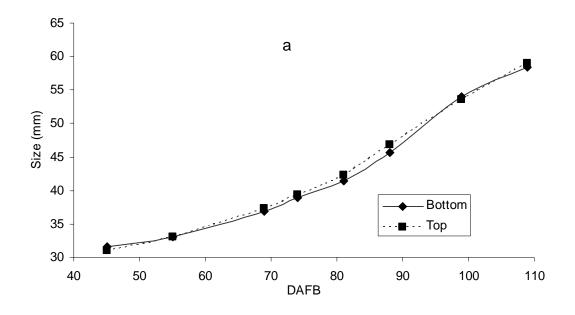


Figure 1.

The three fruit bearing positions and the combinations of them. (1) terminal, (2) middle, (3) basal, (4) middle and terminal, (5) basal and terminal and (6) basal and middle.

Pectora roborant cultus recti



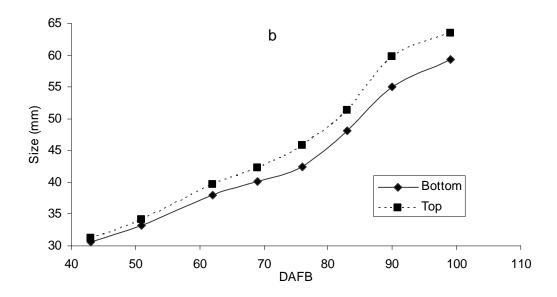
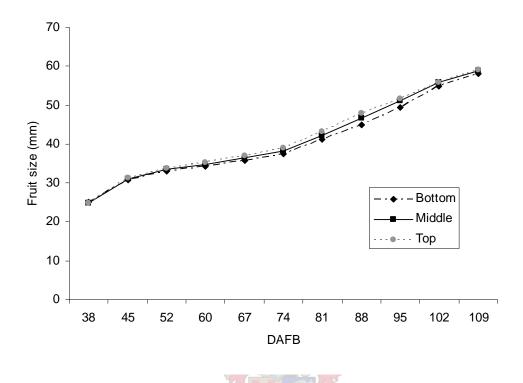


Figure 2.

Fruit growth curves for fruit from two sections in the tree canopy, viz., the bottom and top of 'Red Jewel' (a) and 'Ruby Diamond' (b) nectarines in the 2003/2004 season.



Fruit growth curves for fruit from three sections in the tree canopy, viz. the bottom, middle and top of 'Red Jewel' nectarine in the 2004/2005 season.

PAPER 2: THE EFFECT OF POST HARVEST CONDITIONING TREATMENTS AND CONTROLLED ATMOPHERE STORAGE ON FRUIT QUALITY OF 'RED JEWEL' AND 'SPRING BRIGHT' NECTARINES (*Prunus persica* (L.) Batsch)

ABSTRACT

The effect of pre-conditioning (PC) and controlled atmosphere (CA) storage was evaluated on 'Red Jewel' and 'Spring Bright' nectarines. Free juice percentage was determined at the end of a simulated export protocol. The severity of woolliness differed between the two seasons for both nectarines cultivars. PC, to a firmness of 6 kg, followed by regular atmosphere (RA) storage increased percentage free juice significantly in 'Spring Bright' and 'Red Jewel' nectarines. However, a PC protocol for each cultivar and each producer must be determined beforehand to ensure fruit quality. CA storage is another technique that can be used to alleviate the symptoms of chilling injury (CI). Both 'Spring Bright' and 'Red Jewel' showed an increase in percentage free juice with the use of CA storage during both seasons. The use of PC followed by CA storage might have been more effective if the PC had been performed to a firmness of 6 kg.

KEYWORDS: Chilling injury (CI), controlled atmosphere (CA), pre-conditioning (PC), 'Red Jewel', 'Spring Bright', woolliness.

1. Introduction

One of the most frequent complaints by supermarkets and consumers is the presence of flesh mealiness and the lack of juice in peaches and nectarines. This physiological disorder is known as woolliness in South Africa (Von Mollendorf et al., 1992). These symptoms are a result of low temperature storage (-0.5°C) for extended periods, resulting in chilling injury (CI). The symptoms only appear once the fruit is ripened after storage (Crisosto et al., 1999). During the development of woolliness in nectarines, free juice becomes bound without any change in the moisture content (Von Mollendorf and De Villiers, 1988). Ben Arie and Sonego (1980) demonstrated that woolliness in 'Elberta' peaches is related to an increase in pectin esterase (PE) activity and a decrease in polygalacturonase (PG) activity. This has recently been confirmed in woolly nectarines

by Zhou et al. (2000a). According to Von Mollendorf and De Villiers (1988) the low PG activity during storage, followed by a sharp increase in activity during ripening, causes an accumulation of pectic substances in the intercellular spaces. These pectins possess gelling properties and form apoplastic pectin gels, resulting in typical woolliness symptoms. Brummell et al. (2004) suggested that cold storage affects the activities of numerous cell wall-modifying enzymes. These changes alter the properties of the primary cell wall and middle lamella, resulting in tissue breakage along enlarged air spaces, which reduces the amount and availability of free juice due to the forming of pectin gels.

Sea freight exported South African nectarines are all exposed to protocols that induce CI. After picking and packing, fruit is forced air cooled in cold rooms to -0.5°C and from then on the cold chain is maintained. After forced air cooling fruit is directly loaded into refrigerated containers or transported to the fruit terminals in refrigerated trucks. This loading and accumulation of containers can take up to 7 days before the ship is ready to sail. The voyage time to the United Kingdom (UK) is usually about 18 days. On arrival in the UK, importers ripen the fruit to about 3 kg (11.2 mm tip) firmness for the supermarkets that require juicy and melting fruit. In the supermarkets fruit need to have a maximum shelf life of 5 days. Various factors can influence the susceptibility of cultivars to CI, like genotype (Von Mollendorf, 1987; Hartman, 1985; Crisosto et al., 1999), maturity (Von Mollendorf, 1987) and orchard factors (Crisosto et al., 1995; Crisosto et al., 1997).

All the major nectarine producing areas in the world suffer from the occurrence of woolliness or CI, whether the fruit is exported or stored to extend local marketing opportunities (Sharkey et al., 1983; Von Mollendorf, 1987; Lill and Van der Mespel, 1988; Lurie, 1992; Burmeister and Harman, 1996; Luchsinger and Walsh, 1996; Zhou et al., 2000a; Choi and Lee, 2001). Delayed cooling or pre-ripeing or pre-conditioning (PC) has been used for more than 50 years. Boyes and De Villiers (1949) demonstrated on South African 'Peregrine' peaches that a delay in the commencement of cooling (48 hours at 24°C) reduced woolliness by 32.4%. Futher studies by Boyes (1952) on 'Elberta' peaches indicated that the delay in cooling needs to be longer than 6 hours to be effective in preventing woolliness. Later studies (Hartman, 1985; Von Mollendorf 1987; Nanos and Mitchell, 1991; Retamales et al., 1992; Truter et al., 1993; Combrink and Visagie, 1997; Zhou et al., 2000b; Choi and Lee, 2001) confirmed that pre-conditioning can

alleviate and prevent woolliness in peaches and nectarines. Crisosto (2001) developed pre-conditioning protocols for Californian peaches and nectarines. He found that a 48 hour cooling delay at 20°C is most effective in preventing woolliness. PC has produced inconsistent results, alleviating the onset of woolliness only in some studies (Wade 1981, Tonini et al., 1989).

The use of controlled atmosphere with elevated CO₂ and reduced O₂ concentrations can also prevent chilling injury symptoms (Kajiura, 1975; Olsen and Shomer, 1975). Wade (1981) concluded that the CO₂ concentration appeared to be the critical component for alleviating the onset of physiological storage disorders. Lill et al. (1989) found that a CO₂ concentration of at least 5% was necessary to prevent the appearance of chilling injury. Other authors confirmed these results (Tonini et al., 1989; Lurie, 1992; Truter and Combrink, 1992; Truter et al., 1993; Streif et al., 1994; Burmeister and Harman, 1996; Zhou et al., 2000b; Choi and Lee, 2001) and CA storage is effectively used in various parts of the world today for export and long term storage.

The objective of this trial was to determine whether pre-conditioning or CA storage or the combination of both, which is successfully used on standard cultivars, could improve the quality of new nectarine cultivars.

2. Materials and methods:

2.1 Plant material

Two new nectarine cultivars (*Prunus persica* cvs. Spring Bright and Red Jewel) were used in the pre-conditioning and CA storage trials during the 2003/2004 and 2004/2005 seasons. Fruit were harvested from mature, commercial orchards in the Warm Bokkeveld area near Ceres (33° 13'S, 19° 20'E, 503 m a.s.l). Harvesting commenced at commercial firmness of about 9 kg (11.2 mm probe). After sorting and packing in the commercial packing facility of Graaff Packing, fruit were transported by refrigerated truck to the ARC Infruitec-Nietvoorbij laboratory in Stellenbosch. Fruit (62 – 65 mm or count 23) were packed in commercial 2.5 kg nectarine export cartons. Two post harvest trials were conducted; pre-conditioning followed by regular atmosphere storage (RA) and pre-

conditioning followed by controlled atmosphere (CA) storage.

2.2 Trial 1: Pre-conditioning followed by RA storage

The control treatment cartons were placed into the RA cold rooms at -0.5°C (95% RH) and stored for 25 days (simulating 7 days accumulation + 18 days shipment). The pre-conditioning was done in terms of fruit firmness. Fruit were put into a ripening room at 18°C (95% RH) and monitored on a regular basis. There were three levels of pre-conditioning: 8 kg, 6 kg and 4 kg. After a fruit sample reached the desired firmness the remainder was directly transferred to -0.5°C RA storage. There was also an intermittent warming (IW) treatment included in this trial, which consisted of 10 days at -0.5°C RA, followed by 1 day at 18°C, then back to -0.5°C RA for the rest of the simulated shipment period of 25 days.

After storage, the control treatment was ripened for 3 days at 18°C and then went into a 5 day shelf life period at 10°C. All the pre-conditioning and the IW treatments went directly into shelf life conditions at 10°C after storage.

2.3 Trial 2: Pre Conditioning followed by CA storage

This trial consisted of six treatments, which included three "control" treatments, a RA control, a CA control (5% $O_2 + 10\%$ CO_2) and a pre-conditioning control (8 kg). A new CA regime which is commercially used in Israel (2% $O_2 + 5\%$ CO_2) was evaluated (Lurie, 1992). In addition, both CA regimes were evaluated in combination with PC to 8 kg.

After storage of 25 days, the "control" treatments were ripened for 3 days at 18°C and then went into a 5 day shelf life period at 10°C. All the pre-conditioning treatments went directly into shelf life conditions after storage.

2.4 Pre-conditioning

In the second season 'Spring Bright' from a different producer in the same area was obtained to determine if fruit from the same area reacted similarly to pre conditioning. The fruit was picked, packed and transported to Stellenbosch on the same day. Fruit was then placed together into the ripening room at 18°C and firmness monitored closely.

2.5 Data recorded

On arrival at the ARC Infruitec-Nietvoorbij a random representative sample of fruit was taken to determine the maturity. Four replicates of ten fruit each were used for the maturity indexing.

Maturity indexing was done as follows: Fruit firmness (kg) was determined using a G üss Fruit Texture Analyzer 20 by inserting the 11.2 mm probe into the fruit flesh after (±2 mm) skin was removed from opposite sides of the fruit. The fruit of each replication were then put into an AEG ESF103 Juice extractor to isolate the juice. The juice was used to determine total soluble solids (TSS) (in °Brix) with an Atago PR32 digital refractometer (0 - 32 °Brix range and temperature calibrated) and titratable acidity (TA) with a Crison Compact Titrator which titrated with 0.1 M NaOH to a pH of 8.2.

Sensory evaluations and maturity indexing were done after pre-conditioning, after storage, after ripening and at the end of shelf life. One carton represented a replicate and 10 fruit were used for maturity indexing and 10 for sensory analysis. Woolliness was always determined in terms of percentage free juice. A scale from 0-4 (0= no juice; 1=very little free juice; 2 = little free juice; 3 = juicy; 4 = very juicy) was used as a subjective rating for juiciness on all 10 fruit. A score above 2 was regarded as acceptable. The total number of "acceptable" fruit was then expressed as a percentage. At the end of shelf life a quantitative method was used to determine the free juice in each treatment (Crisosto and Labavitch, 2002). The same fruit that was used for sensory evaluation was peeled and cut into smaller pieces. Six fruit of each replicate were randomly chosen for this analysis. The fruit tissue was individually homogenised with a Braun MR 400 mini hand blender. Thirty grams of fruit pulp from each fruit/replicate and 30 ml of distilled water were then mixed with a Vortex Genie 2 (Scientific Industries) and centrifuged with a Heraeus Sepatech Biofuge 17 RS at 6000 g for 10 min (in the second season only 4000 g was used). After centrifugation the supernatant weight was used to determine the percentage free juice based on the initial fruit tissue sample weight. The percentage of free juice that related to unacceptable woolliness was not determined.

2.6 Trial layout and data analysis

Both trials were completely randomised designs, with 5 or 6 treatments and 4 replicates consisting of 20 fruit per evaluation.

Statistical Analysis Systems (SAS), Enterprise Guide was used to perform the analysis of variance (ANOVA). Single degree of freedom, orthogonal polynomial contrasts were used where applicable. Logit data transformation was used on percentages.

3 Results and discussion

3.1 Pre-conditioning

Cultivars differ in how they react to pre-conditioning (Fig. 1), confirming the results of Crisosto (2001). 'Spring Bright' needed 36 and 38 hours, respectively, to reach the desired firmness of 4 kg in the two seasons. In both seasons 'Spring Bright' responded very consistently to pre-conditioning. On the other hand, 'Red Jewel' needed 24 and 26 hours, respectively to decrease to the firmness of 4 kg. Fruit of both cultivars increased slightly in firmness during the first four hours of pre-conditioning.

Not only cultivars differ in their response to pre-conditioning, but fruit of the same cultivar but from different producers also differ in their response (Fig. 2). At arrival in the laboratory 'Spring Bright' nectarines from two producers were at the same firmness (Table 1), but fruit from Graaff Packing needed 28 hours to reach a firmness of 6 kg, while fruit from Verdun reached 6 kg after 19 hours. These results imply that the time required to reach a specific firmness cannot be generalised for a given cultivar.

3.2 Pre-conditioning followed by RA storage

The severity of woolliness, expressed as percentage free juice, differed between the two seasons (Table 2 and 3) which is in agreement with results obtained by Truter and Combrink (1992). The 2003/2004 season was severely affected by woolliness, while in the 2004/2005 season more free juice was observed in all the treatments. Free juice levels in 'Spring Bright' nectarines (Table 2) in the first season never exceeded 25 %, whereas in 2004/2005 the free juice percentage of all treatments was higher than 30 % (Table 2). In the case of 'Spring Bright' the control RA stored fruit had a significantly lower percentage free juice than any of the treatments in 2003/2004 (Table 2). The percentage

free juice decreased linearly with an increase in the length of PC (Table 2), while the IW treatment did not differ significantly from any PC treatments. However, the subjective ratings suggest that the decrease in free juice with the length of PC is quadratic. Subjective ratings for the amount of free juice for 'Spring Bright' also show the RA control treatment had significantly less juice than the other treatments in 2003/2004. In the 2004/2005 season 'Spring Bright' fruit exposed to IW had significantly less free juice than the other treatments except for fruit receiving the short PC (8 kg). The PC treatments resulted in a linear increase in percentage free juice (Table 2) with an increase in the length of the PC treatment which is contradictory to the results obtained during the previous season (Table 2). The subjective rating of woolliness verifies the linear increase in amount of free juice with length of PC. The subjective results for the 2004/2005 season confirm that the short PC and IW treatments had significantly less available juice than the other treatments.

In both seasons fruit from the treatment with the least available free juice, were also significantly firmer than any other treatment (Table 2), confirming the symptoms of chilling injury (Crisosto et al., 1999). In both seasons there were no significant differences in TSS (Table 2). The TA response differed between the treatments and over the seasons. In the first season the 8 kg PC had significantly less acid than all other treatments, resulting in a significantly higher TSS:TA ratio (Table 2), while in the 2004/2005 season the RA control stored fruit had a significantly higher acid content (Table 2).

PC increased percentage free juice in 'Spring Bright' nectarines in both seasons. PC to a firmness of 6 kg seems to be the best treatment. Longer PC in 2003/04 aggravated the woolliness problem while in 2004/05 the longer PC gave good results in terms of woolliness, but softer fruit will be more difficult to handle and be more prone to bruises and injuries. Crisosto et al. (1999) concluded that 'Spring Bright' nectarine, grown in California, does not develop CI symptoms, even after 5 weeks of storage at 0°C. Hartman (1987) did a comparative study on 'Fantasia' and 'Flavortop' nectarines grown in South Africa and in California. He found that South African grown fruit develop woolliness after storage, but Californian fruit ripened normally, which might be indicative thereof that South African nectarines are inherently more susceptible to woolliness.

The difference in severity of woolliness during the two seasons was also evident in 'Red Jewel' nectarines (Table 3). The percentage free juice for 'Red Jewel' during 2003/2004 season never exceeded 22 %, while in the following season free juice levels of all treatments were above 34 % (Table 3). In the 2003/2004 season 'Red Jewel' from the RA control stored fruit did not differ significantly from the longer PC (6 kg and 4 kg) treatments in terms of percentage free juice, while the shorter PC (8 kg) and the IW treatment had significantly lower free juice percentage than the other treatments. The percentage free juice increased quadraticaly with an increase in length of PC (Table 3). The same quadratic trend was observed in the subjective rating (Table 3). Results for the second season were very similar in the case of 'Red Jewel'. The percentage free juice again increased quadraticaly with the increase in length of PC (Table 3), while the IW treatment had significantly less juice than any other treatment. The subjective rating of the degree of woolliness indicates that the increase in free juice was linear for the 2004/2005 season.

There was no correlation between the amount of free juice and the firmness at the end of shelf life for 'Red Jewel' nectarine (Table 3). The TSS showed no significant differences in 2003/04, while in 2004/05 the control fruit had significantly higher TSS. In the first season the IW treatment and PC 4 kg and in the second season the PC 6 kg had significantly higher TA levels than any other treatment, which resulted in significantly lower TSS/TA ratios (Table 3).

PC increased percentage free juice in 'Red Jewel' nectarines in both seasons. PC to a firmness of 6 kg seems to be the best treatment, while the longer PC gave good results in terms of woolliness. On the other hand softer fruit will be more difficult to handle and be more prone to bruises and injuries

3.3 Pre-conditioning followed by CA storage

The severity of woolliness, expressed as percent free juice, also differed between the two seasons in the PC followed by CA storage trial (Table 4 and 5). For 'Spring Bright' percentage free juice in the first season never exceeded 28 %, compared to free juice levels in the following season that were never below 28 %. For 'Spring Bright' in the

2003/2004 season the PC followed by CA storage combination gave the best results and there was a significant increase in percent free juice for fruit that received PC (Table 4). The RA and CA control had significantly less juice than any other treatment (Table 4). Subjective ratings also confirm that PC resulted in a significant increase in free juice. In the 2004/2005 season the RA control, CA control and CA 2 (2% O₂ + 5% CO₂) were significantly juicier than the rest of the treatments and there was a significant decrease in percentage of free juice for all treatments receiving PC (Table 4), which is contradictory to the results obtained during the previous season. The PC control (8 kg) had significantly lower free juice percentage than any other treatment, confirming the results of the PC followed by RA storage trial (Table 2). Again, the subjective results for woolliness confirm that the PC treatments significantly decreased free juice percentage. Only in the 2003/2004 season the subjective ratings showed a significant difference between CA control and CA 2.

In the first season fruit from the PC followed by CA treatments were significantly firmer than the CA treatments without PC (Table 4). In the 2004/2005 season the three treatments with the highest percentage free juice (RA control, CA control and CA 2), were significantly softer than the rest, indicating normal fruit ripening and softening (Crisosto et al., 1999). In the 2003/2004 season the PC followed by CA 2 storage had a significantly lower TA value, resulting in a significantly higher TSS/TA ratio. Lurie (1992) found that CA storage retards respiration and consequently inhibits TA decline in storage. In the second season the CA control and CA 2 had significantly higher TA values, resulting in significantly lower TSS:TA ratios (Table 4).

Variable results were obtained in response to PC and CA storage for the two seasons on 'Spring Bright' nectarines. In the first season PC followed by CA storage increased percentage free juice significantly, while in the second season CA storage with no PC increased percentage free juice. Unfortunately PC was only performed to a firmness of 8 kg which we know from the previous trial was not long enough. Zhou et al. (2000b) also found that both PC and CA storage can alleviate or prevent woolliness in nectarines.

In the 2003/2004 season the maximum free juice level was 28 % for 'Red Jewel', whereas in the subsequent season the minimum level was 37 % (Table 5). In the case of 'Red Jewel' the PC control fruit had the lowest percentage free juice in both seasons. In

the 2003/2004 season the CA control significantly increased the free juice percentage (Table 5), while PC did not show any beneficial effects. This confirms the results of Wade (1981) and Lurie (1992) that higher CO₂ levels are more effective in controlling CI symptoms. In the following season there was no difference between the CA control and the CA 2 (Table 5), but all the treatments that were stored in CA, without PC, had a significantly higher free juice percentage than PC fruit. Subjective ratings of free juice also confirm that CA stored fruit was less affected by woolliness (Table 5) at the end of shelf life.

In both seasons there was no obvious correlation between free juice and firmness. No significant differences in TSS values were observed in either season (Table 5). In the 2004/2005 season CA stored fruit had significantly higher TA values, confirming the results of Lurie (1992).

For 'Red Jewel' nectarine CA storage significantly increased percentage free juice in both seasons, confirming the results of Lurie (1992), Zhou et al. (2000b) and Choi and Lee (2001). In the 2003/2004 season, when woolliness was more severe, the higher CO₂ concentration (10 %) was significantly better than the lower level (5 %), which is similar to the results of Watada et al. (1979), Wade (1981) and Lurie (1992). Lill et al. (1989) and Olsen and Schomer (1975) reported that nectarines stored in high CO₂ (15 % - 50 %) developed off-flavours at such high CO₂ levels.

4. Conclusion

The severity of woolliness differed between the two seasons for both 'Spring Bright' and 'Red Jewel' nectarines. PC, to a firmness of 6 kg, followed by regular atmosphere (RA) storage can increase percentage free juice significantly for 'Spring Bright' and 'Red Jewel' nectarines, respectively. However, a PC protocol for each cultivar and each producer must be determined beforehand to ensure fruit quality. CA storage is another method that can be used to prevent the symptoms of CI. Both 'Spring Bright' and 'Red Jewel' showed an increase in percentage free juice with the use of CA storage during both seasons. The use of PC followed by CA storage might have been more effective if the PC had been performed to a firmness of 6 kg.

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<u>Table 1.</u>
The maturity of 'Spring Bright' nectarines from Graaff Packing and Verdun Estates on arrival in the 2004/2005 season and of 'Spring Bright' and 'Red Jewel' for the 2003/2004 and 2004/2005 seasons.

	Verdun Estate	Graaff Packing
Firmness (kg)	9.73	9.42
TSS (°Brix)	11.7	14.65
Titratable acidity (g 100 ml ⁻¹)	1.8	2.00
TSS/TA Ratio	6.5	7.33
Spring Bright	2003/2004	2004/2005
Firmness (kg)	8.87	9.42
TSS (°Brix)	15.43	14.65
Titratable acidity (g 100 ml ⁻¹)	2.00	2.00
TSS/TA Ratio	7.72	7.33
<u>Red Jewel</u>	2003/2004	2004/2005
Firmness (kg)	9.07	9.72
TSS (°Brix)	15.33	14.48
Titratable acidity (g 100 ml ⁻¹)	1.90	2.40
TSS/TA Ratio	8.07	6.03

Table 2.

The means and significance levels of % free juice, % juicy fruit, firmness, total soluble solids (TSS), titratable acidity (TA) and total soluble solids / titratable acidity ratio for the pre-conditioning (PC) + RA storage trial on 'Spring Bright' for the 2003/2004 and 2004/2005 seasons at the end of shelf life.

Treatment	% Free Juice	% Juicy fruit ^{xyz}	Firmness (kg)	TSS (°Brix)	TA (% Malic)	TSS/TA Ratio
<u>2003/2004:</u>						
RA Control	14.44 c	$24.17 c^{z}$	2.60 a	15.5 ns	0.41 b	38.08 ns
PC 8 kg	25.32 a	34.17 ab	1.90 b	16.2	0.17 c	111.70
PC 6 kg	24.38 ab	43.33 a	1.55 b	15.7	0.45 b	37.35
PC 4 kg	19.91 b	30.83 bc	1.47 b	15.9	0.55 b	29.54
IW	21.56 ab	22.50 bc	1.30 b	14.5	1.38 a	10.81
Significance level: Pr>F						
Treatments	0.0040	0.0086	0.0100	0.0950	0.0001	0.0024
Contrast PC linear	0.0327	0.4935	0.0700	0.5628	0.0042	0.0011
Contrast PC quadratic	0.3735	0.0333	0.8606	0.5053	0.3408	0.0616
LSD (5%)	4.87	-	0.66	ns	0.23	ns
<u>2004/2005:</u>		THE G				
RA Control	37.09 a	81.24 ab	0.84 c	15.5 ns	1.50 a	10.30 b
PC 8 kg	30.35 b	32.09 d	1.49 b	15.6	0.89 b	18.98 a
PC 6 kg	39.03 a	76.03 b	1.38 b	14.5	0.79 b	19.04 a
PC 4 kg	40.53 a	93.74 a	1.01 c	15.3	0.85 b	18.92 a
IW	31.34 b	44.79 cd	2.03 a	16.0	0.90 b	18.63 a
Significance level: Pr>F						
Treatment	0.0010	0.0001	0.0001	0.4530	0.0006	0.0712
Contrast PC linear	0.0005	0.0003	0.0114	0.6737	0.7867	0.9864
Contrast PC quadratic	0.0937	0.8552	0.3644	0.1958	0.4882	0.9751
LSD (5%)	4.94	-	0.36	ns	0.29	7.06

x - % Acceptable fruit determined with subjective scale (0 = no juice; 4 = very juicy).

y - Logit Data Transformation used on percentages.

z – LSD's for logit data transformation used within column

Table 3. The means and significance levels of % free juice, % juicy fruit, firmness, total soluble solids (TSS), titratable acidity (TA) and total soluble solids / titratable acidity ratio for the pre-conditioning (PC) + RA storage trial on 'Red Jewel' for the 2003/2004 and 2004/2005 seasons at the end of shelf life.

Treatment	% Free Juice	% Juicy Fruit ^{xy}	Firmness (kg)	TSS (°Brix)	TA (%Malic)	TSS/TA Ratio
<u>2003/2004:</u>						
RA Control	19.12 a	43.33 b	1.49 ab	15.5 ns	0.33 b	47.50 a
PC 8 kg	10.55 b	15.00 c	1.95 a	16.2	0.32 b	50.31 a
PC 6 kg	20.18 a	50.83 ab	1.16 b	14.8	0.32 b	47.67 a
PC 4 kg	21.69 a	57.50 a	1.48 ab	16.5	0.58 a	29.90 b
IW	14.36 b	15.83 с	1.30 b	15.7	0.73 a	22.07 b
Significance level: Pr>F						
Treatment	0.0004	< 0.0001	0.0712	0.2925	< 0.0001	0.0017
Contrast PC linear	< 0.0001	<0.0001	0.0844	0.5845	0.0239	0.0052
Contrast PC quadratic	0.0224	0.0072	0.0248	0.0455	0.1653	0.1584
LSD (5%)	3.86		0.54	ns	0.22	12.77
<u>2004/2005:</u>						
RA Control	38.95 b	76.04 b	1.04 b	16.2 a	0.91 b	17.92 a
PC 8 kg	37.64 b	46.87 c	1.71 a	15.0 b	0.85 b	17.86 a
PC 6 kg	42.44 a	73.96 b	1.70 a	14.5 b	1.21 a	12.29 b
PC 4 kg	40.59 ab	68.73 a	1.40 ab	15.0 b	0.99 b	15.32 ab
IW	34.25 c	32.27 d	1.37 ab	14.7 b	0.92 b	18.90 a
Significance level: Pr>F						
Treatment	0.0010	< 0.0001	0.0160	0.0100	0.0100	0.0170
Contrast PC linear	0.0757	<.0001	0.1247	1.0000	0.1428	0.0640
Contrast PC quadratic	0.0253	0.2520	0.4022	0.1760	0.0022	0.0014
LSD (5%)	3.30		2.13	0.9	0.20	3.88

x – Woolliness % determined with subjective scale (0 = no juice; 4 = very juicy).

y - Logit Data Transformation used on percentages.

z – LSD's for logit data transformation used within column

Tabel 4. The means and significance levels of % free juice, % juicy fruit, firmness, total soluble solids (TSS), titratable acidity (TA) and total soluble solids / titratable acidity ratio for the pre-conditioning (PC) + CA storage trial on 'Spring Bright' for the 2003/2004 and 2004/2005 seasons at the end of shelf life .

Storage trial on 'Spring B' Treatment	% Free Juice	% Juicy Fruit ^{xyz}	Firmness (kg)	TSS (°Brix)	TA (% Malic)	TSS/TA Ratio
<u>2003/2004:</u>						
RA Control	12.76 c	23.33 с	2.07 ns	15.0 ns	0.43 b	34.91 bc
CA Control	14.82 c	29.16 bc	1.67	15.8	0.41 bc	39.28 b
CA 2	21.02 b	47.50 a	1.80	14.8	0.30 bc	50.43 b
PC Control	20.50 b	34.17 b	1.84	14.7	1.01 a	15.67 d
PC+CA Control	27.04 a	51.67 a	2.02	15.3	0.85 a	18.15 cd
PC+CA 2	23.37 ab	44.17 a	1.77	15.5	0.15 c	104.61 a
Significance level:Pr>f						
Treatments	0.0006	0.0002	0.5474	0.1800	0.0035	0.0001
PC vs CA	< 0.0001	0.0008	< 0.0001	0.1269	< 0.0001	<.0001
CA Conrol vs CA2	0.4648	0.0328	0.1589	0.1571	0.4978	0.6586
LSD (5%)	5.16		ns	ns	0.26	17.67
<u>2004/2005:</u>						
RA Control	41.76 a	70.83 b	0.91 c	15.3 ns	1.48 a	10.38 b
CA Control	46.38 a	91.45 a	1.07 c	14.6	1.45 a	10.13 b
CA 2	46.52 a	98.75 a	1.07 c	14.7	1.53 a	9.66 b
PC Control	28.15 c	38.33 c	1.53 b	15.7	0.67 b	24.51 a
PC+CA Control	33.13 b	63.54 c	1.86 a	14.7	0.71 b	21.28 a
PC+CA 2	33.39 b	64.59 c	1.65 ab	16.0	0.70 b	23.11 a
Significance level:Pr>f						
Treatments	0.0001	0.0020	0.0001	0.1790	0.0001	0.0001
PC vs CA	<0.0001	< 0.0001	< 0.0001	0.1269	< 0.0001	< 0.0001
CA Control vs CA2	0.9040	0.5522	0.1589	0.1571	0.4978	0.6586
	4.83		0.21		0.15	4.52

x – Woolliness % determined with subjective scale (0 = no juice; 4 = very juicy).

y - Logit Data Transformation used on percentages.

z – LSD's for logit data transformation used within column

Table 5. The means and significance levels of the % free juice, % juicy fruit, firmness, total soluble solids (TSS), titratable acidity (TA) and total soluble solids / titratable acidity ratio for the pre-conditioning + CA storage trial on 'Red Jewel' nectarine for the 2003/2004 and 2004/2005 season at the end of shelf life.

Treatment	% Free Juice	% Juicy Fruit ^{xy}	Firmness (kg)	TSS (°Brix)	TA (% Malic)	TSS/TA Ratio
<u>2003/2004:</u>						
RA Control	16.32 c	35.83 d	1.88 bc	14.4 ns	0.31 c	46.92 ab
CA Control	22.92 b	54.17 bc	1.427 c	15.2	0.43 a	35.09 d
CA 2	23.97 b	56.67 ab	1.62 bc	15.4	0.35 bc	44.70 bc
PC Control	11.21 d	27.50 e	1.78 bc	14.7	0.38 ab	54.04 a
PC+CA Control	28.37 a	67.50 a	2.75 a	15.4	0.29 c	42.67 cd
PC+CA 2	18.61 c	47.50 cd	2.34 a	14.5	0.34 bc	38.70 cd
Significance level:Pr>f						
Treatments	0.0001	0.0001	0.0003	0.6806	0.0035	0.0034
PC vs CA	0.1061	0.2442	0.0001	0.8059	0.1708	0.1895
CA Conrol vs CA 2	0.0030	0.0111	0.4486	0.5498	0.4794	0.7363
LSD (5%)	3.62		0.44	ns	0.06	7.90
<u>2003/2004:</u>						
RA Control	40.15 bc	78.75 b	1.26 bc	13.6 ns	1.70 ab	8.01 b
CA Control	42.61 ab	93.13 a	1.12 c	14.3	1.65 ab	8.64 b
CA 2	45.81 a	97.50 a	1.07 c	13.9	1.73 ab	8.12 b
PC Control	37.82 c	34.37 c	1.43 ab	13.8	1.85 a	7.52 b
PC+CA Control	41.91 b	41.25 b	1.56 a	14.1	1.63 b	8.77 b
PC+CA 2	41.45 b	41.04 b	1.48 ab	13.9	1.23 c	11.47 a
Significance level Pr>f						
Treatments	0.0023	< 0.0001	0.0022	0.7900	0.0001	0.001
PC vs CA	0.0134	< 0.0001	< 0.0001	0.9273	0.0447	0.0272
CA Conrol vs CA 2	0.2305	0.3060	0.4611	0.4822	0.0343	0.0454
LSD (5%)	3.27	-	0.3	ns	0.21	1.50

x – Woolliness % determined with subjective scale (0 = no juice; 4 = very juicy).

y - Logit Data Transformation used on percentages.

z – LSD's for logit data transformation used within column

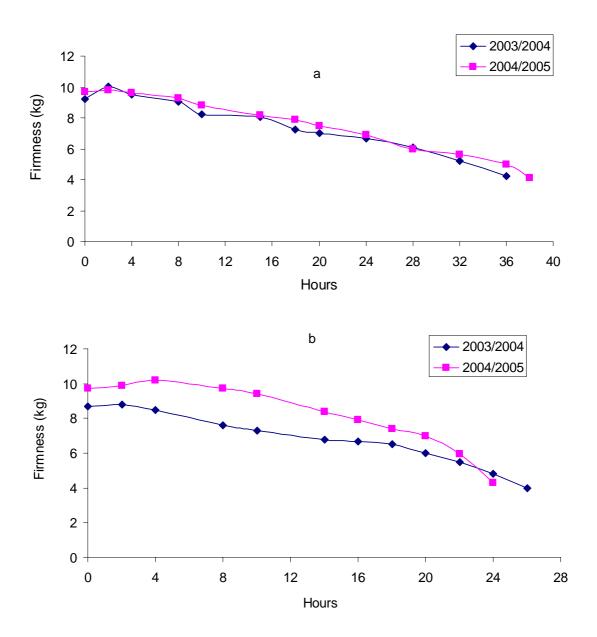


Fig. 1: Results for the pre-conditioning of 'Spring Bright' (a) and 'Red Jewel' (b) nectarines for the 2003/2004 and 2004/2005 seasons.

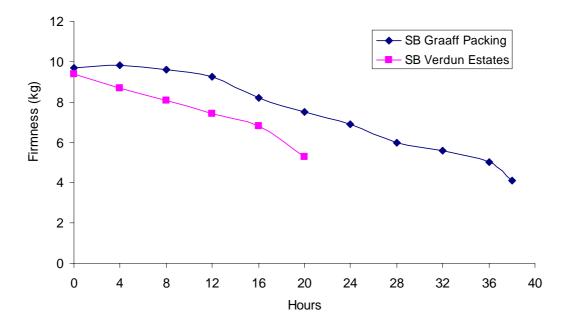


Fig. 2: Results for the pre-conditioning of 'Spring Bright' nectarine from two different producers for the 2004/2005 season.

PAPER 3: CHANGES IN SUGAR AND ORGANIC ACID CONCENTRATIONS DURING MATURATION AND STORAGE OF NECTARINES (*Prunus persica* (L.) Batsch) GROWN IN SOUTH AFRICA

ABSTRACT

Concentrations of individual sugars and organic acids during maturation and storage of nectarines grown in South Africa, were determined with HPLC. Sucrose was found to be the main sugar in all cultivars at maturity and increased significantly as maturity progressed. Sucrose decreased significantly during storage in 'Mayglo' nectarine. Of the reducing sugars, fructose was present at slightly higher levels than glucose and both of their concentrations decreased significantly in all cultivars as fruit matured. In storage, glucose and fructose levels increased significantly for 'Mayglo' and 'Red Jewel', whereas only fructose increased significantly for 'Spring Bright'. A significant accumulation of sorbitol was observed for 'Big Top' and 'Red Jewel' nectarines as maturity progressed, while sorbitol decreased significantly for 'Mayglo' and 'Spring Bright'. Sorbitol levels decreased significantly from harvest to the end of shelf life for all nectarine cultivars, except 'Zeeglo'. Malic acid significantly increased as maturity progressed and became the main acid in all cultivars at optimum maturity. The malic acid concentration decreased significantly during storage in all cultivars, but it remained the dominant acid. Citric acid was one of the dominant acids in immature fruit, but its concentration decreased significantly towards maturity. Citric acid decreased significantly throughout the simulated export and shelf life for 'Spring Bright', 'Red Jewel' and 'Zeeglo'. Immature fruit had high quinic acid levels, but it also decreased significantly towards maturity. Ouinic acid content increased significantly during storage for 'Red Jewel and 'Spring Bright'.

KEYWORDS: Citric acid, malic acid, *Prunus persica*, quinic acid, reducing sugars, sorbitol, sucrose.

1. Introduction

Consistent high yield, good fruit size, sufficient colour development, firmness and resistance to the most common pathogens are only some of the characteristics of

new nectarine cultivars (Dirlewanger et al., 1999). In the past few years the eating quality of fruit and especially stone fruit, has become a very important quality trait which has necessitated producers to plant new improved cultivars (Esti et al., 1997; Crisosto et al., 2002). Demand is for juicy, melting fruit but the flavour and taste components are also very important. Therefore, sugars, organic acids, and pectins, and the structure and ratios of these have become important in determining quality (Selli and Sansavini, 1995). According to Chapman and Horvat (1990), fruit taste and flavour properties are largely defined by the composition of these compounds and the sugar-acid ratio. In order to improve the organoleptic quality of peach and nectarine cultivars, breeding programmes were recently introduced with the aim of producing genotypes with excellent taste, high sugar levels, and balanced sugar/acid ratios (Esti et al., 1997).

After water (85 – 90%), sugars are the next most abundant constituent of peaches and nectarines (Wills et al., 1983). Sugar content of peaches and nectarines may vary considerably with cultivar, maturity and environmental conditions. According to Génard and Souty (1996) the eating quality of peaches and nectarines to a great extent depends on the sweetness, which is related to total sugar content. Crisosto et al. (2002) concluded during an 'in-store' consumer acceptance trial that for 'Elegant Lady' peach and 'Spring Bright' nectarine, consumer acceptance is significantly affected by ripe soluble solids content (RSSC). Later studies by Crisosto and Crisosto (2005) reported that consumer acceptance reached a plateau for the two acidic cultivars 'Elegant Lady' and 'Spring Bright' at a RSSC level of 11 – 12 %, but for the low-acid cultivars 'Ivory Princess' and 'Honey Kist', consumer acceptance increased as RSSC increased without flattening off.

Organic acids are minor components in peach and nectarine fruits, but they make an important contribution to organoleptic quality, in combination with sugars and aromatic compounds (Wang et al., 1993). Sweeney et al. (1970) and Esti et al. (1997) found that consumers are more sensitive to the SSC (soluble solids content): TA (titratable acidity) ratio than to fruit acidity on its own. Fruit acidity is determined by many factors such as cultivar, environmental conditions, canopy position, crop load, ripening, fruit maturity and rootstock (Crisosto et al., 1997). The three major non-volatile organic acids in peaches and nectarines are malic-, citric-, and quinic acid

(Byrne et al., 1991; Wang et al., 1993; Génard et al., 1999; Wu et al., 2002). Very low levels of succinic (Bassi and Selli, 1990; Chapman and Horvat, 1990; Selli and Sansavini, 1995), shikimic (Wang et al., 1990; Wu et al., 2002), fumaric, oxalic and ascorbic acid (Sweeney et al., 1970; Liverani and Cangini, 1991; Wang et al., 1993) have also been reported.

Comparisons in how standard (acidic) and new low-acid cultivars differ in their metabolism have received some attention the past few years (Moing et al., 1998). They reported that malic acid levels during development in low acid cultivars are characterised by the absence of malate accumulation near maturity. At maturity, 'Fantasia' fruit (acidic cultivar) had 3 times higher malic acid content compared to 'Jalousia' (low acid) fruit. Picha et al. (1989) concluded that low acid cultivars contain less malic acid than standard cultivars at any stage of development.

The majority of research on sugar and organic acid concentrations in peaches and nectarines focussed on the quantification during fruit development and at optimum maturity. Very little information is available on how sugars and organic acids change during storage. The aim of this study was to determine the levels of individual sugars and organic acids during maturation and simulated export of new imported nectarine cultivars.

2. Materials and Methods

2.1 Harvest trial

One early season ('Mayglo') and three mid season ('Spring Bright', 'Big Top', 'Red Jewel') nectarine cultivars were used to compare the sugar and organic acid levels in fruit flesh during maturation. These cultivars are all standard acidic, yellow flesh cultivars, except 'Big Top', which is a yellow flesh, low-acid cultivar as described by its breeder (Zaiger Genetics USA, 1219 Grimes Avenue, Modesto, CA. 95358, USA).

'Mayglo' nectarines were obtained from Valence Farm, a commercial stone fruit farm in the Agter Paarl area (33° 40'S, 18° 54'E, 190 m.a.s.l.) in South Africa. Trees on Kakamas seedling rootstock were planted in 1985 at a spacing of 5 m x 3 m and are trained to a closed vase system.

All the other cultivars were obtained from Lushof Farms, a commercial farm in the Warm Bokkeveld area near Ceres (33° 13'S, 19° 20'E, 503 m.a.s.l) in South Africa. 'Spring Bright', 'Big Top' and 'Zeeglo' trees on SAPO 778 clonal rootstock were all planted in 2000 at a spacing of 5 m x 3 m. These three cultivars are all trained and pruned to the Spanish Bush system. 'Red Jewel' trees on the same rootstock were planted in the same year, but are trained to central leader trees and planted 1.5 m x 4.5 m.

Fruit were harvested weekly for four weeks from pre-optimum to post-optimum maturity from six randomly chosen trees in each orchard. Optimum picking maturity is ±9 kg (11.2 mm probe) and was usually between the second and third harvest. At each harvest date, ten fruit were picked from each of six replications (trees). Fruit were then taken to our laboratory in Stellenbosch. The stylar one third of the fruit flesh (including the skin) of each of the ten fruit per replicate was pooled and then immersed in liquid nitrogen and ground to a fine powder in a Waring Blender. The powder was then stored in plastic vials at -80°C until later analysis.

2.2 Storage trial

One early season ('Mayglo'), two mid season ('Spring Bright' and 'Red Jewel') and one late season ('Zeeglo') nectarine cultivar were used in the storage trial. 'Mayglo' nectarines were obtained from Windmill Farm in the Agter Paarl area. 'Spring Bright' and 'Zeeglo' were obtained from Verdun Estates and 'Red Jewel' from Graaff Packing, both in the Warm Bokkeveld area.

Fruit were harvested at commercial harvest maturity (firmness ±9 kg, 7.2 mm probe). On arrival at our laboratory fruit were stored in regular atmosphere (RA) cold storage at -0.5°C and 95 % relative humidity (RA) for 25 days (simulating 7 days accumulation + 18 days shipment). After storage fruit was ripened for 3 days at 18°C

and then went into a 5 day shelf life period at 10°C. Fruit for analysis were sampled on the harvest date, after storage, after ripening and at the end of shelf life. At each stage six replicates of 10 fruit each were used for analysis. Fruit was prepared for analysis as described above.

2.3 Sample extraction and purification

Ten millimetres of distilled water were added to 5 g of frozen fruit sample to extract the sugars and organic acids. The fruit pulp was removed by filtering through a Whatman 4 filter paper and 2 ml of this filtrate was filtered through a 0.8 g C18 cartridge filter (Waters Bulk Packaging Part nr. WAT 020594) to remove all apolar components (anthocyanins, phenols etc.). This filtrate was made up to 10 ml with distilled water and filtered with a 0.45 µm Millipore filter and analysed by high pressure liquid chromatography (HPLC).

2.4 HPLC analysis

The soluble sugars and organic acids were determined using an Agilent 1100 series HPLC with photo-diode array detector (DAD) and refractive index detector (RID) in series. The sugars and acids were separated using an ion exclusion column (300 x 7.8 mm Transgenomic ION 300 column). The column temperature was kept at a constant 29°C with 17 mM H₂SO₄ as eluent and flow rate of 0.4 ml/min. Injection volume was 10 µl. Citric, malic, succinic, shikimic, fumaric and quinic acids were identified and quantified by comparing their retention times and integrated peak heights with that of external acid standards using DAD output at 230 nm (Figure 1).

During the same analysis, the elution of sugars (and acids) was followed by the refractive index detector. Sucrose, glucose, fructose, and sorbitol were identified and quantified by comparing their retention times and integrated peak heights to external sugar standards (Figure 2 and 3).

The running conditions were chosen to minimise the overlap of peaks of the sugars and acids. With these conditions the overlap of peaks at their maximum height was minimal, except for malic acid and sorbitol, which overlapped perfectly. The

concentration of malic acid could, however, be calculated based on the UV-chromatogram. The theoretical contribution of malic acid to the height of the combined malic acid-sorbitol peak could therefore be calculated. The concentration of sorbitol could then be calculated using the peak height (RID) corrected for the presence of malic acid.

To verify the results of the ION 300 column a Phenomenex CapCell Pak C18 MG column (250 mm x 4.6 mm ID, 5 µm particle size) was also used to determine the organic acid content. Results were very similar to those obtained by the ION 300 column (data not shown), except for quinic acid which had a 10% higher concentration on the ION 300.

2.5 Data analysis

A complete randomised design was used for both trials. Data were analysed using the GLM (general linear models) procedure in the Statistical Analysis Systems (SAS), Enterprise Guide 3.0.

3. Results and Discussion

3.1 Harvest trial

Sucrose was the dominant sugar at all four harvest dates and accounted for between 62 – 76 % of total sugars (Figure 4), which is in agreement with Chapman et al. (1991). These results indicate that the fruit was already in the final phase of fruit growth and near optimum maturity (Moriguchi et al., 1990; Vizzotto et al., 1996; Dirlewanger et al., 1999). All cultivars were also characterised by a significant accumulation of sucrose as maturity progressed (Figure 4, Table 1), as was found by Selli and Sansavi (1995), Vizzotto et al. (1996) and Génard et al. (1999). In 'Mayglo' however, the sucrose concentration flattened off towards the final harvest date (Figure 4). The low-acid cultivar, 'Big Top', had significantly higher sucrose levels than any other cultivar, and 'Mayglo' the lowest (Table 2).

Of the reducing sugars, fructose was present in slightly higher concentrations than glucose in all cultivars (Figure 4), as was found by Esti et al. (1997), Versari et al. (2002) and Wu et al. (2003). Fructose and glucose each amounted to less than 15 % of total sugars (Table 2) and both decreased significantly during maturation of all cultivars (Figure 4, Table 1), which is in agreement with the results of Chapman and Horvat (1990), Liverani and Cangini (1991) and Selli and Sansavini (1995). Since glucose and fructose are substrates for growth and respiration, the decrease in concentration during maturation is to be expected (Génard et al., 1999). 'Mayglo' fruit had significantly lower glucose and fructose than any other cultivar, while 'Spring Bright' and 'Red Jewel' contained the highest amount of reducing sugars (Table 2). Robertson et al. (1989) reported that "high quality" peaches contained higher levels of fructose and lower levels of glucose and sorbitol than "low quality" fruit.

Sorbitol levels in 'Big Top' and 'Red Jewel' nectarines were higher than levels of the reducing sugars (Figure 4) and were significantly higher than levels in other cultivars (Table 2). For both these cultivars sorbitol levels increased significantly towards maturity (Figure 4), as was found by Selli and Sansavini (1995). Sorbitol levels varied between 3 – 14 % for different cultivars (Table 2), which is fairly high as Génard et al. (1999), Versari et al. (2002) and Wu et al. (2003) found levels of between 1-5 %. Moing et al. (1998) reported that the phloem sap of peach trees contains equal amounts of sorbitol and sucrose and these are the only sugars in the phloem. The fact that sorbitol content is always low in peach and nectarine fruit suggests that sorbitol is metabolised into reducing sugars by sorbitol oxidase and sorbitol dehydrogenases (Moriguchi et al., 1990). The high sorbitol levels in 'Big Top' and 'Red Jewel' can possibly be explained by an over supply of sorbitol and not enough enzyme activity to metabolise these high levels or by the fact that different cultivars metabolise sorbitol differently (Moriguchi et al., 1990). Sorbitol levels in 'Mayglo' and 'Spring Bright' decreased significantly as maturity progressed (Figure 4, Table 1), confirming the results of Liverani and Cangini (1991), Chapman and Hovat (1990) and Brooks et al. (1993).

Malic, citric and quinic acid were found to be the main acids in nectarines (Figure 5), confirming results published by Sweeney et al. (1970), Wills et al. (1983), Brady (1993), Wang et al. (1993) and Wu et al. (2003). Malic acid was found to be

the dominant acid in all cultivars at optimum maturity (Figure 5) and accounted for more 30 - 48 % of total acids (Table 3) as was found by Liverani and Cangini (1991) and Versari et al. (2002). Malic acid became the principal acid in nectarines as its levels significantly increased towards maturity (Figure 5, Table 1), confirming the results of Liverani and Cangini (1991), Moing et al. (1998) and Wu et al. (2005). Malic acid also accumulated towards maturity in the low-acid cultivar 'Big Top' (Figure 5), which is contrary to what Picha et al. (1989), Byrne et al. (1991) and Moing et al. (1998) found. Malic acid concentration in 'Big Top' was not significantly lower than in 'Red Jewel' (Table 3).

Citric acid was a dominant acid in immature fruit (Figure 5), but levels decreased significantly towards maturity in all cultivars (Chapman and Horvat, 1990; Liverani and Cangini, 1991; and Wu et al., 2002). Citric acid accounted for 30 – 40 % of total acids in standard cultivars, but only for 16 % in the low-acid cultivar 'Big Top' (Table 3), which was significantly lower than any other cultivar (Esti et al., 1997). Pangborn (1963) reported that the taste perception of citric acid appears before that of malic acid and it is perceived as more acidic. This, together with low citric acid levels in 'Big Top', can maybe explain the low-acid taste. Citric acid levels in 'Big Top' were even lower than that of quinic acid (Figure 5, Table 3) (Wu et al., 2003). Génard et al. (1999) concluded that there is an analogy in the sucrose-reducing sugars and malic acid-citric acid pairs, since sucrose and malic acid are storage components, whereas reducing sugars and citric acid are used as substrates for growth and respiration.

Quinic acid accounted for 15 - 35 % of the organic acids in the four cultivars evaluated (Table 3). As mentioned before, for 'Big Top' quinic acid was the second most abundant acid during all harvest dates (Figure 5). 'Red Jewel' had significantly higher quinic acid levels than any other cultivar (Table 3). Quinic acid also significantly decreased towards maturity (Figure 5, Table 1) (Chapman and Horvat, 1990; Chapman et al., 1991; Wang et al., 1993; Wu et al., 2002) and was one of the main acids in immature fruit (Figure 5). This high initial concentration could be responsible for the astringent flavour in immature fruit (Robertson et al., 1989). Quinic and shikimic acid are both important intermediary metabolites connecting carbohydrate metabolism and aromatic biosynthesis (Jensen, 1985). Wu et al. (2002)

also reported that assimilate supply strongly accelerated the decrease in quinic acid concentration, which could be beneficial to the formation of fruit aroma.

Minor acids like succinic, shikimic and fumaric acid were also observed (Figure 5), but were present in very small quantities (<1.0% of total acids) (Table3). Succinic acid was also observed by Bassi and Selli (1990), Chapman and Horvat (1990) and Selli and Sansavini (1995) in similar low concentrations. Wang et al. (1993) reported fumaric acid levels of less than 0.38 %. Fumaric acid levels increased towards maturity in all cultivars (Figure 5). The presence of shikimic acid in all cultivars confirms the results of Wang et al. (1993) and Wu et al. (2002). As fruit matured, the shikimic acid concentration decreased (Figure 5), confirming the results of Wu et al. (2002). Jensen (1985) emphasised the importance of shikimic and quinic acid in the biosynthesis of aromatic compounds, therefore the decreasing shikimic acid levels may be indicative of the accumulation of aromatic compounds (Wu et al., 2002).

3.2 Storage trial

Sucrose was the dominant sugar in all nectarine cultivars at the day of harvest as was found in the harvest trial (Chapman and Horvat, 1990; Chapman et al., 1991; Esti et al., 1997) and remained the main sugar throughout the entire simulated export process (Table 4). 'Mayglo' had a significant decrease in sucrose levels from harvest to the end of shelf life (Table 4).

Fructose was again present in slightly higher concentrations than glucose for all four cultivars (Table 4), as was found by Versari et al. (2002) and Wu et al. (2003). Fructose increased significantly from harvest to the end of shelf life for all cultivars, except 'Zeeglo'. For 'Mayglo' fructose and glucose increased significantly after shipping and then again at the end of shelf life. Fructose levels of 'Spring Bright' increased significantly during ripening and then stayed unchanged during shelf life. 'Red Jewel' also had a significant increase in fructose and glucose during shipping and ripening, but levels of both sugars decreased significantly during shelf life. Higher fructose levels will increase the sweetness and improve the eating quality of nectarines (Pangborn, 1963).

Sorbitol was the least abundant sugar in all cultivars at harvest, except for 'Red Jewel' where sorbitol levels were higher than the reducing sugars (Selli and Sansavini, 1995). Sorbitol levels decreased significantly from harvest to the end of shelf life for all cultivars except 'Zeeglo' (Table 4). For 'Mayglo' and 'Spring Bright' sorbitol levels decreased significantly during ripening and then stayed unchanged during shelf life. Sorbitol levels in 'Red Jewel' decreased significantly during storage and again during shelf life. This could indicate that sorbitol is metabolised to reducing sugars during the simulated export process.

Malic acid was the dominant acid in all cultivars at harvest as was found in the harvest trial (Table 5) (Liverani and Cangini, 1991; Versari et al., 2002). Malic acid concentration decreases significantly from harvest to the end of shelf life for all cultivars (Table 5). For 'Mayglo' and 'Red Jewel' malic acid decreased significantly during shipping and then during shelf life. Malic acid levels in 'Spring Bright' and 'Zeeglo' decreased significantly during shipping and then stayed unchanged for the rest of the simulated export. Malic acid remained the main acid throughout the simulated export for all cultivars, except in 'Red Jewel' where quinic acid became more dominant after shelf life (Table 5).

Citric acid was the second most abundant acid at harvest for all cultivars, except 'Red Jewel' (Table 5) (Chapman and Horvat, 1990; Liverani and Cangini, 1991; and Wu et al., 2002). The citric acid concentration decreased significantly during the simulated export to the end of shelf life for all cultivars, except 'Mayglo' (Table 5). For 'Spring Bright' and 'Zeeglo' citric acid decreased significantly during ripening and then remained stable during shelf life. Citric acid levels for 'Red Jewel' decreased significantly during shipping and ripening.

Quinic acid was the least abundant major acid in all cultivars (Chapman et al., 1991; Wang et al., 1993; Wu et al., 2002), except in 'Red Jewel'. For 'Red Jewel' quinic acid was present at higher levels than citric acid (Table 5). Quinic acid levels increased significantly during the shipping period in 'Spring Bright'. Robertson et al. (1989) and Wu et al. (2003) reported that high quinic acid levels can impart a slight sour and bitter taste to fruit.

The following minor organic acids were observed in samples; shikimic (< 0.3 % of total acids), fumaric (< 0.1 % of total acids) and succinic acid (< 0.9 % of total organic acids) (Table 5). Their concentrations were too low to have any significant influence on flavour and taste properties. The decrease in shikimic acid levels during the export process (data not shown) could indicate the accumulation of aromatic compounds (Wu et al., 2002).

4. Conclusion

The composition of sugars and organic acids are important factors that influence the eating quality of nectarines. Sucrose was the dominant sugar at optimum maturity in all the evaluated cultivars and accumulated significantly during fruit maturation. 'Big Top' had significantly higher sucrose levels than any other cultivar. Of the reducing sugars, fructose was present in slightly higher levels than glucose. Reducing sugar content decreased significantly in all cultivars towards maturity. Sorbitol was usually the least abundant sugar at maturity, except for 'Big Top' and 'Red Jewel' nectarines, where sorbitol was present in higher concentration and accumulated towards maturity, while it significantly decreased for 'Mayglo' and 'Spring Bright'.

Malic acid significantly increased as maturity progressed and became the main acid in all cultivars at optimum maturity. Malic acid accumulation was also observed in the low-acid cultivar 'Big Top'. 'Mayglo' had significantly higher malic acid content than any other cultivar. Citric acid was one of the dominant acids in immature fruit, but decreased significantly towards maturity. Citric acid levels were significantly lower in the low-acid cultivar 'Big Top'. Immature fruit had high quinic acid levels, but it also decreased significantly towards maturity. Quinic acid was the least abundant of the main acids, except in 'Big Top'. 'Red Jewel' contained significantly more quinic acid than any other cultivar. Shikimic, fumaric and succinic acid were also observed in very small quantities.

Sucrose remained the dominant sugar in all cultivars during the simulated export and shelf life, but decreased significantly in 'Mayglo'. Glucose and fructose levels increased significantly for 'Mayglo' and 'Red Jewel', whereas only fructose increased significantly for 'Spring Bright'. Sorbitol levels decreased significantly from harvest

to the end of shelf life for all nectarine cultivars, except 'Zeeglo'. This could indicate that sorbitol is metabolised to reducing sugars during storage.

The malic acid concentration decreased significantly during storage in all cultivars, but remained the dominant acid. Citric acid decreased significantly throughout the simulated export and shelf life for 'Spring Bright', 'Red Jewel' and 'Zeeglo'. Quinic acid content increased significantly during storage for 'Red Jewel and 'Spring Bright'. Shikimic, fumaric and succinic acid were again observed in very small quantities.

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Table 1.

Least significant difference (LSD 5 %) values and significance levels for sugar and organic acids for the harvest trial data presented in Figures 4 and 5.

Cultivar	Malic Acid (g/100g)	Citric Acid (g/100g)	Quinic Acid (g/100g)	Sucrose (g/100g)	Glucose (g/100g)	Fructose (g/100g)	Sorbitol (g/100g)
Mayglo							
LSD (5%)	0.02	0.03	0.03	0.62	0.05	0.06	0.04
<i>Pr>f</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Big Top							
LSD (5%)	0.04	0.03	0.04	1.11	0.08	0.09	0.29
<i>Pr>f</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Spring Bright							
LSD (5%)	0.06	0.04	0.05	0.67	0.10	0.11	0.28
<i>Pr>f</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0004	0.0177
Red Jewel				,			
LSD (5%)	0.05	0.02	0.05	0.41	0.06	0.07	0.16
Pr>f	< 0.0001	< 0.0001	0.0007	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 2.

The average soluble sugars and percentage of total sugars for 'Mayglo', 'Big Top', 'Spring Bright' and 'Red Jewel' nectarines over the four weekly harvest dates from pre-optimum to post-optimum maturity.

Cultivar	Sucrose	Glucose	Fructose	Sorbitol	
	(g/100g)	(g/100g)	(g/100g)	(g/100g)	
Mayglo	3.83 d (68%)	0.81 d (14%)	0.86 c (15%)	0.17 c (3%)	
Big Top	11.17 a (76%)	1.02 c (7%)	1.10 b (7%)	1.45 a (10%)	
Spring Bright	7.45 b (67%)	1.22 a (11%)	1.33 a (12%)	1.20 b (10%)	
Red Jewel	6.15 c (62%)	1.11 b (11%)	1.29 a (13%)	1.36 a (14%)	
LSD (5%)	0.36	0.05	0.05	0.14	
<i>Pr>f</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	



Table 3.

The average concentration and percentage of total acids of organic acids for 'Mayglo', 'Big Top', 'Spring Bright' and 'Red Jewel' nectarines over the four weekly harvest dates from pre-optimum to post-optimum maturity.

Cultivar	Malic	Citric	Quinic	Shikimic	Fumaric	Succinic
	Acid	Acid	Acid	Acid	Acid	Acid
	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)
Mayglo	0.562 a	0.513 b	0.199 c	0.003 ns	0.001 ns	0.010 ns
Maygio	(43%)	(39%)	(15%)	(<1%)	(<1%)	(<1%)
Dig Top	0.476 c	0.159 d	0.353 b	0.003	0.001	0.009
Big Top	(48%)	(16%)	(35%)	(<1%)	(<1%)	(<1%)
Spring Bright	0.519 b	0.400 c	0.380 b	0.003	0.001	0.005
	(40%)	(31%)	(29%)	(<1%)	(<1%)	(<1%)
Red Jewel	0.469 c	0.611 a	0.508 a	0.004	0.000	0.005
	(30%)	(37%)	(32%)	(<1%)	(<1%)	(<1%)
LSD (5%)	0.031	0.023	0.031	ns	ns	ns
Pr>f	<0.0001	<0.0001	<0.0001	0.4133	0.1863	0.0628

Table 4.

The average soluble sugars for 'Mayglo', 'Spring Bright', 'Red Jewel' and 'Zeeglo' nectarines during the simulated export and shelf life.

C. Iv	Sucrose	Glucose	Fructose	Sorbitol
Cultivar	(g/100g)	(g/100g)	(g/100g)	(g/100g)
Mayglo				
Harvest	5.68 a	0.71 c	0.69 c	0.08 a
After shipping ^x	5.38 ab	0.80 b	0.86 b	0.09 a
After ripening ^y	4.92 c	0.78 b	0.86 b	0.04 b
End of shelf life ^z	5.04 bc	0.84 a	0.97 a	0.04 b
LSD (5%)	0.44	0.33	0.05	0.01
Pr>f	0.0069	< 0.0001	< 0.0001	< 0.0001
Spring Bright				
Harvest	6.78 ab	1.01 ns	1.15 a	0.35 a
After shipping ^x	6.18 b	0.99	1.18 a	0.29 a
After ripening ^y	7.03 a	1.06	1.32 b	0.21 b
End of shelf life ^z	6.79 ab	1.01	1.28 b	0.16 b
LSD (5%)	0.68	ns	0.07	0.07
Pr>f	0.0932	0.2288	0.0002	<.0001
Red Jewel		Contract of the second		
Harvest	8.30 ab	1.00 c	1.17 d	1.36 a
After shipping ^x	8.22 ab	Pectura culu au 13 b ti	1.39 c	1.19 b
After ripening ^y	8.59 a	1.29 a	1.62 a	1.11 b
End of shelf life ^z	7.89 b	1.12 b	1.51 b	0.71 c
LSD (5%)	0.50	0.07	0.08	0.13
Pr>f	0.0594	<.0001	<.0001	<.0001
Zeeglo				
Harvest	8.71 ns	0.84 ns	0.96 ns	0.33 ns
After shipping ^x	8.43	0.83	0.99	0.28
After ripening ^y	8.12	0.81	1.01	0.26
End of shelf life ^z	8.56	0.85	1.06	0.25
LSD (5%)	ns	ns	ns	ns
Pr>f	0.7919	0.8650	0.4444	0.2526

x – Storage for 25 days (7 days accumulation + 18 days shipment) at -0.5°C

y – Ripening for 3 days at 18°C

z - Simulated shelf life for 5 days at 10°C

Table 5.

The average organic acid concentration for 'Mayglo', 'Spring Bright', 'Red Jewel' and 'Zeeglo' nectarines during the simulated export and shelf life.

Cultivar	Malic	Citric	Quinic	Shikimic	Fumaric	Succinic
	Acid	Acid	Acid	Acid	Acid	Acid
	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)
Mayglo						<u> </u>
Harvest	0.707 a	0.460 ab	0.224 a	0.002	0.000	0.013 a
After shipping ^x	0.614 b	0.498 a	0.186 b	0.002	0.000	0.003 b
After ripening ^y	0.588 bc	0.452 b	0.174 b	0.002	0.000	0.003 b
End of shelf life ^z	0.562 c	0.466 ab	0.230 a	0.002	0.000	0.010 a
LSD (5%)	0.040	0.037	0.020	-	-	0.003
Pr>f	< 0.0001	0.1355	< 0.0001	-	-	< 0.0001
Spring Bright						
Harvest	0.468 a	0.318 a	0.227 b	0.002	0.000	0.004 b
After shipping ^x	0.363 b	0.280 a	0.218 b	0.002	0.000	0.003 b
After ripening ^y	0.359 b	0.238 b	0.247 a	0.001	0.000	0.009 a
End of shelf life ^z	0.354 b	0.196 b	0.251 a	0.001	0.000	0.006 a
LSD (5%)	0.040	0.037	0.019	-	-	0.004
<i>Pr>f</i>	< 0.0001	< 0.0001	0.0028	-	-	< 0.0001
Red Jewel		C				
Harvest	0.783 a	0.398 a	0.433 b	0.002	0.000	0.001 c
After shipping ^x	0.462 b	0.299 b	0.441 b	0.002	0.000	0.001 c
After ripening ^y	0.433 b	0.234 c	0.477 a	0.002	0.000	0.004 b
End of shelf life ^z	0.371 c	0.224 c	0.451 b	0.002	0.000	0.007 a
LSD (5%)	0.037	0.032	0.023	-	-	0.003
<i>Pr>f</i>	< 0.0001	< 0.0001	0.0054	-	-	< 0.0001
Zeeglo						
Harvest	0.597 a	0.351 a	0.147 ns	0.003	0.001	0.008 a
After shipping ^x	0.465 b	0.292 ab	0.149	0.002	0.000	0.005 b
After ripening ^y	0.431 b	0.247 b	0.151	0.002	0.000	0.008 a
End of shelf life ^z	0.437 b	0.234 b	0.166	0.002	0.000	0.001 c
LSD (5%)	0.071	0.063	ns	-	-	0.003
<i>Pr>f</i>	0.0002	0.0043	0.1175	-	-	< 0.0001

x-Storage for 25 days (7 days accumulation + 18 days shipment) at -0.5 $^{\circ}C$

y – Ripening for 3 days at 18°C

z - Simulated shelf life for 5 days at $10^{\circ}C$

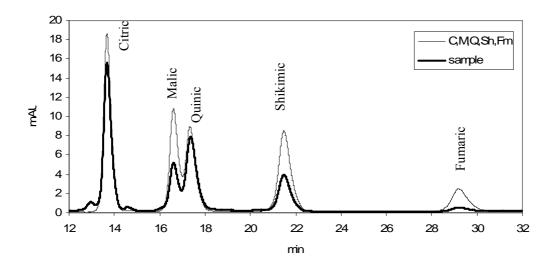


Figure 1.

Chromatogram (DAD) of citric, malic, quinic, shikimic and fumaric acids standards (C, M, Q, Sh, Fm) superimposed over a chromatogram of a typical nectarine sample. (ION 300 column)

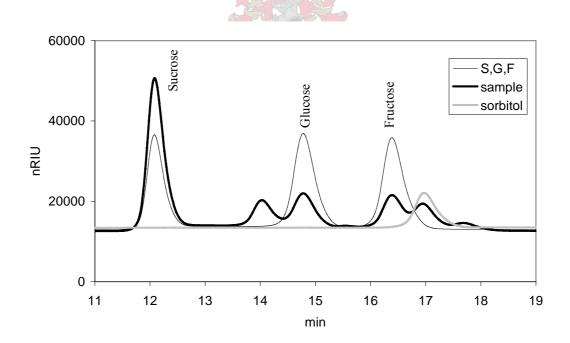


Figure 2.

Chromatogram (RID) of sucrose, glucose, fructose and sorbitol standards (S, G, F) superimposed over a chromatogram of a typical nectarine sample. (ION 300 column)

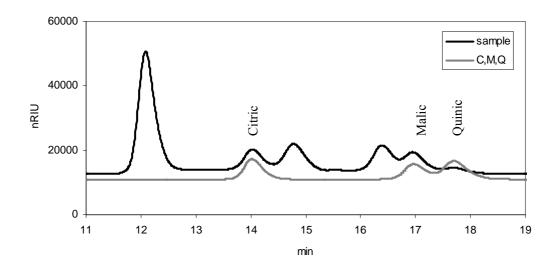


Figure 3.

Chromatogram (RID) of citric, malic and quinic acids standards (C, M, Q) superimposed over a chromatogram of a typical nectarine sample. (ION 300 column)



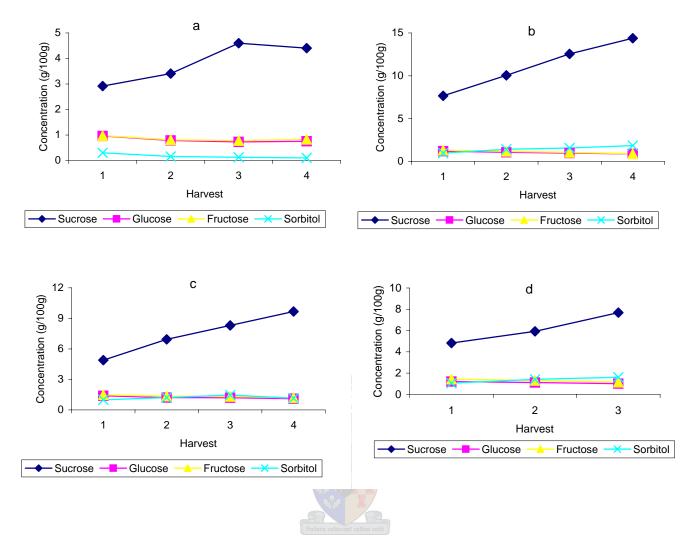


Figure 4. Soluble sugar concentrations for four nectarine cultivars viz. (a) Mayglo, (b) Big Top, (c) Spring Bright and (d) Red Jewel. Harvest dates were from pre-optimum (1) to post-optimum (4) maturity.