Assessing the effect of a new growth regulator on the storage life of Pink Lady apples

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Assessing the effect of ReTain (AVG) on the storage life of Pink Lady™ Apples - AP02031.

Horticulture Australia Project No. AP02031

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16th July 2005

Executive Summary

ReTain® plant growth regulator is a commercial formulation of aminoethoxyvinylglycine hydrochloride (AVG). This compound is known to competitively inhibit the activity of the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) which is the rate limiting enzyme in the ethylene biosynthetic pathway. By inhibiting the activity of ACS, ethylene mediated ripening processes can be delayed.

Pink Lady™ apples were sourced from two growing districts (Shepparton, Victoria and Lenswood, South Australia). These fruit were treated with ReTain® at two application times, 21 and 7 days before the commercial harvest date and harvested at two maturities; maturity 1 (starch pattern index 2, a harvest index for long term storage) and maturity 2 (starch pattern index 4, a harvest index not suited for long term storage and better suited to the fresh market). The samples were stored at 0°C and assessed for quality attributes at harvest and after 2, 4 and 6 months storage in air and after 2, 4, 6, 8 and 10 months in CA storage (2% O_2 and 1% CO_2). The quality attributes assessed at each removal included internal ethylene concentration (IEC), flesh firmness, starch pattern index (SPI), %TSS, background colour and skin greasiness. Naturally occurring fruit volatiles were also measured after 6 months storage. A shelf life assessment was also carried out at 6 months for the first harvest of fruit from Lenswood, South Australia.

The results indicated that treatment with ReTain® significantly reduced the IEC of fruit from both districts which correlated to higher flesh firmness throughout storage than was observed in untreated control fruit. ReTain® treatment 21 days before harvest (DBH) resulted in an extended harvest period in both Shepparton and Lenswood which can be a significant commercial benefit for apple growers. Treatment with ReTain® 7 DBH for fruit grown in Lenswood and harvested at maturity 1 had the additional benefit of a 2 month extension of storage life due to the retention of flesh firmness. The benefits of treatment with ReTain® were maintained during the 14 day shelf life assessment. The research results showed that the main advantages of applying ReTain® for Pink Lady™ apple growers were the improvement of harvest management as well as the improvement of postharvest fruit quality.

During 6-11 June 2004, Dr John Golding presented this work at the 5th International Postharvest Symposium in Verona Italy, with funding from Horticulture Australia Ltd. (Project AP02031) and Valent BioSciences as a voluntary contribution. NSW Agriculture also supported his time in this project. His presentation entitled, 'ReTain™ Maintains Pink Lady™ Fruit Quality during Long Term Storage', drew large interest both at the presentation and after the allotted time. Many pertinent questions were fielded, particularly from researchers from Europe.

This was the largest postharvest conference ever held and provided a valuable and worthwhile forum for discussion of the current trends and future directions in postharvest science. A major focus of the conference was product quality and ripening physiology. Apples were the major crop of interest at the conference with 74 presentations, but stonefruit, citrus and cutflowers were also well represented.

Contents

Introduction

Apples are commercially produced worldwide and production has increased dramatically over the last ten years (FAO, 2003; O'Rourke, 2003). Over the last ten years there has also been a rise in the number of varieties grown. Varieties such as Pink Lady™, Gala and Fuji are increasing in popularity (O'Rourke, 2003). Production throughout Australia remains low on the worldwide scale with the main areas of production being Victoria and New South Wales who's combined production accounts for over half of Australia's total apple production (ABS, 2003).

It is important to note that the premium new variety Pink Lady™ was developed in Western Australia from a cross between the Lady Williams and Golden Delicious cultivars (Cripps et al., 1993). The fruit are medium in size (70 to 75 mm in diameter) with 40% to 70% pink/red blush over a green-yellow background colour (Cripps et al., 1993). The flesh of the fruit is firm and perceived by consumers to be crunchy and crisp (Cripps et al., 1993). It also has a distinct flavour being fruity and sweet with a balance of acid flavour. Pink Lady™ apples are late maturing with a long storage life in air at 0 to 1^{o}C of up to 4 months.

Apples are climacteric fruit which means that they experience a burst of ethylene production after harvest (Wills et al, 1998). Ethylene triggers ripening responses such as softening of the flesh and the development of volatile flavour compounds (Jerie et al, 1978). Ethylene is also responsible for regulating the ripening process. Ripening in apples involves the coordination of a number of biochemical and physiological changes which include changes in the sugar and acid balance and cell wall composition as well as changes in the pigmentation and the generation of aroma related organic compounds and changes in metabolism and enzyme activity associated with fruit ripening (Phan-Thien et al., in review). The ripening process in apples can be quantified using a number of maturity indices including the rate of ethylene production, changes in flesh firmness, starch distribution, percentage soluble solids, background colour and skin greasiness (Little and Holmes, 2000).

The stage of fruit ripening is an important parameter of fruit quality. In apples it is also an indicator of the optimal time of harvest (Huybrechts et al, 2003) which is of economic importance for both fruit growers and consumers. The timing of the apple harvest is an extremely critical time as the physiological age of apples at harvest determines their storage life. It is often difficult to harvest the entire crop at the right time for optimal quality and storage. Delaying harvest can lead to increases in fruit quality characteristics, most importantly the amount of red blush for Pink Lady™ apples. Improved colour can lead to greater economic returns. However, a delay in harvest will mean a reduction in the storage potential of the fruit (Byers and Eno, 2002). For long term storage, fruit need to be harvested before they begin producing ethylene. This compromise is a difficult practical and economic issue for all apple growers.

Ethylene is a key component of apple ripening. Ethylene is a plant hormone that controls many plant developmental processes (McKeon et al, 1995; Fluhr and Mattoo, 1996). The biosynthesis of ethylene follows a well established pathway from methionine → S-adenosylmethionine → 1-aminocyclopropane-1-carboxylic acid → ethylene (Kende, 1993; McKeon et al, 1995; Fluhr and Mattoo, 1996; Alexander and Grierson, 2002). The process is catalysed by several enzymes. The 2 main enzymes being 1-aminocyclopropane-1-carboxylic acid oxidase and 1-aminocyclopropane-1-carboxylic acid synthase. The formation of 1-aminocyclopropane-1-carboxylic acid is understood to be the rate limiting step in the biosynthesis pathway (Roberts and Hooley, 1988).

Aminoethoxyvinylglycine hydrochloride (AVG) is a plant growth regulator commercially registered as ReTain®. It's application competitively inhibits 1-aminocyclopropane-1-carboxylic acid production and consequently inhibits ethylene production (Roberts and Hooley, 1988; Dussi et al, 2002; Green, 2003). The application of ReTain® offers the potential for delaying the onset of fruit ripening. ReTain® has been demonstrated to give several benefits for apple growers. It can reduce preharvest fruit drop, delay ripening and reduce the loss of quality in storage (Byers, 1997; Park et al, 1999; Brackman and Waclawovsky, 2001; Valent BioSciences, 2001; Phan-Thien et al., in review). There has been a significant amount of research work done using AVG in the 1980's (Bramlage et al., 1980; Autio and Bramlage, 1982; Halder-Doll and Bangerth, 1987) and later on with the commercial application of ReTain®. Never the less, there are still issues relating to the optimum timing of application for maximum benefit as well as the possible differences in response between different districts.

The aim of this study was to assess the postharvest effect of ReTain® on quality characteristics (internal ethylene concentration (IEC), firmness, starch pattern index (SPI), percentage total soluble solids (%TSS), background colour and skin greasiness) of Pink Lady apples™ stored in air for 6 months at 0^oC as well as evaluating its sensitivity to application time, harvest maturity and growing district.

Material and Methods

Source of fruit and treatments

The apples used in this experiment were harvested from fifteen year old trees grown on M106 rootstocks on a straight trellis and were the cultivar Pink Lady™ grown in Shepparton, Victoria and Lenswood, South Australia. Agrisearch Services applied the AVG treatment in the orchard. ReTain® (soluble powder; 150 g aminoethoxyvinylglycine/kg, Valent Biosciences Corporation) was applied as a rate of 83 g/100 L together with the 0.1% MAXX organosilicone surfactant (Sumitomo Chemical Company Pty Ltd).

For both districts, ReTain® was applied to part blocks at 21 DBH and to the remaining blocks at 7 DBH (25/03/2003 and 09/04/2003 in Shepparton, Victoria and 31/03/2003 and 15/04/2003 in Lenswood, South Australia) a third block was left as an untreated control (UTC). The application date was determined using district historical commercial harvest data as well as a combination of quality factors including fruit size, colour, firmness and the SPI of samples of fruit. ReTain® was applied using a power-operated hand gun incorporating a single Spraying Systems GC 3009 solid cone nozzle. The hand gun used a pressure of 1500 kPa and was calibrated to deliver 6.5 L/min. Each plot was sprayed at a rate of 1000 L/ha.

Each treatment was harvested at 2 maturities; maturity 1 (M1) at a SPI of 2 and maturity 2 (M2) with a SPI of 4, as measured on the ENZA 7 point starch chart. The harvest dates for all treatments of the 2 districts are given in table 1. The fruit were packaged into single layer fruit trays and cooled to 2^oC. The samples were transported to Sydney Postharvest Laboratory overnight and the next morning the fruit were set up in the storage experiment. On arrival at Sydney Postharvest Laboratory, one set of fruit were stored in air and the other set was stored in CA.

The CA storage trial was carried out at the NSW Department of Agriculture Research facility at Gosford. Pink Lady™ fruit were stored at 0^oC in a flow through CA system at 2% O_2 and 1% CO_2 with each replicate in a separate 60L steel drum. The gas atmospheres were established by mixing N_2 produced by an air separator (Calor™) with CO_2 from a compressed industrial CO_2 cylinder (BOC Gases, Sydney). Any ethylene contamination in the flow through system was scrubbed from the inlet gas streams by passing through a series of 4 x 5 L chambers filled with Purafil®. The gas mixture was humidified twice by ventilating through water in drums and distributed to CA drums using a capillary flow board system at a flow rate sufficient to provide 0.4 chamber void volumes per hour. A flow through system using a paramagnetic O_2 transducer (Servomex Series 1100) and an infra-red gas analysis (Horibal PIR 2000) were employed to monitor drum O_2 and CO_2 levels, respectively. Calibrations were performed at regular intervals throughout the experiment using standard CO_2 mixture, air, and CO_2 and O_2 free air.

For both the air and CA storage regimes 80 fruit from each treatment for the air trial and 120 for the CA trial were randomly selected and divided into replicates of 20 fruit. For each harvest date, a sample of 20 fruit was assessed on arrival and the rest of the fruit were placed in storage at either 0°C in air for 6 months or in controlled atmosphere storage (2% oxygen and 1% carbon dioxide) at 0°C for 10 months. Samples of 20 fruit were removed from storage and assessed at 2, 4 and 6 months after harvest in the air trial and after 2, 4, 6, 8 and 10 months. All the samples were left for 3 hours to warm to room temperature (21°C) before evaluation.

Table 1 The dates of application of ReTain® and the harvest dates for the 2 maturities at Shepparton, Victoria and Lenswood, South Australia.

Treatment	Shepparton, Victoria	Lenswood, South Australia
Application of ReTain® applied 21 DBH	25/03/2003	31/03/2003
Application of ReTain® applied 7 DBH	09/04/2004	15/04/2003
Harvest of M1, UTC	16/04/2003	17/04/2003
Harvest of M1, ReTain® applied 7 DBH	16/04/2003	17/04/2003
Harvest of M1, ReTain® applied 21 DBH	23/04/2003	23/04/2003
Harvest of M2, UTC	29/04/2003	30/04/2003
Harvest of M2, ReTain® applied 7 DBH	01/05/2003	02/05/2003
Harvest of M2, ReTain® applied 21 DBH	05/05/2003	02/05/2003

Fruit quality assessment postharvest

At each removal time, samples of 20 fruit from each treatment were assessed for flesh firmness, %TSS, SPI, background colour and skin greasiness, for the assessment of IEC, samples of 10 fruit were used.

The concentration of internal ethylene was measured using a 0.5 ml gas sample extracted from the core space of the apple and analysed using a gas chromatograph (Flame ionisation detector, Shimadzu GC-17A, GS-Q column J & W Scientific with helium as the carrier gas). As the measurement was non-destructive, the same 10 fruit were included as part of the 20 fruit used in the additional quality assessments.

Flesh firmness was measured using a drill-press mounted Effegi penetrometer fitted with an 11 mm tip (the measures of firmness (kg) were converted to Newtons: N = kg x 9.807). Two measurements were taken for each piece of fruit, from the blushed and unblushed sides of the fruit equator, after the skin was removed. A sample of juice was also taken from each piece of fruit and the %TSS was measured using a bench top refractometer.

Background colour was measured using a Ctifl Pink Lady™ Colour chart (produced by Ctifl, France) for samples of 20 fruit. A score of 2 indicated a green background colour and a score of 7 indicated a yellow background colour. Previous work has indicated that measuring hue angle using a Minolta colour meter is less accurate for blushed apples such as the Pink Lady™ (Jobling, pers. comm.).

SPI was rated for samples of 20 fruit using the Enza Fruit SPI 7 point chart for apples. A score of 0 indicated a high level of starch distribution and a score of 6 being a low level of starch distribution. Skin greasiness was recorded for each fruit using a 3 point scale (0 indicating no greasiness, a score of 1 indicating slight greasiness and a score of 2 indicating high greasiness). Any storage or internal disorders were also recorded.

Volatile analysis

After the 6 months removal for both air and CA storage samples were prepared for volatile analysis. A 5 gram section from five fruit per treatment was taken. The sample was quickly crushed into a saturated sodium chloride solution to prevent enzyme activity and then the samples were frozen at -20°C for later analysis.

Each sample was frozen in a 40mL glass centrifuge vial. The samples were thawed at room temperature prior to analysis. The concentration of volatiles in the headspace was analysed using the solid phase micro-extraction (SPME) method as described by Matich *et al*, 1996. The samples for time 0 were measured by Sydney Postharvest Laboratory using a Shimadzu GC-17A flame ionisation chromatograph with a SGE vitreous silica capillary chromatography column (30m, 0.25 mm *i.d*) and helium as the carrier gas at a flow rate of 0.5 mL min^{-1}. The oven temperature was 50°C for 2 min, increasing by 10°C min^{-1} to 220°C and held for 2 mins. The injector port and detector temperatures were 200 and 300°C respectively. Standard volatile mixtures were used to identify retention times of key peaks.

The 4 and 7 day samples were analysed by Food Science Australia. They used the same gas chromatograph conditions using a Hewlett Packard 5890 GC and the volatiles were identified by GC/MS analysis and peaks were identified using the library spectra (VG70-250S mass spectrometer, VG Instruments, Manchester, UK).

Data analysis

The IEC, flesh firmness, %TSS and volatile concentrations were analysed using an analysis of variance and least significant differences (5%) calculated using the general linear model procedure in SimStat (Provalis Corporation. IEC was transformed using a log transformation due to the enzyme nature of the reaction. For measurements that involved scoring data, background colour, SPI and greasiness, an ordinal logistic regression was performed in Minitab statistical software (release 13.32, Minitab Incorporated) for the air stored samples.

The results for each district have been presented on figures with the same Y axis scale to allow for easier comparison of the data.

Results for the Storage Trial – combined data for the districts

Effect of growing district on the storage quality of Pink Lady™ apples treated with ReTain®

The following data compares the effect of treatment with ReTain® between the two districts in the trial Shepparton, Victoria and Lenswood, Victoria. There was a significant district effect for most of the parameters measured. The only factor which didn't have a significant district effect was the level of internal ethylene for the fruit stored in air (Figure 1).

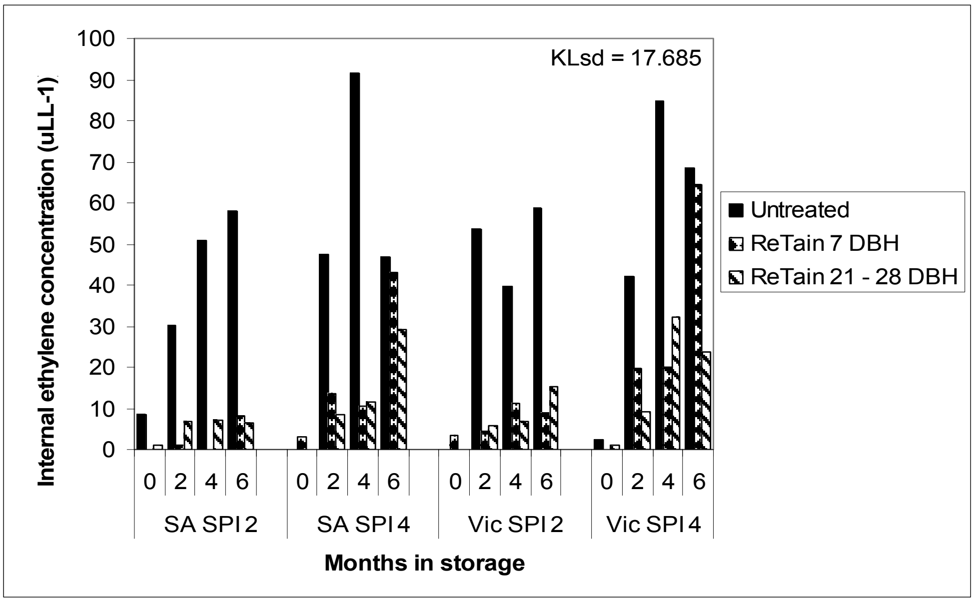

Figure 1. Mean IEC of Pink Lady™ apples grown in Lenswood, South Australia (SA) and Shepparton, Victoria (Vic) harvested at two maturities (SPI = 2 and SPI =4) during postharvest storage period in air at 0°C for 6 months. Values represent means of 60 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

Treatment with ReTain® at 7 and 21 – 28DBH significantly reduced the level of internal ethylene compared to the untreated control fruit. After 6 months storage in air at 0°C there was no significant difference between the 7 DBH treatment and the untreated fruit for the second harvest of fruit (SPI = 4). The reduction in ethylene production was more significant for the CA stored fruit (Figure 2), the reduction of ethylene production by ReTain® continued for 10 months under CA.

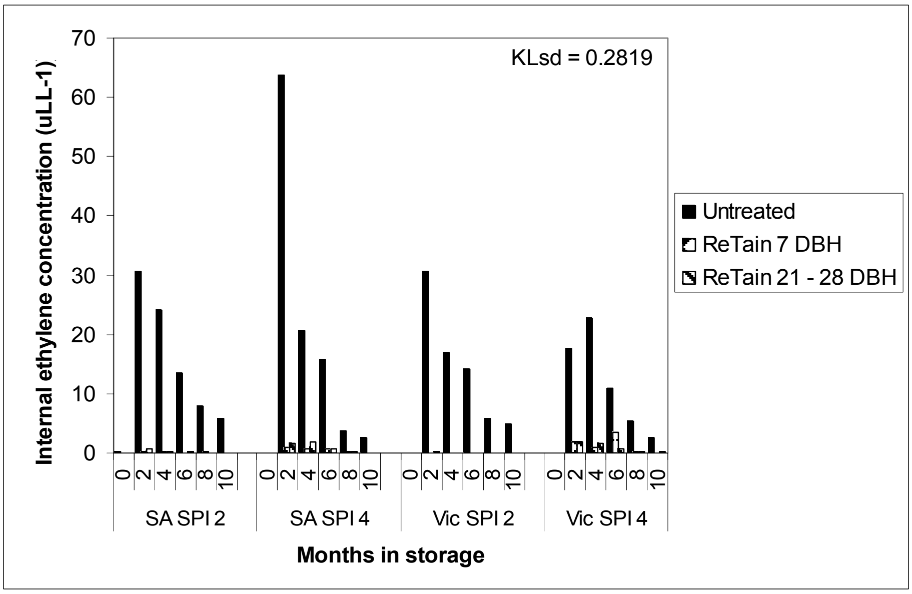

Figure 2. Mean IEC of Pink Lady™ apples grown in Lenswood, South Australia(SA) and Shepparton, Victoria (Vic) harvested at two maturities (SPI = 2 and SPI =4) during postharvest storage period in CA (2% O_2 and 1% CO_2) at 0°C for 10 months. Values represent means of 60 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

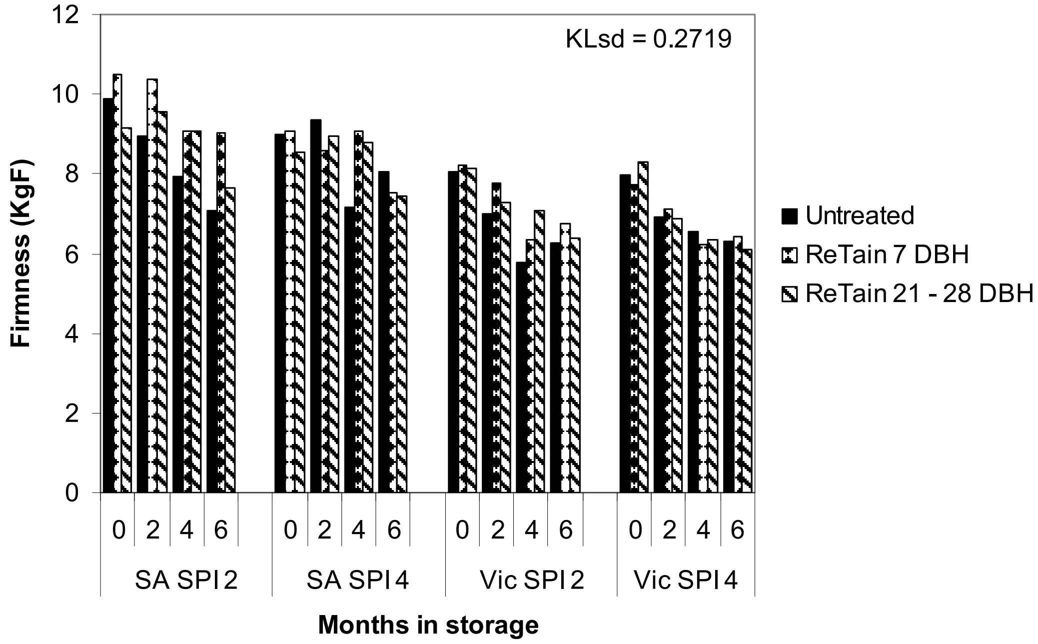

Figure 3. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia(SA) and Shepparton, Victoria (Vic) harvested at two maturities (SPI = 2 and SPI =4) during postharvest storage in air at 0°C for 6 months. Values represent means of 120 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

There was a significant effect of district, treatment and maturity on the flesh firmness of fruit stored in air (Figure 3). Treatment with ReTain® had a significant effect on reducing the loss of firmness of fruit harvested at an SPI 2 (Figure 4). There was no difference in firmness of the treated and untreated harvested at SPI 4 (Figure 5).

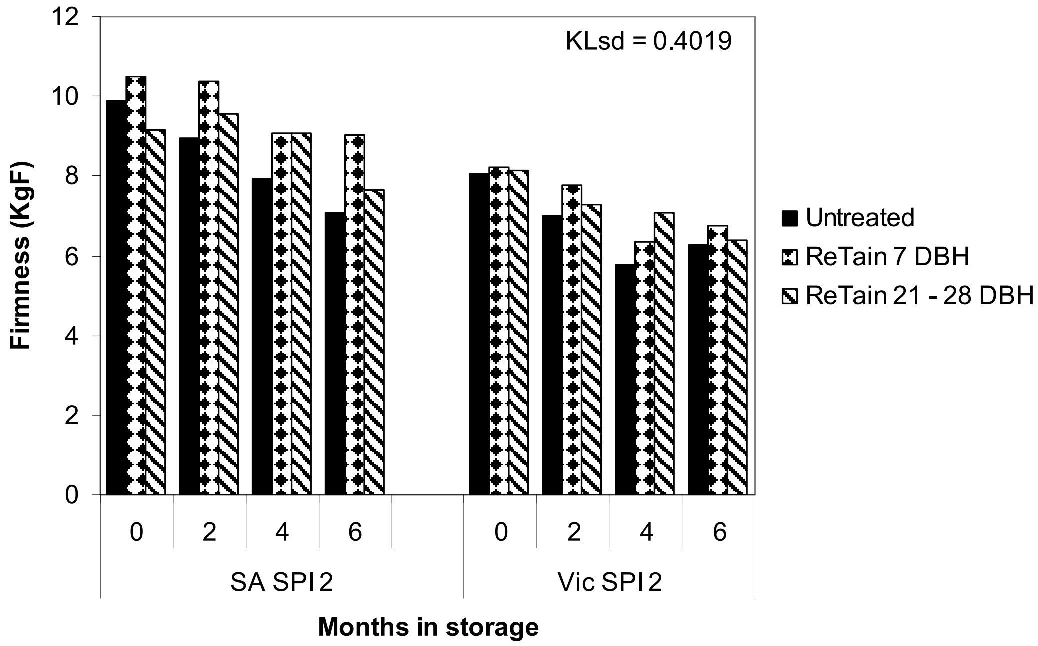

Figure 4. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria harvested at a SPI 2 during postharvest storage in air at 0^oC for 6 months. Values represent means of 120 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

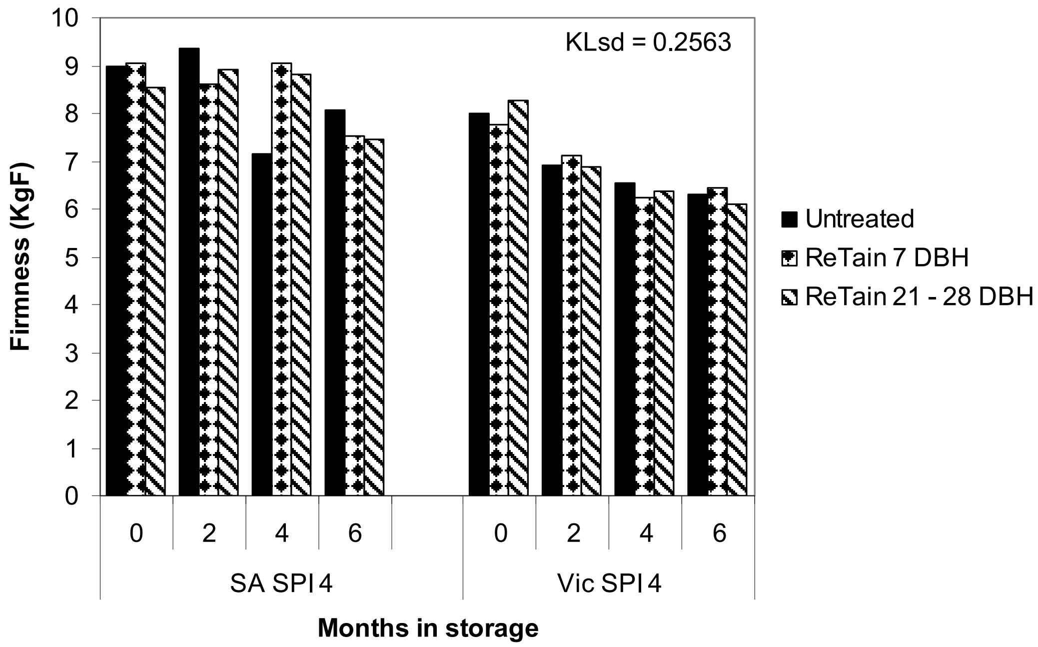

Figure 5. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria harvested at a SPI 4 during postharvest storage in air at 0^oC for 6 months. Values represent means of 120 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

There was also a similar relationship for firmness of CA stored fruit (Figure 6).

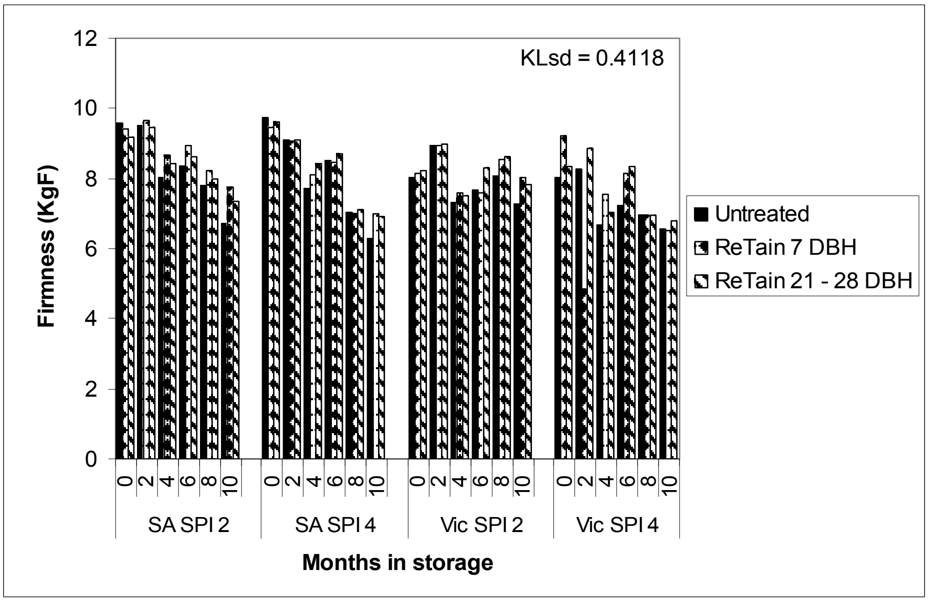

Figure 6. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria during postharvest storage in CA (2% O_2 + 1% CO_2) at 0^oC for 10 months. Values represent means of 120 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

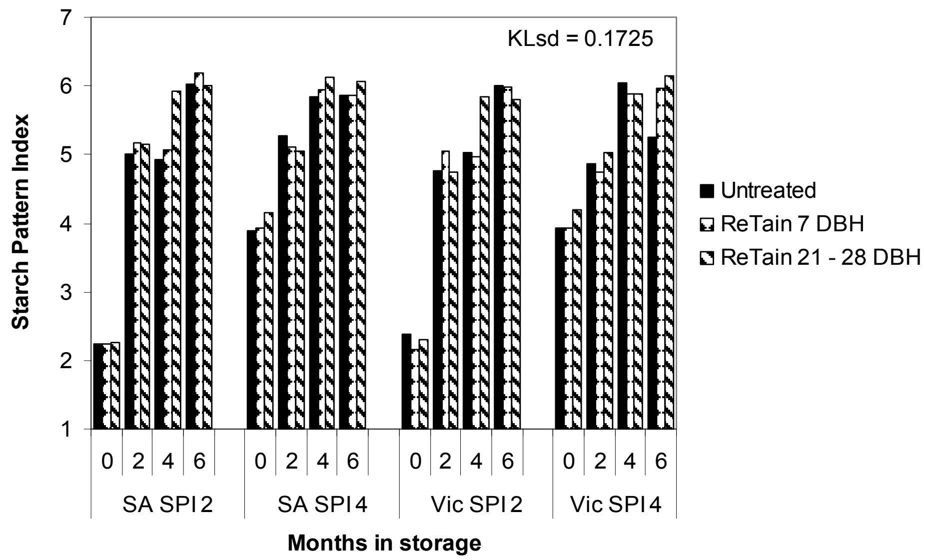

Figure 7. Mean SPI score of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria during a postharvest storage period in air at 0^oC for 6 months. Values represent means of 120 replicates. SPI was measured on the ENZA fruit 7 point scale, a score of 0 indicated high starch distribution and a score of 6 indicated a low starch distribution. Data analysed using an ordinal logistic regression.

ReTain® didn't have much effect on the rate of change in starch score (Figure 7). There was a significant district effect in the rate of starch loss especially at 2 months.

The main difference was between SPI levels, with SPI of 2 there was a large increase in %TSS after 2 months due to conversion of starch to sugar and then a drop in %TSS at 4 months. There was a district effect at SPI with fruit from South Australia declining in %TSS after 2 months but for Victorian fruit the %TSS stayed relatively constant.

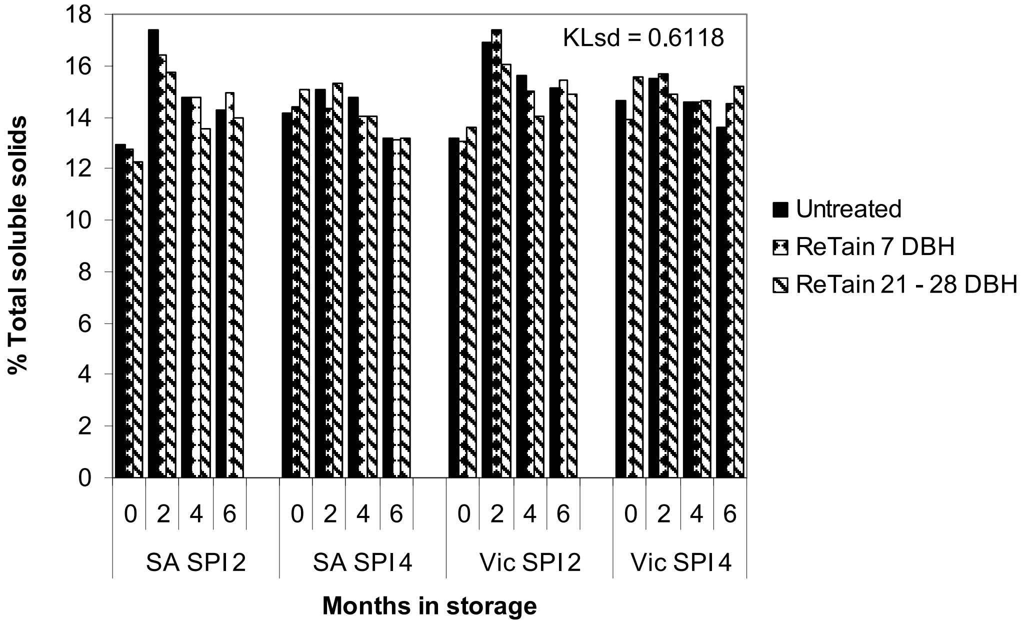

Figure 8. Mean %TSS of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria during a postharvest storage period in air at 0°C for 6 months. Values represent means of 120 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

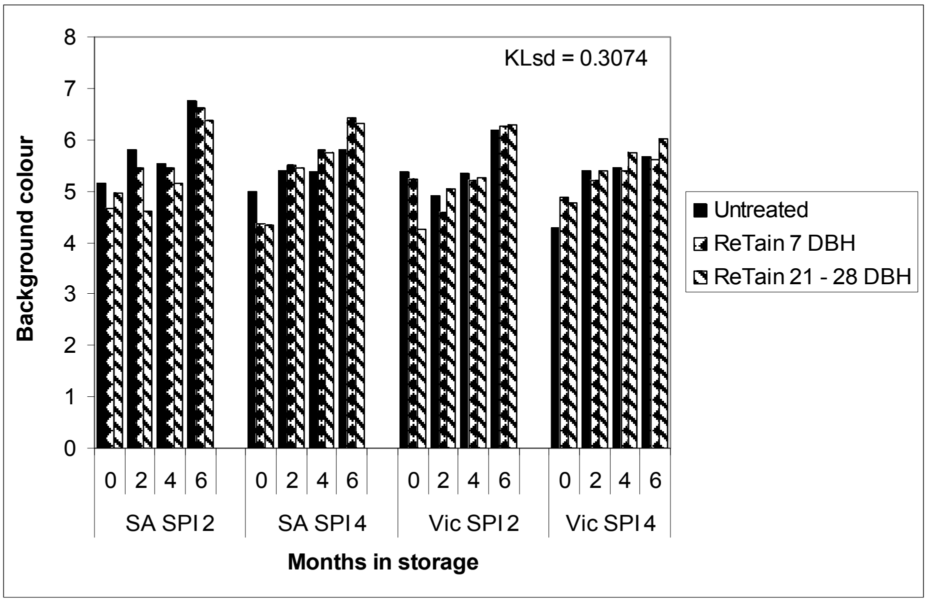

Figure 9. Mean background colour score of Pink Lady™ apples grown in Lenswood, South Australia and Shepparton, Victoria during a postharvest storage period in air at $0^{o}C$ for 6 months. Values represent means of 120 replicates. Background colour was assessed using a Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour.

There was no significant effect of harvest date or Retain in terms of changes to background colour (Figure 9). There was a trend for yellowing to increase over storage time. Also, there was a district effect, with the South Australian fruit having a faster rate of yellowing of the background colour than the Victorian fruit. The effect was most dramatic for the fruit harvested at an SPI 2.

These results show that the main effect of ReTain® for both districts is to control the maturity on the tree prior to harvest. The 21 – 28 DBH treatment resulted in an extended harvest period in both Shepparton and Lenswood which can be a significant commercial benefit for apple growers. Treatment with ReTain® 7 DBH for fruit grown in Lenswood and harvested at maturity 1 had the additional benefit of a 2 month extension of storage life due to the retention of flesh firmness. This effect was true for both the air and CA stored fruit (not all data shown) with the effect being more dramatic for the air stored fruit.

There was a significant district effect for most parameters and so the districts were also analysed separately for all parameters. The results are discussed in the following sections.

Effect of ReTain® on quality parameters of Shepparton, Victoria fruit

The ANOVA showed a significant difference between districts. The data was re-analysed for each district separately in an effort to understand the differences in response of the fruit from Victoria and South Australia to the ReTain® treatments. The ANOVAs performed for IEC, firmness and %TSS all showed a significant three way interaction between maturity, time and treatment ($p<0.001$ for all parameters). Significant 2 way interactions were also found. The following results present the findings when the main effects were analysed as well as significant individual data points from the ANOVA which included all interactions.

For the fruit grown in Shepparton, the results showed a significant overall difference in internal ethylene content (IEC) between the 2 maturities, with the level of IEC for M2 being significantly higher than the level for M1 ($p<0.001$) (Figure 7). The IEC increased significantly over time ($p<0.001$). The greatest increase was from 0 to 2 months where the IEC increased from 0.35 ppm to 10.35 ppm (Figure 7). After 4 months in storage, the IEC of the treated fruit harvested at M1 were significantly lower than any other treatment, at this time the UTC fruit showed the highest mean IEC (Figure 7). The IEC continued to increase to a maximum level after 6 months in air storage at $0^{o}C$ (16.75 ppm) however this increase was not significantly different to the IEC level after 4 months storage. Treatment with ReTain® was found to have a significant effect on IEC ($p<0.001$), with treated fruit having a significantly lower IEC than the UTC fruit. The UTC fruit produced the highest overall treatment mean IEC (16.41 ppm). Treatment with ReTain® 7 DBH produced the lowest overall treatment mean IEC (3.13 ppm), however, this was not statistically different from treatment 21 DBH which resulted in an overall treatment mean IEC of 3.51 ppm.

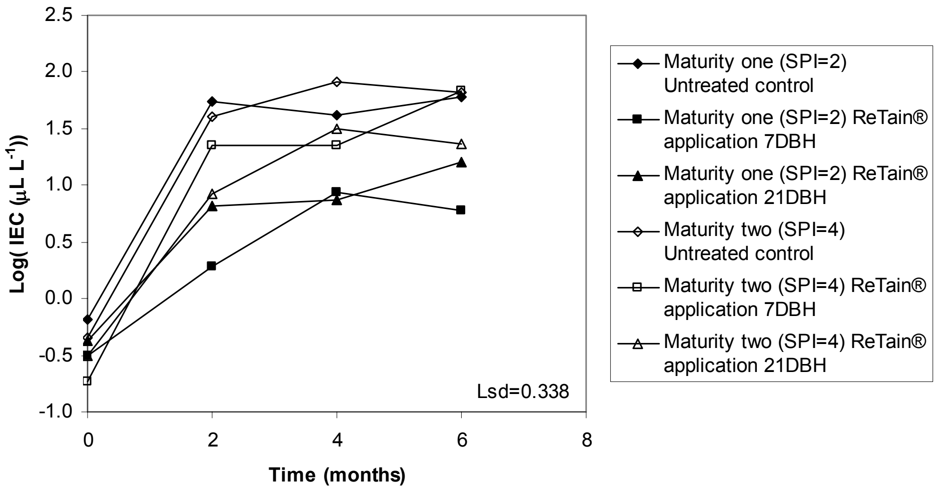

Figure 7. Mean IEC of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at $0^{o}C$ for 6 months. Values represent means of 10 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Flesh firmness is often related to the IEC. Fruit with a high IEC is usually softer than those with a lower IEC (Little and Holmes, 2000). When the main effects of flesh firmness only were analysed, the results showed that a significant difference in flesh firmness was found between the 2 maturities with M1 being significantly firmer than M2 ($p=0.007$) (Figure 8). After 2 months in storage, fruit treated 7 DBH and harvested at M1 had the highest mean firmness than any other treatment. Flesh firmness of all treatments decreased significantly throughout the experiment ($p<0.001$). The mean flesh firmness of the fruit decreased significantly from a mean of 78.5 N at time 0 to 69.2 N after 2 months in air storage at $0^{o}C$. The mean flesh firmness further significantly decreased after 4 months in storage to a mean value of 62.8 N and remained steady thereafter (Figure 8). Treatment with ReTain® was found to have a significant effect on flesh firmness ($p=0.003$). The UTC fruit had the lowest mean firmness (66.7 N) and the treatment with ReTain® 7 DBH resulted in the highest overall treatment mean flesh firmness (70.6 N). Treatment with ReTain® 21 DBH was also significantly different from the UTC and resulted in an overall treatment mean flesh firmness of 68.6 N which was not statistically different from treatment 7 DBH.

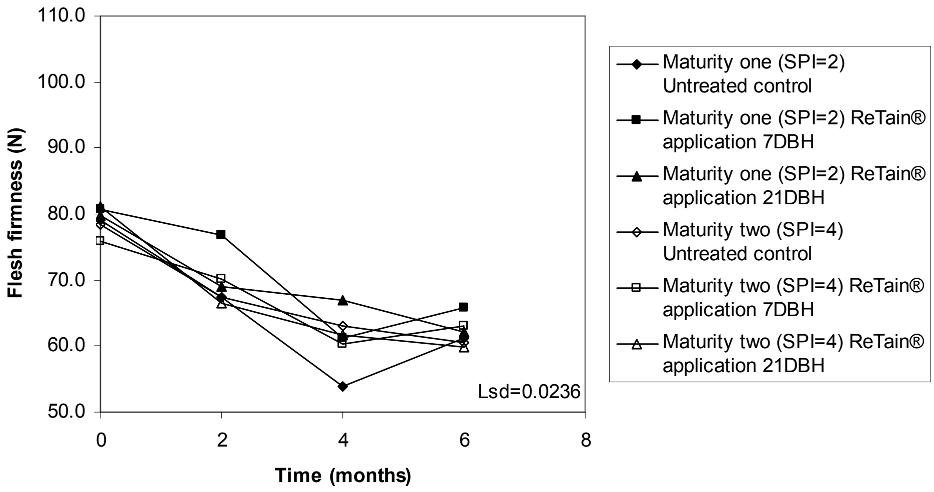

Figure 8. Mean flesh firmness of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at 0°C for 6 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Figure 9 shows the changes in the SPI. The results showed that a significant difference overall in SPI was found between the 2 maturities (p=0.000) with M2 being significantly higher than M1. The main difference was at harvest when fruit were specifically harvested at M1 with an SPI of 2 and M2 with an SPI of 4 (Figure 9). SPI increased significantly over time, the greatest increase was after 2 months in air storage at 0°C where the overall mean SPI increased from 3.2 to 4.9 units. After this time SPI increased at a more moderate rate, reaching a maximum mean level after 6 months in storage (5.85 units). Treatment with ReTain® significantly affected the SPI. Treatment with ReTain® at both 21 DBH and 7 DBH resulted in a higher overall treatment mean SPI than the UTC, however fruit treated 21 DBH resulted in the highest overall treatment mean SPI (5.02 units).

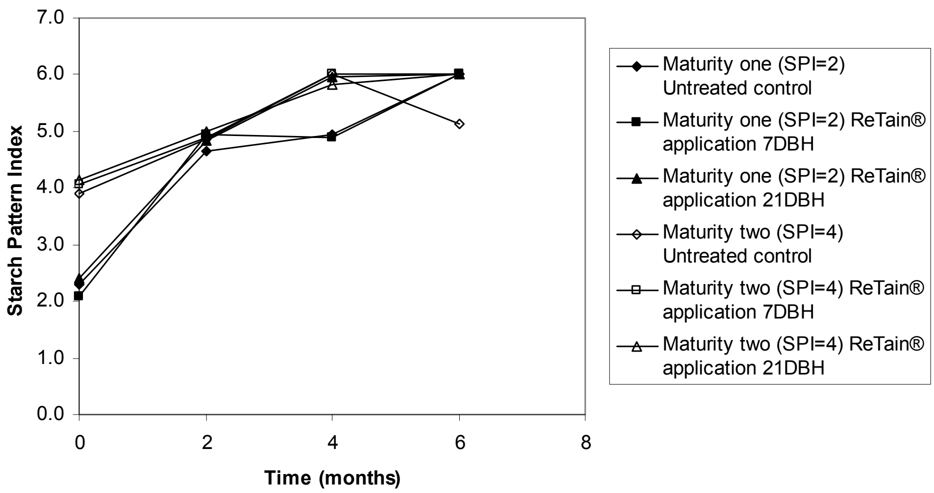

Figure 9. Mean SPI score of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at 0°C for 6 months. SPI was measured on the ENZA fruit 7 point scale, a score of 0 indicated high starch distribution and a score of 6 indicated a low starch distribution. Values represent means of 20 replicates. Data analysed using an ordinal logistic regression.

The %TSS for the fruit showed some significant differences (Figure 10). The results showed a significant overall difference between the 2 maturities with M1 having a significantly higher %TSS than M2 ($p<0.001$). The %TSS changed significantly over time ($p<0.001$) and increased to a maximum of 16.1 % after 2 months in air storage at 0^oC and decreased significantly thereafter to 14.8 % after 6 months storage, which was not significantly different from the level after 4 months. Treatment with ReTain® did not significantly affect %TSS, however treatment with ReTain® 7 DBH resulted in the highest overall treatment mean %TSS (14.9%).

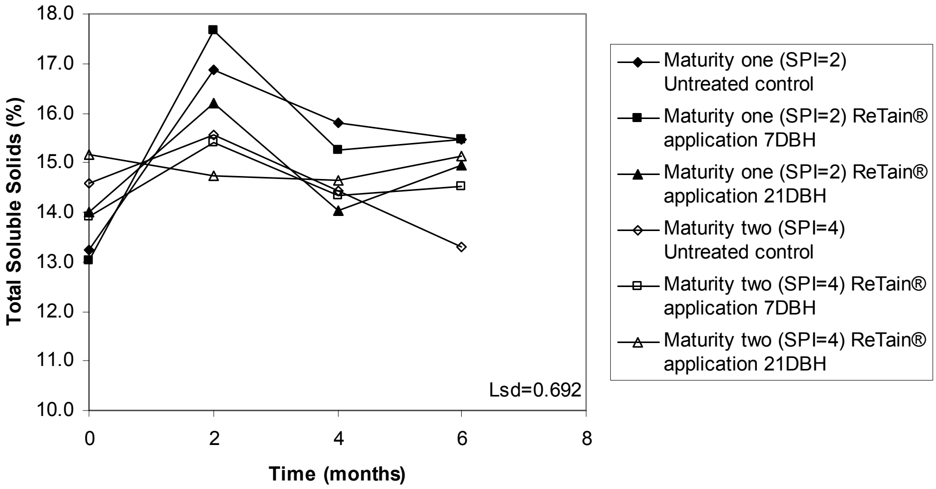

Figure 10. Mean %TSS of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at 0^oC for 6 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

The analysis of the change in mean background colour showed no significant difference between the 2 maturities ($p=0.999$). However, the overall mean background colour increased significantly over time from a mean level of 4.8 units at time 0 to 6.0 units after 6 months in air storage at 0^oC (Figure 11). Treatment with ReTain® did not have a significant effect on background colour.

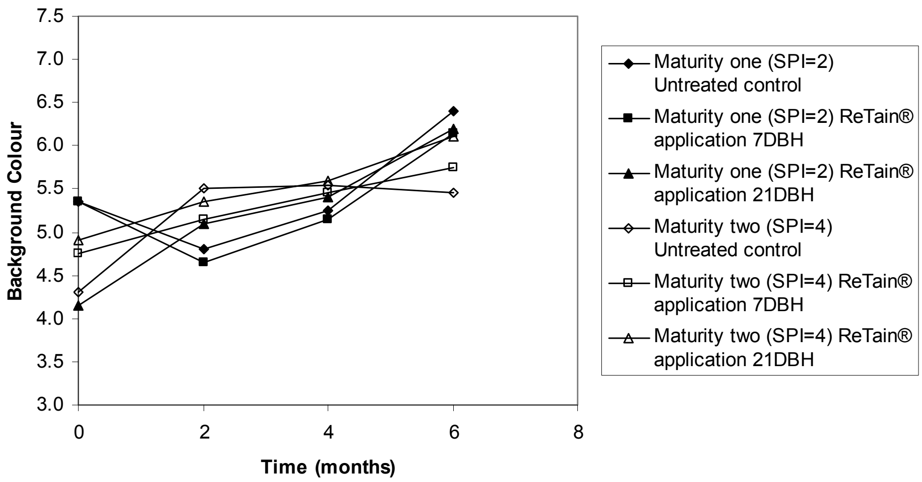

Figure 11. Mean background colour score of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at 0^oC for 6 months. Background colour was assessed using a Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour. Values represent means of 20 replicates. Data analysed using an ordinal logistic regression.

There was a significant overall difference in skin greasiness between the 2 maturities (p=0.000) with M2 being greasier than M1 (Figure 12). The skin greasiness increased over time reaching a maximum mean level (0.56) after 6 months in air storage at 0^{o}C. Treatment with ReTain® was found to have a significant effect on greasiness, the UTC showed the lowest overall treatment mean incidence (0.04) compared to treatment with ReTain® 21 DBH having the highest overall treatment mean score (0.254). The incidence for all treatments is very low representing a difference in the range of none to slightly greasy and as such should be interpreted with care.

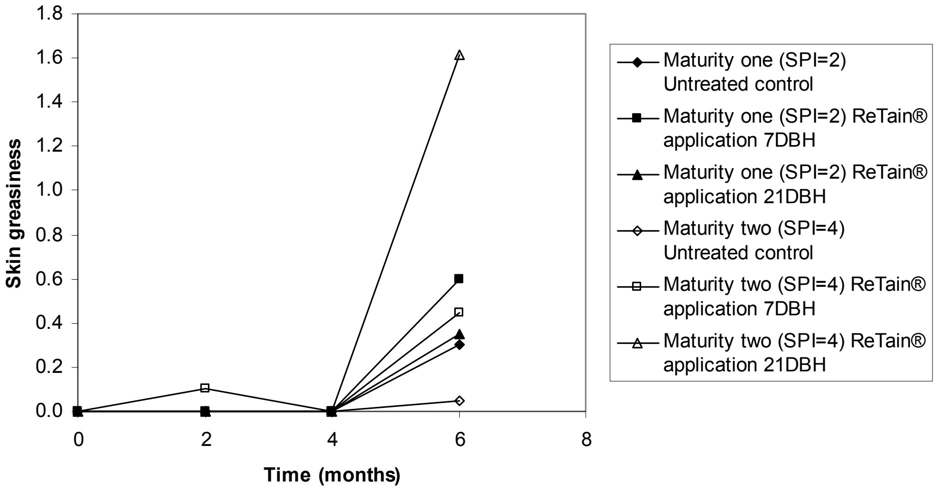

Figure 12. Mean skin greasiness score of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in air at 0^{o}C for 6 months. Values represent means of 20 replicates. Skin greasiness was assessed on a 3 point scale, 0 indicated low greasiness and 2 indicated high skin greasiness. Data analysed using an ordinal logistic regression.

Effect of ReTain® on quality parameters of Lenswood, South Australia fruit

The treatment effects were quite different for the South Australian grown fruit compared to the Victorian fruit. The ANOVAs performed for IEC, firmness and %TSS all showed a significant three way interaction between maturity, time and treatment (p=0.046, p<0.001 and p=0.055 respectively). Significant 2 way interactions were also found. The following results present the findings when the main effects were analysed as well as significant individual data points from the ANOVA which included all interactions.

The results showed that there was a significant overall difference in internal ethylene concentration (IEC) between the 2 maturities, with M2 being significantly higher than M1 ($p=0.007$) (Figure 13). The IEC increased significantly over time ($p<0.001$) for all treatments. The greatest increase was from 0 to 2 months, where the overall mean IEC increased from 0.64 ppm to 9.10 ppm. The IEC continued to increase to a maximum level after 6 months in air storage at 0^oC (14.66 ppm) however this increase was not significantly different to the IEC level after 2 or 4 months. After 6 months in storage ReTain® treated fruit (both 7 DBH and 21 DBH) and harvested at M1 showed a significantly lower mean IEC than any other treatment (Figure 13). Treatment with ReTain® was found to have a significant effect on IEC ($p<0.001$) with treated fruit having a significantly lower IEC than the UTC fruit. The UTC produced the highest overall treatment mean IEC (18.16 ppm). Treatment with ReTain® 21 DBH produced the lowest overall treatment mean IEC (2.86 ppm) however this was not statistically different from treatment 7 DBH which resulted in an overall treatment mean IEC of 3.05 ppm.

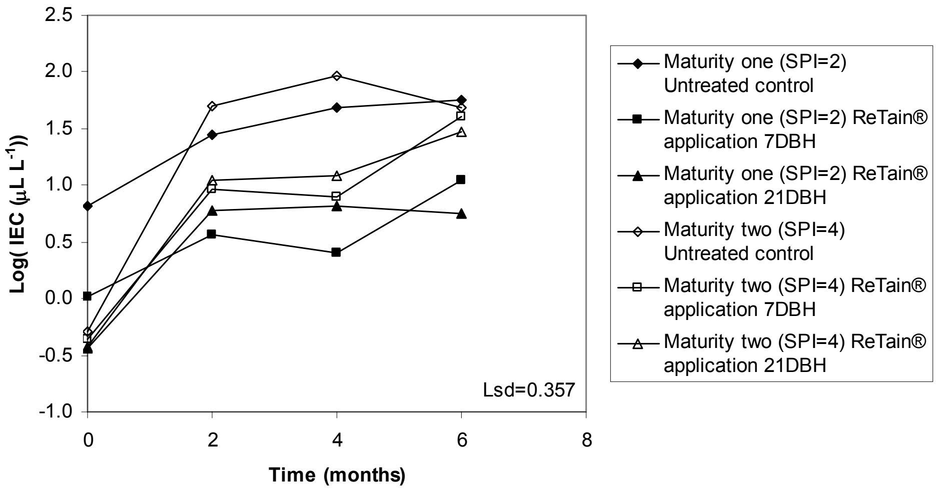

Figure 13. Mean IEC of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at 0^oC for 6 months. Values represent means of 10 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Figure 14 shows that there was a significant overall difference in the overall mean flesh firmness between the 2 maturities with M1 being significantly firmer than M2 ($p<0.001$). Flesh firmness decreased significantly over time ($p<0.001$) for all treatments. For both maturities the flesh firmness remained steady for the first 2 months in air storage at 0^oC with a loss of only 1 N being observed. After 4 months in storage, the overall mean flesh firmness had significantly reduced from 91.2 N at time 0 to 83.4 N. At this time, the UTC fruit from both maturities had the lowest mean firmness while the treated fruit had a significantly higher firmness. After 6 months storage, the overall mean flesh firmness reached its lowest value of 76.5 N. At this time fruit treated with ReTain® 7 DBH and harvested at M1 were significantly firmer than any other treatment (Figure 14) .Treatment with ReTain® was found to have a significant effect on firmness ($p<0.001$). The UTC fruit had the lowest overall treatment mean firmness and treatment with ReTain® 7 DBH resulted in the highest overall treatment mean firmness (89.2 N). Treatment with ReTain® 21 DBH was also significantly different from the UTC and resulted in an overall treatment mean flesh firmness of 83.4 N.

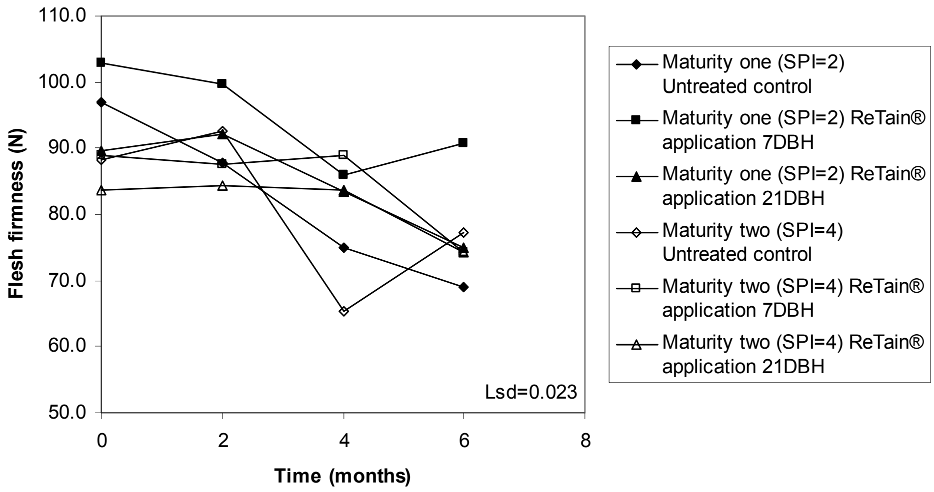

Figure 14. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at $0^{o}C$ for 6 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

The starch pattern index (SPI) showed a significant overall difference between the 2 maturities (p=0.000) with M2 being significantly higher than M1 (Figure 15. The main difference between the two maturities was a rapid increase in the SPI for M1 fruit harvested at SPI2 between 0 and 2 months. From 2 to 6 months storage, basically all the fruit behaved the same with a gradually increase in SPI.

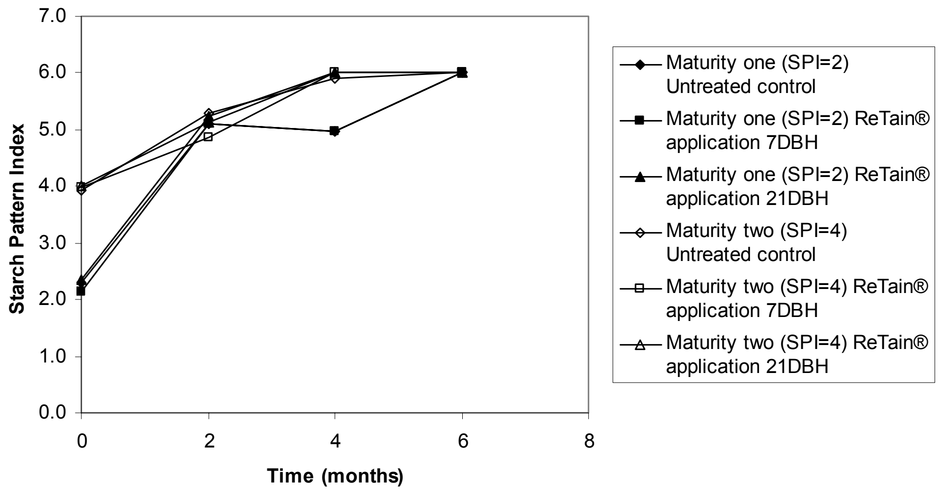

Figure 15. Mean SPI score of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at $0^{o}C$ for 6 months. SPI measured on the ENZA fruit 7 point scale, a score of 0 indicated high starch distribution and a score of 6 indicated a low starch distribution. Values represent means of 20 replicates. Data analysed using an ordinal logistic regression.

There was no overall significant difference in %TSS between the 2 maturities (p=0.926). However, there was a rapid incease in %TSS at 2 months for SPI2 fruit as starch converted to sugars, this dropped by 4 months and 4 and 6 months %TSS were similar. Maturity one fruit had higher %TSS at 6 months with Retain treated fruits having higher %TSS than the UTC.

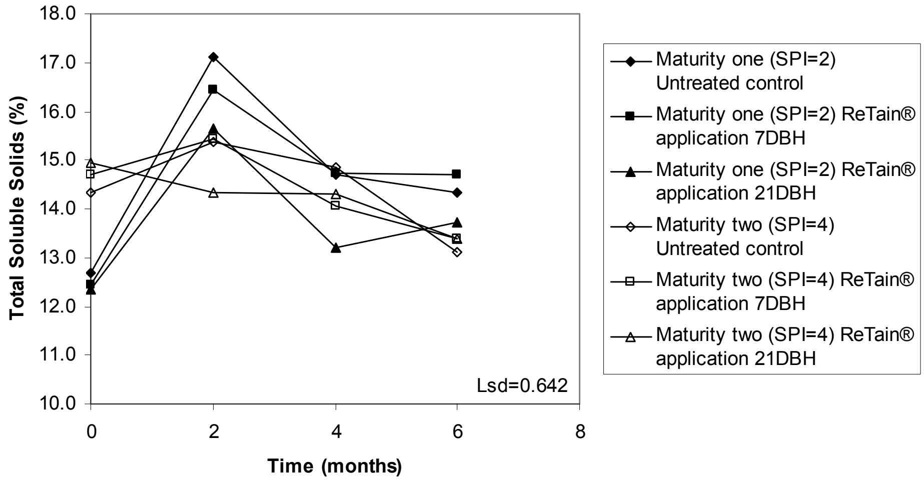

Figure 16. Mean %TSS of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at 0°C for 6 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Figure 17 shows the changes in background colour. The results showed that there was no significant overall difference in background colour between the 2 maturities (p=0.119). The background colour increased significantly over time from a mean level of 4.75 units at time 0 to 6.4 units after 6 months in air storage at 0°C. Treatment with ReTain® had a significant effect on background colour with treatment 21 DBH having a significantly lower overall treatment mean background colour (5.4 units) than UTC (5.6 units) and fruit treated 7 DBH (5.5 units) (p=0.001).While the treatment effect was statistically significant, a difference in fruit background colour of 0.2 units does not represent a highly significant difference on a background colour score ranging from 2 to 7 units.

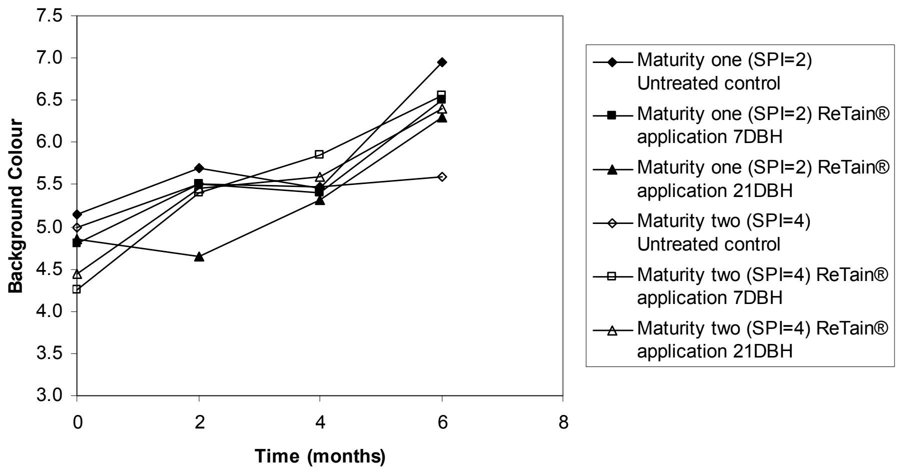

Figure 17. Mean background colour score of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at 0°C for 6 months. Background colour assessed using Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour. Values represent means of 20 replicates. Data analysed using an ordinal logistic regression.

The skin greasiness of the fruit from South Australia changed significantly over time and there was also a significant difference between the 2 harvest maturities (Figure 18). The results showed that a significant difference in greasiness was found between the 2 maturities (p=0.000) with M2 showing a higher score than M1. The greasiness increased over time reaching a maximum mean level after 6 months in air storage at 0^{o}C (1.05 units). Treatment with ReTain® was found to have a significant effect on greasiness with treatment 7 DBH having a lower overall treatment mean greasiness (0.338 units) than UTC (0.619) (p=0.000), while fruit treated 21 DBH showed the highest overall treatment mean incidence (0.66 units). However, after six months storage for M2 the most greasy fruit were the UTC and 21 DBH Retain, suggesting that Retain may not have a strong effect. Similarly to the results from Victoria, the incidence for all treatments is very low, representing a difference in the range of none to slightly greasy and as such should be interpreted with care.

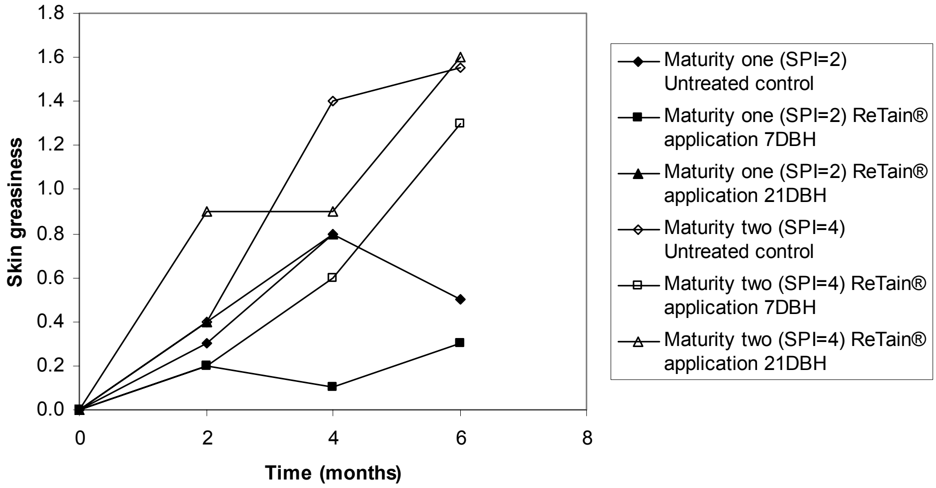

Figure 18. Mean skin greasiness score of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in air at 0^{o}C for 6 months. Values represent means of 20 replicates. Skin greasiniess was assessed on a 3 point scale, 0 indicated low greasiness and 2 indicated high skin greasiness. Data analysed using an ordinal logistic regression.

Difference between the Shepparton, Victoria and Lenswood, South Australia fruit

One factor that could play a role in determining fruit structure and ripening is district seasonal climate. The daily minimum, maximum and mean temperatures, the difference between the daily minimum and maximum daily temperature, the mean daily evaporation rate and the mean monthly rainfall show interesting differences between the 2 sites.

There was a higher mean daily maximum temperature in Shepparton, Victoria compared to Lenswood, South Australia for all months of the year (Figure 19). The highest mean daily maximum temperature in Shepparton was in February (29.4°C), while the lowest was in July (12.8°C). The highest mean daily maximum temperature for Lenswood was also in February (25.7°C), while the lowest was also in July (11.4°C), both of these temperatures were lower than in Shepparton, Victoria. Shepparton had a higher mean daily minimum temperature for the summer months, December, January and February while Lenswood had a higher mean daily minimum temperature for the rest of the year. The highest mean daily minimum temperature for Shepparton was in February (14.3°C), while the lowest was in July (2.8°C).

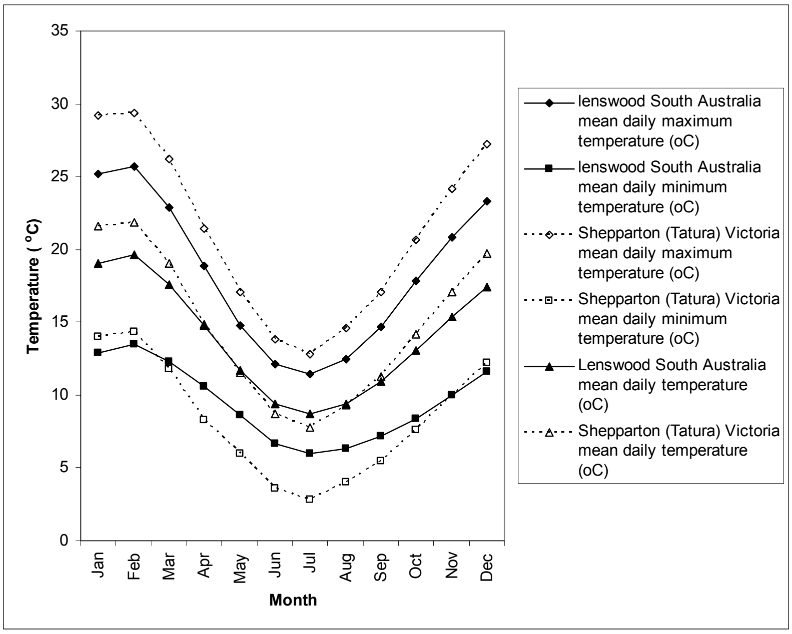

Figure 19. Mean daily minimum and maximum temperature and mean daily temperature for Lenswood, South Australia and Shepparton, Victoria (source: Commonwealth Bureau of Meteorology).

The diurnal temperature difference is an important factor around harvest. A large difference between the maximum and minimum daily temperature encourages the development of red blush (Little and Holmes, 2000). The development of red blush between the 2 districts showed some differences (Figures 20, 21, 22 and 23). The climate data showed that Shepparton had a greater difference between the daily minimum and maximum temperature than Lenswood for all months of the year (Figure 24). The difference between the daily minimum and maximum temperature for Shepparton in January was 15.2°C while for Lenswood in January it was 12.3°C. This would suggest that the fruit from Shepparton should have a higher percentage of blush than the fruit from Lenswood. This was not found to be the case (Figures 20, 21, 22 and 23).

Figure 20. Red blush development of Pink Lady™ apples grown in Shepparton, Victoria and harvested at a SPI score of 2.

Figure 21. Red blush development of Pink Lady™ apples grown in Shepparton, Victoria and harvested at a SPI score of 4.

Figure 22. Red blush development of Pink Lady™ apples grown in Lenswood, South Australia and harvested at a SPI score of 2.

Figure 23. Red blush development of Pink Lady™ apples grown in Lenswood, South Australia and harvested at a SPI score of 4.

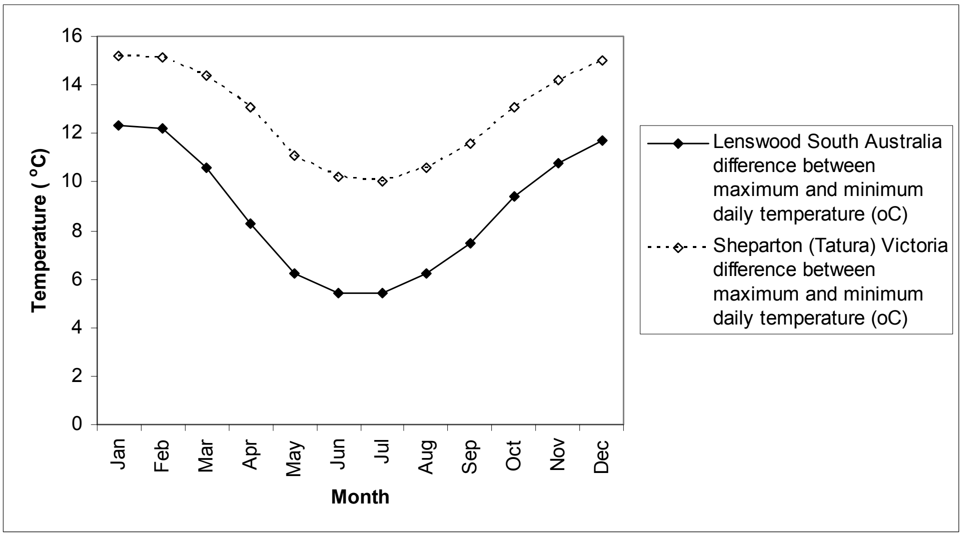

Figure 24. Mean difference between mean daily minimum and maximum temperature for Lenswood, South Australia and Shepparton, Victoria (source: Commonwealth Bureau of Meteorology).

The year 2002/2003 was a drought year for both of these districts, with evaporation being higher than rainfall in the summer months. However, both sites were irrigated. The climate data showed that Shepparton had a higher mean daily evaporation than Lenswood for the months from September through to April (Figure 25). The highest mean daily evaporation for Shepparton was in 7.1 mm in January and the highest mean daily evaporation for Lenswood was 6.3 mm, also in January. Lenswood had a higher mean monthly rainfall than Shepparton from March to December and a higher mean monthly rainfall overall. The highest mean monthly rainfall for Shepparton was in July (49.3 mm) while the lowest was in February (30.8 mm). The highest mean monthly rainfall for Lenswood was in August (148.8 mm) while the lowest was in February (28.3 mm). This data was taken from climate averages for both districts (BOM, 2003).

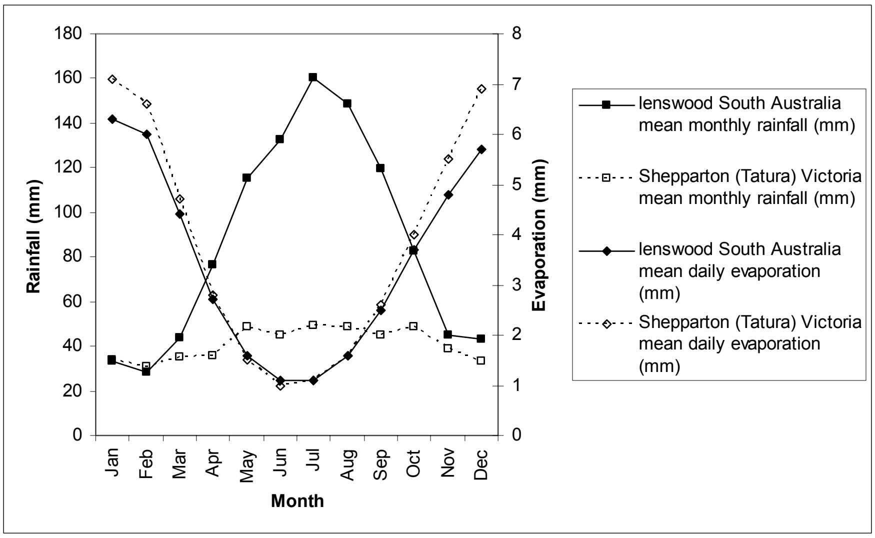

Figure 25. Mean monthly rainfall and mean daily evaporation for Lenswood, South Australia and Shepparton, Victoria (source: Commonwealth Bureau of Meteorology).

Results for the Controlled Atmosphere Storage Trial

Effect of growing district on the storage quality of Pink Lady™ apples treated with ReTain®

The results showed significant differences between the two growing districts for all of the quality parameters measured in this experiment. There were significant differences in internal ethylene levels, flesh firmness, %TSS background colour and skin greasiness between the two districts. There were also significant two way and three way interactions between treatment, maturity and time in storage. The trends between districts are similar to those reported for the air stored fruit (Section 3.1).

Effect of ReTain® on quality parameters of Shepparton, Victoria fruit

In CA storage, the untreated control (UTC) fruit of both harvest maturities displayed the characteristic ethylene climacteric behaviour, with a peak of ethylene production during 2 months storage at 0C (Figure 26). A peak internal ethylene concentration of about 30 - 50 $\mu L.L^{-1}$ was followed by the commensurate decline during storage. This is typical ripening behaviour for non-treated apple fruit during cold storage.

The internal concentration of ethylene in ReTain® treated fruit during storage was very low, generally not rising above 0.5 $\mu L.L^{-1}$ in CA throughout the storage period. However in ReTain® treated fruit, those from harvest maturity 2 had higher levels of internal ethylene than those fruit harvested at maturity 1. This was consistent through CA storage, except near the end of the storage period (8 months) where the differences were not significant. The 7DBH maturity 1 fruit contained lower levels of internal ethylene than the earlier ReTain® treated fruit during long term CA storage. By 8 months storage, there were no differences in IEC.

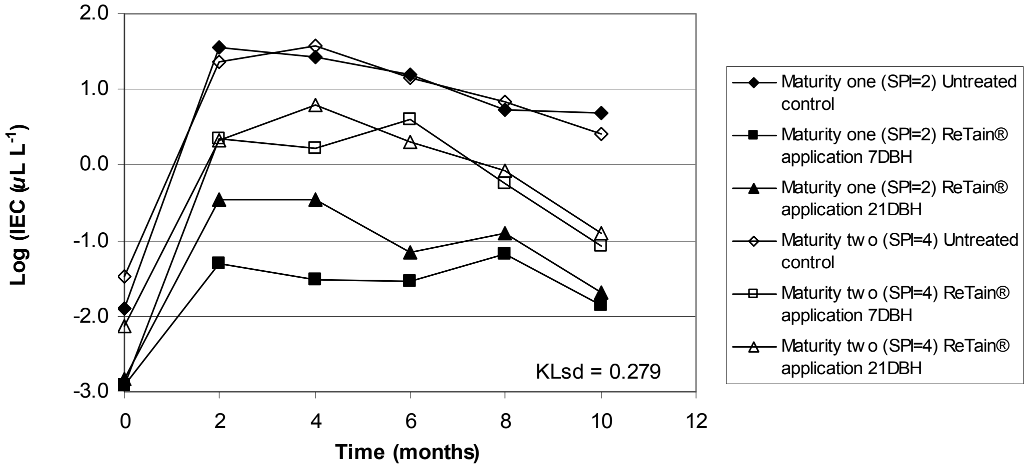

Figure 26. Mean IEC of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Orchard location and timing of harvest had a significant effect on firmness retention during storage in controlled atmosphere storage. For example, maturity 1 fruit from Victoria was softer and there were no difference between treatments during the storage period. However in the later harvested fruit, the untreated control fruit were always softer than the ReTain® treated fruit during CA storage (Figure 27).

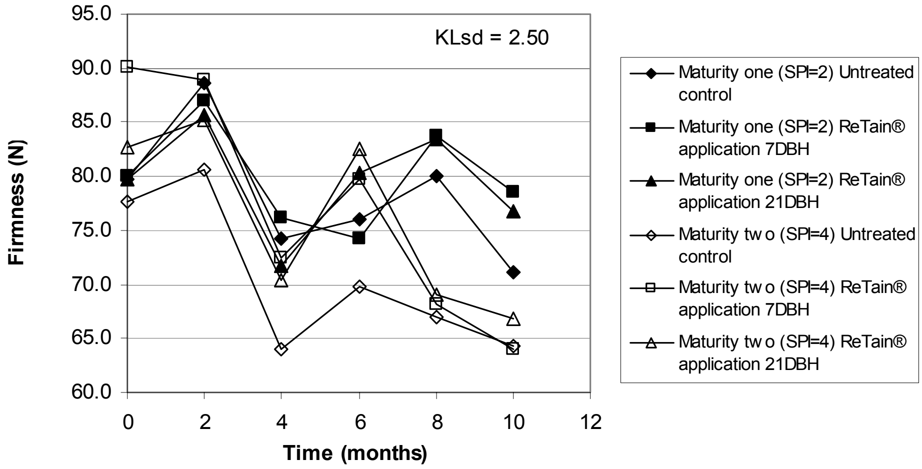

Figure 27. Mean flesh firmness of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Values represent means of 40 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

There were significant differences in the starch pattern index (SPI) between harvest maturities at harvest, with maturity 1 fruit containing more starch (Figure 28). During CA storage there were no differences in maturity 2 fruit SPI, but early harvest 21 DBH ReTain® treated fruit had a lower SPI than 7 DBH ReTain® treated fruit at two months storage only. All fruit had reached maximum starch conversion by four months CA storage.

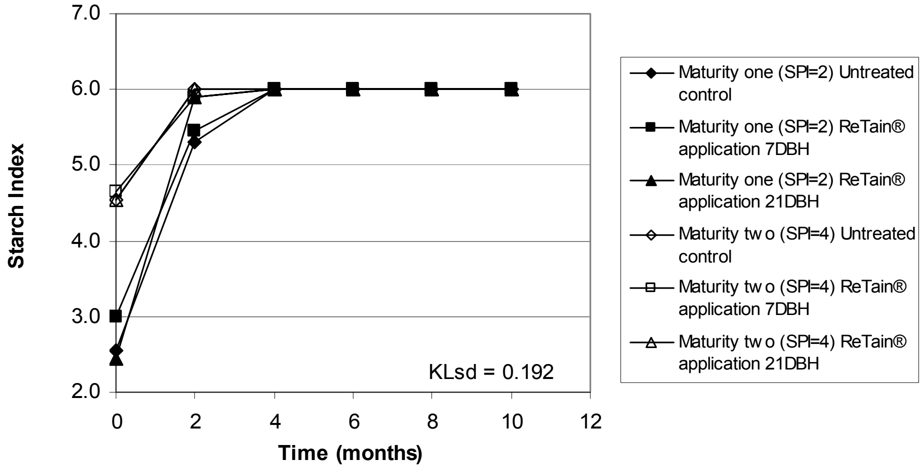

Figure 28. Mean SPI score of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in controlled atmosphere at 0°C for 10 months. SPI measured on the ENZA fruit 7 point scale, a score of 0 indicated high starch distribution and a score of 6 indicated a low starch distribution. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

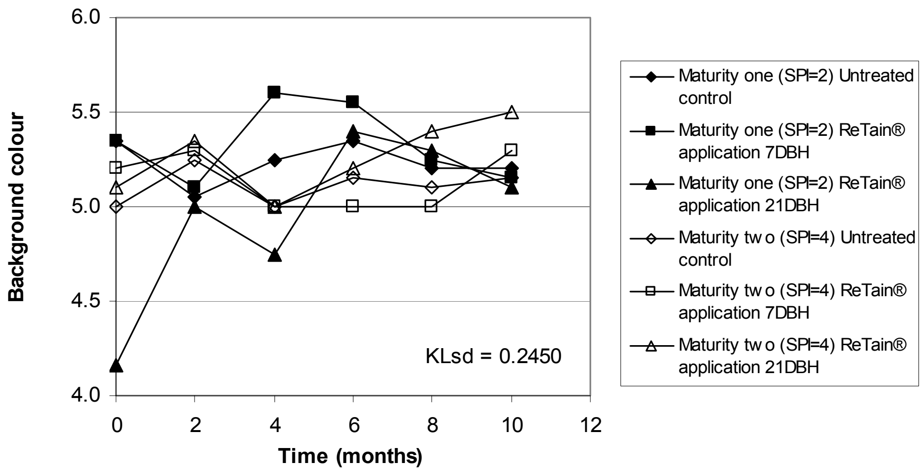

Figure 29. Mean background colour score of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in CA at 0°C for 10 months. Background colour was assessed using a Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

The background scores of Pink Lady™ apples grown in Shepparton remained relatively stable during CA storage, i.e. between scores 5 and 5.5 (Figure 29). There were no commercial differences between the maturity treatments. CA successfully suppressed de-greening during storage.

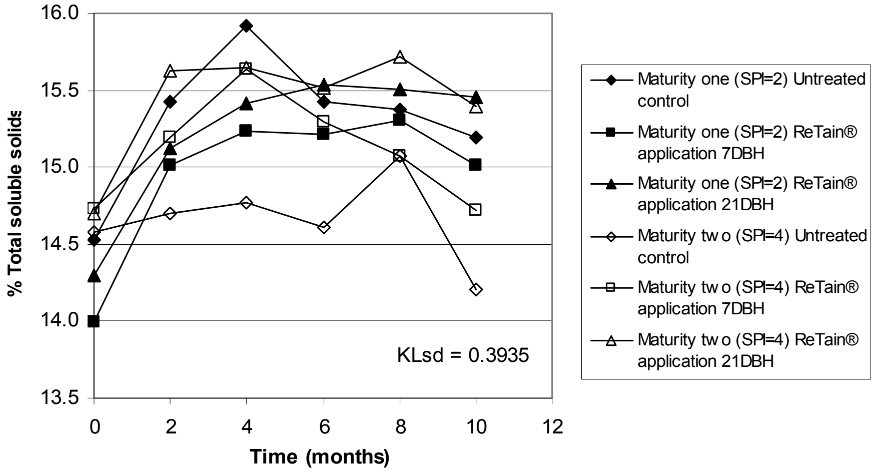

Figure 30. Mean %TSS of Pink Lady™ apples grown in Shepparton, Victoria during a postharvest storage period in CA at 0^{o}C for 10 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

The general increase in TSS during the early stages of CA storage (Figure 30) reflects the conversion of starch to sugars during the early stages of storage (compare Figure 28).

Effect of ReTain® on quality parameters of Lenswood, South Australia fruit

Similar trends and magnitude of internal ethylene concentration response of Lenswood fruit (Figure 31) were observed as those from Shepparton (Figure 26). However the untreated control fruit of maturity 1 had higher internal ethylene concentrations (>1ppm) at harvest and were probably into their ethylene climacteric when placed into CA storage. Maturity 2 UTC fruit rapidly (within 2 months of CA storage) also produced high levels of internal ethylene, where as all ReTain® treated fruit produced very low levels of internal ethylene, also peaking within 2 – 4 months CA storage.

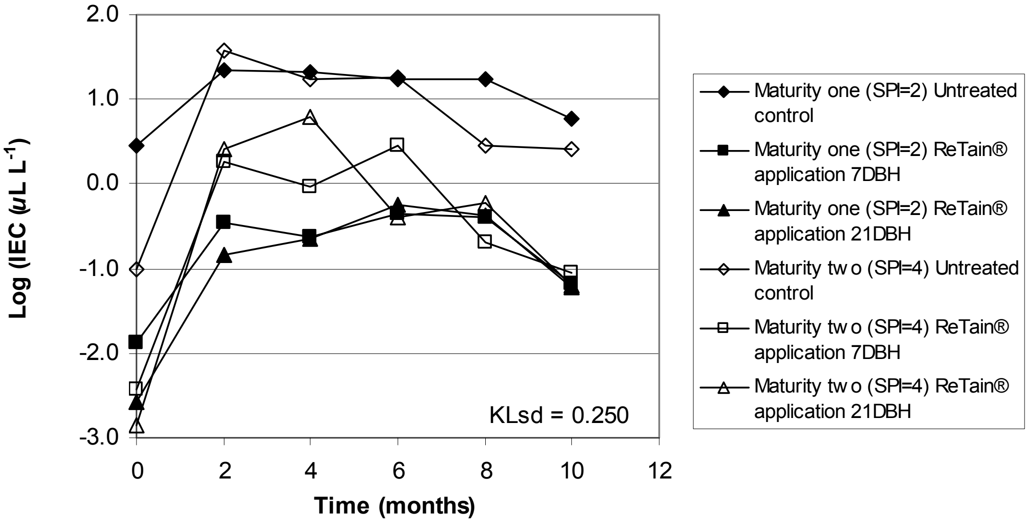

Figure 31. Mean IEC of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

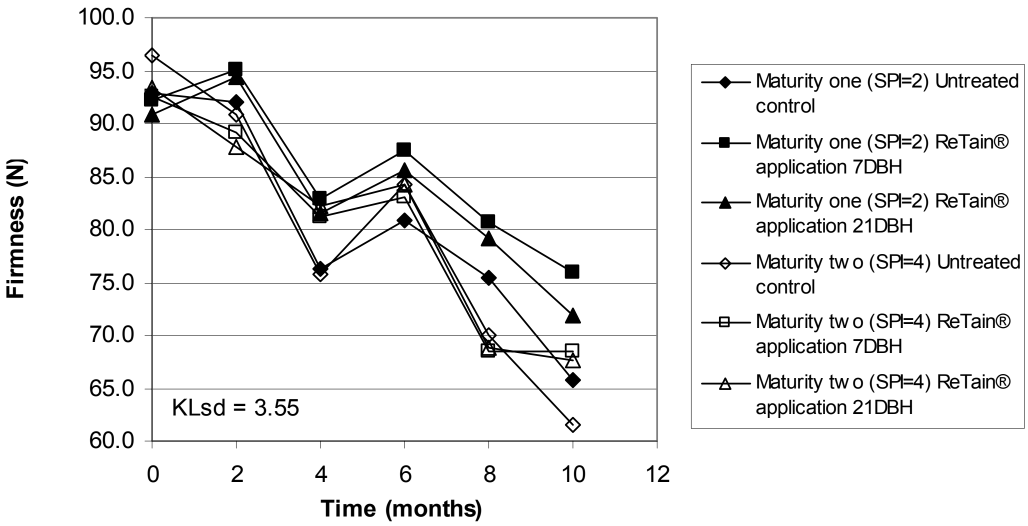

Figure 32. Mean flesh firmness of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Values represent means of 40 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Flesh firmness declined with CA storage (Figure 32). Maturity 1 South Australian fruit that were treated with ReTain® were firmer after 8 months CA storage than the untreated control fruit. However in the later mature fruit, the differences were not so clear, and tended to soften at a faster rate than early harvested fruit. However by 8 months CA storage, fruit from maturity 1 were firmer than from maturity 2.

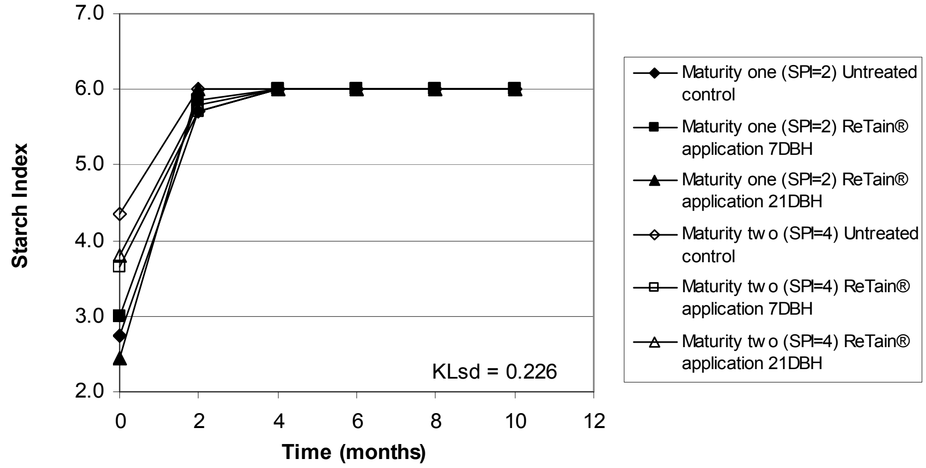

Figure 33. Mean SPI score of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in controlled atmosphere at 0°C for 10 months. SPI measured on the ENZA fruit 7 point scale, a score of 0 indicated high starch distribution and a score of 6 indicated a low starch distribution. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

At harvest, fruit from maturity 1 had lower SPI than fruit from maturity 2 (Figure 33). By 2 months CA storage nearly all starch had been converted to sugar (SPI 6).

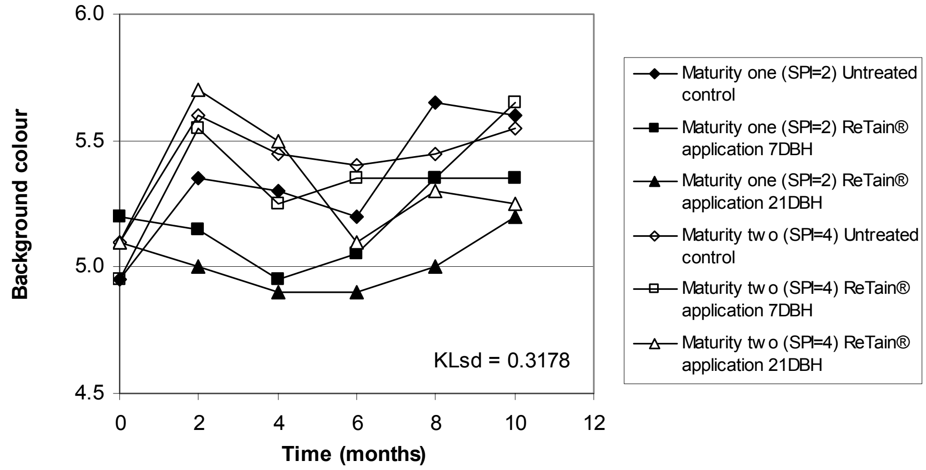

Figure 34. Mean background colour score of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Background colour was assessed using a Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Background colour was repressed by CA storage in all treatments (Figure 34), where maturity 1 fruit generally maintained a greener background colour than fruit of maturity 2. ReTain® treated fruit of maturity 1 had lower (greener) background colour scores than the untreated control fruit.

There were significant differences in the level of soluble solids for the Lenswood fruit with interactions between treatment, storage duration and maturity (Figure 35). All treatments showed an increase in soluble solids in the first 2 months on storage and this corresponds with the changes in starch index (Figure 33) and after 4 months the levels drop off as the fruit metabolise some of the sugars during storage.

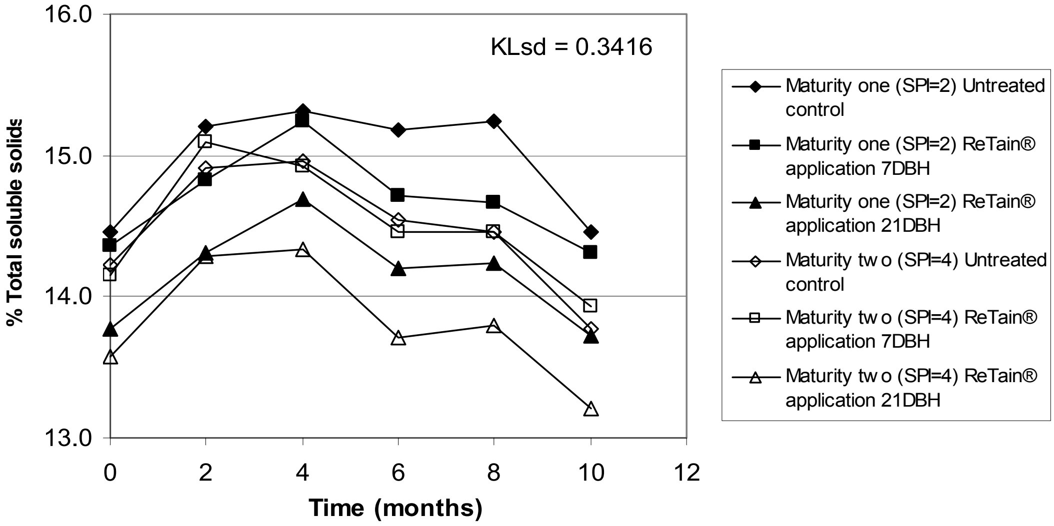

Figure 35. Mean %TSS of Pink Lady™ apples grown in Lenswood, South Australia during a postharvest storage period in controlled atmosphere at 0°C for 10 months. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Assessment of Volatiles after 6 months in storage

Volatile compounds are responsible for the development of aroma and significantly contribute to the overall sensory quality of apple fruit. Over 300 volatile compounds have been identified in apples, and the main groups are alcohols, aldehydes, carboxylic esters, ketones and ethers (Dimick and Hoskin, 1983).

Analysis of volatile compounds from fruit is a complex process which can be conducted using a direct headspace or a dynamic headspace method. Each method has its benefits and problems, but one of the major considerations of selecting a method is its reliability and time to collect and analyse volatiles. This is particularly important when conducting horticultural research, where the time of collecting and analysing aroma is often short. The method used in this experiment for volatiles collection employed solid phase microextraction (SPME). This method is relatively quick and reliable for the qualitative determination of small molecular weight volatiles (such as those in apple aroma) (Matich *et al*., 1996). The SPME method involves sampling the headspace above an apple sample and absorbing the volatile compounds from the apple headspace onto a polymer coated silica fibre. The volatiles are then thermally desorbed into a gas chromatograph (GC) column when the fibre is inserted into the injection port of a GC. The volatile compounds are then measured by GC.

The volatile analysis conducted in this trial was a comparative rather than quantitative. It is therefore misleading to quantitatively compare peak areas between Figures 36 and 37. However within the treatment times (assessments), the results show that on the day of removal from storage there was a significantly higher level of volatile production for the untreated control fruit stored in air compared to all other treatments, including the untreated controlled atmosphere stored fruit. Indeed all other air stored fruit and all the CA stored fruit had lower levels of volatile production (Figure 36). This pattern of volatile production parallels the level of ethylene production (Figure 13 – air stored and Figure 31 – CA stored).

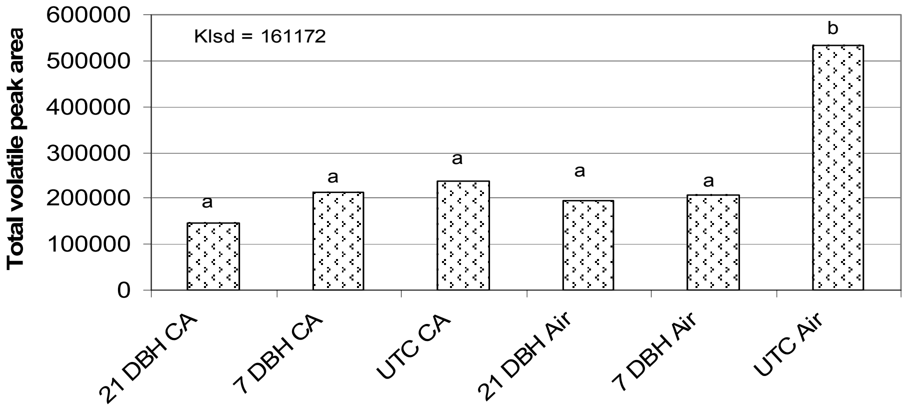

Figure 36. The mean total volatile peak area of samples of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage. Values represent means of 3 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Figure 37 shows the level of volatile production at 20°C 4 days after removal from either air or CA storage. There was no significant difference between the levels of volatile production for the air stored fruit. However there was a significantly lower level of volatile production for all the CA stored fruit and the ReTain® treated CA stored fruit had lower levels of total volatiles than the untreated control fruit. Again these levels show similar patterns to the level of ethylene production (Figure 31).

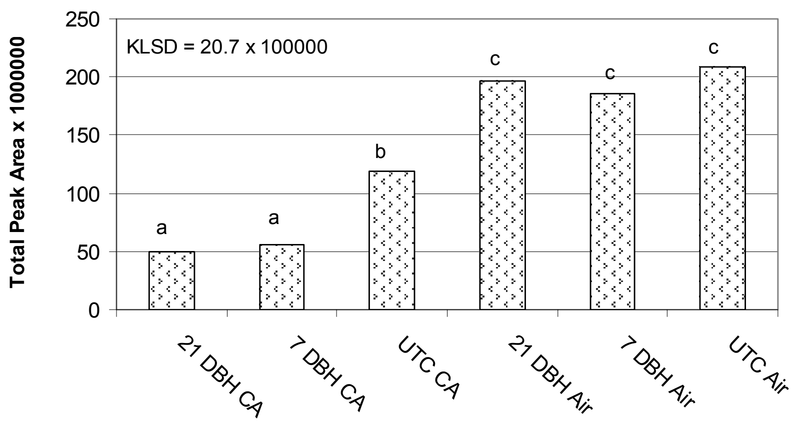

Figure 37. The mean total volatile peak area of samples of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 4 days at 20°C. Values represent means of 3 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

In apples the major volatile that consumers associate with ripe fruit is ethyl 2-methylbutyrate and it is detectable at concentrations as low as 0.001µL/L (Wills *et al.*, 1998). This compound was present in all treatment however it is not possible to accurately quantify the amount using this analysis. It is possible that the lower levels of total volatiles present in the samples may be enough to provide adequate flavour, although further work is required to confirm this.

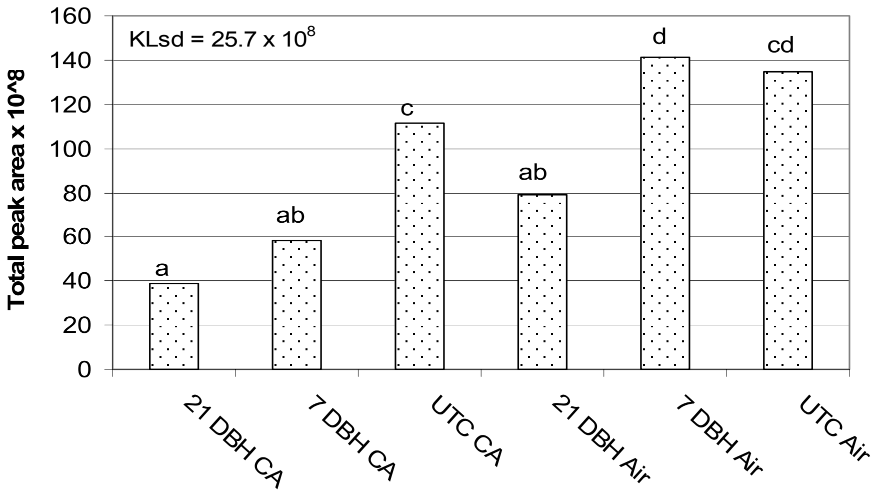

Figure 38. The mean total volatile peak area of samples of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 7 days at 20°C. Values represent means of 5 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

The pattern of volatile production follows the pattern of ethylene production. The internal ethylene and volatile levels of the ReTain® treated fruit in CA don't recover to the same level as the untreated control fruit.

Similarly the level of internal ethylene and total volatiles drops off after 7 days at 20°C for the 21 DBH ReTain® treated fruit.

Assessment of Shelf Life after 6 months in storage

For the 6 month removal from storage, extra samples of fruit from the first maturity (SPI=2) from Lenswood South Australia were kept at 20°C to assess the shelf life of the fruit after storage. Quality parameters were measured at intervals over a 14 day period to simulate the consumer shelf life.

Figure 38 shows the changes in internal ethylene concentration after storage. Even after 14 days shelf life at 20C after 6 months CA storage, the internal ethylene concentration was suppressed in ReTain® treated fruit, whereas the untreated fruit were producing large amounts of ethylene. Indeed the ReTain® treated fruit that were stored in CA showed no increase in ethylene production during storage at 20°C. Treatment with ReTain® inhibited ethylene production and this inhibition was maintained during the shelf life study. The untreated fruit from both air and CA storage had the highest levels of internal ethylene and both treatments showed an increase in internal ethylene over the 14 day period at 20°C.

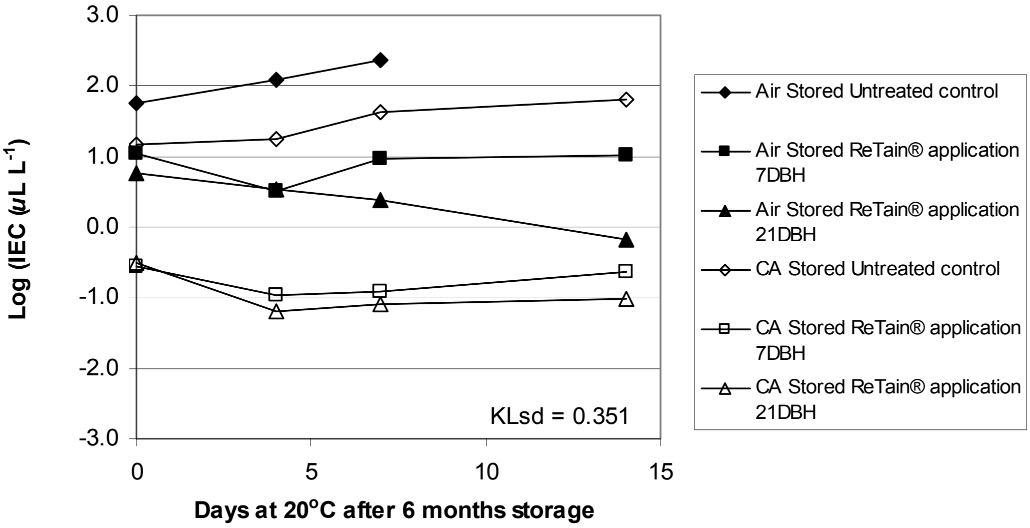

Figure 38. Changes in internal ethylene levels of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 14 days at 20°C. Values represent means of 10 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level)

After 6 months storage and a simulated shelf life at 20°C, CA treated fruit were firmer than air stored fruit. The changes in flesh firmness have a similar pattern to the changes in internal ethylene (Figure 28). The fruit stored in CA maintained their firmness after storage whereas the air stored fruit softened. The untreated fruit stored in air softened the most. The ReTain® treated air stored fruit were significantly firmer after 14 days at 20°C compared to the untreated air stored fruit. The difference between the treatments for the CA stored fruit was significant on removal from storage but not after 14 days at 20°C.

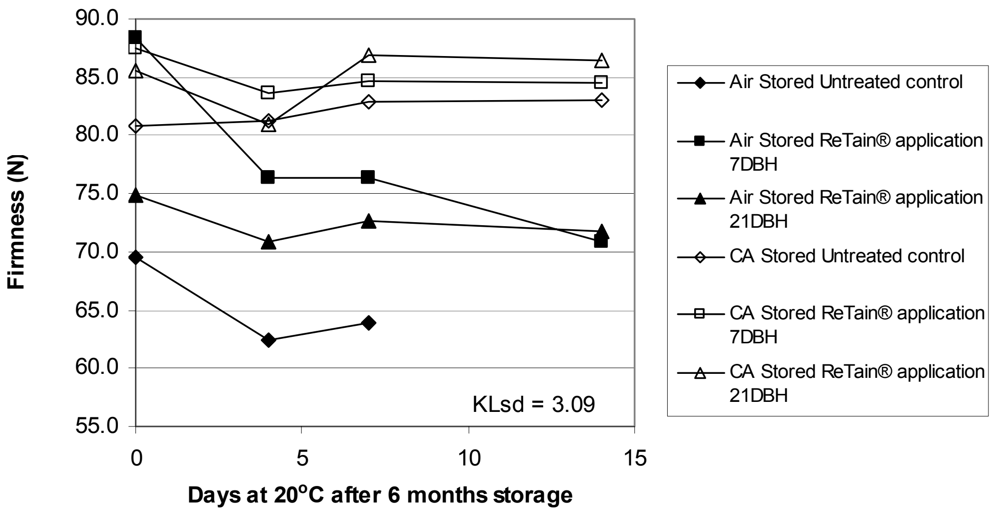

Figure 39. Changes in the flesh firmness of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 14 days at 20°C. Values represent means of 40 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

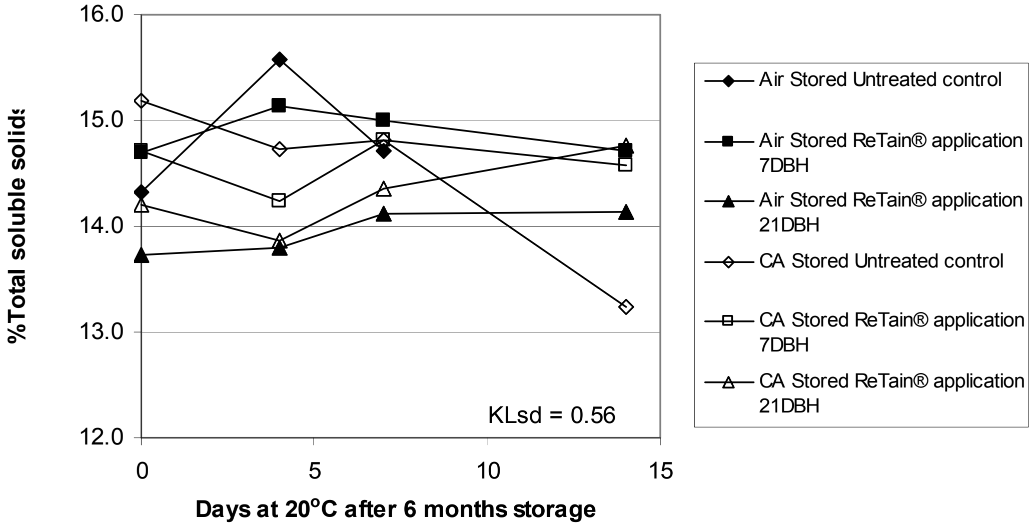

Figure 40. Changes in the %TSS of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 14 days at 20°C. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

Figure 40 shows the changes in the percentage of soluble solids. This is an indication of the sweetness of the fruit. The sugars are metabolised once the fruit begins to age and the level of soluble solids can decrease over time for this reason. The untreated control fruit stored in both air and CA showed a significant decrease in %TSS after 14 days at 20°C. The other Retain treated fruit maintained a similar level throughout the shelf life trial. Treatment with ReTain® slows the rate of fruit ripening as illustrated by the level of internal ethylene (Figure 38) and as a result delays the loss of soluble solids.

Another parameter that is often used by consumers as a guide to apple quality is the change in background colour. Figure 41 shows those changes after removal from storage. The untreated control was not measured at 14 days due to postharvest rots. A colour score of 2 indicates a green background colour and a score of 7 indicates a yellow colour. The more yellow the skin colour the riper the apple is.

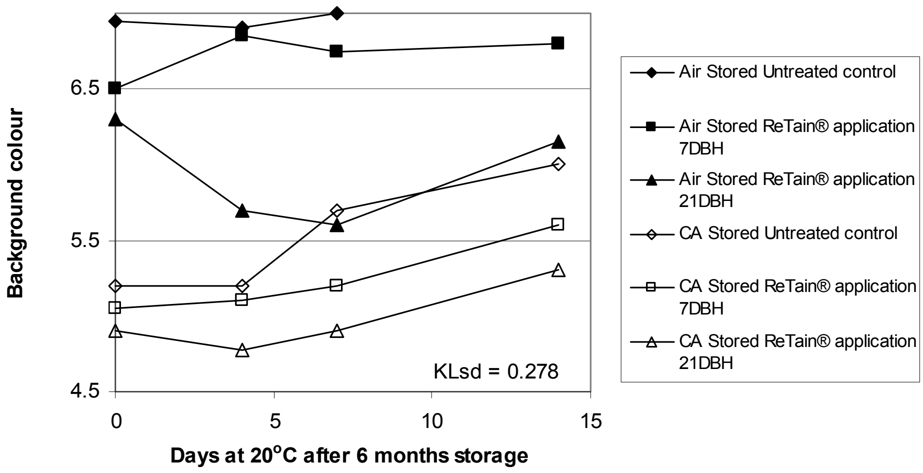

Figure 41. Changes in the background colour of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A after 6 months storage and 14 days at 20°C. Background colour was assessed using a Ctifl Pink Lady™ colour chart, a score of 2 indicated a green colour and a score of 7 indicated a yellow colour. Values represent means of 20 replicates. Significance tested by k Ratio LSD rule at k=100 level (~ 5% significance level).

As expected on removal from storage the CA stored fruit were significantly greener that the air stored fruit. This is because CA inhibits ripening and the subsequent loss of chlorophyll and green colour. The fruit stored in air had already lost most of their green colour during storage and so the changes during the shelf life trial were small. The fruit treated with ReTain® 21 days before harvest and stored in air maintained a greener colour than the other air stored treatments. For the CA stored fruit both ReTain® treatments were significantly greener than the untreated control fruit.

Treatment of Pink Lady™ apples with ReTain® before harvest reduces the rate of quality loss on removal from either air or CA storage when fruit are then held at 20°C for 14 days. Treatment with ReTain® particularly for air stored fruit would give consumers a much better quality apple with a longer shelf life than untreated fruit.

Discussion

The plant growth regulator ReTain® applied 7 days before the first possible pick for long term storage ('Go Date') does not delay the first pick of the fruit, but rather suppresses the maturation and over-maturation of later harvested fruit as well as giving benefits in storage (Rath, 2003). This was particularly true for the Lenswood fruit, where the 7 DBH treatment was applied on the 15/04/2003 and the fruit reached a SPI of 2 (which is the harvest maturity for long term storage) on the 17/04/2003. In Shepparton the timing of the application was more accurate. It is important to highlight the fact that the comparison of the fruit between districts is a physiological comparison based on the SPI at harvest rather than a chronological comparison from the application date of ReTain®.

Timing the application of ReTain® can be a difficulty for growers. They use the ten year district average date for fruit reaching an SPI of 2 which is referred to as the 'Go Date' for harvest (Frost, Agrisearch Services, pers. comm.). ReTain® application at 7 days before the 'Go Date' also has a physiological indicator, being the SPI (Rath, 2003). On the ENZA SPI chart 7 days before an SPI of 2 is approximately and SPI of 0.5, therefore when half the fruit in an assessment sample are at SPI 0 and the other half SPI 1.0 it is recommended to apply ReTain® (Rath, 2003). In 2003, the maturity of the fruit changed more quickly in Lenswood than the ten year average and consequently the timing of the application of ReTain® was not optimum. The differences in response for the 2 districts could in part be due to the timing of the application as well as the climatic differences between the 2 districts.

The 2 application times of 7 DBH and 21 DBH are going to be recommended on the new ReTain® product label (Rath, 2003). The benefits for growers for the 7 DBH application include improving fruit quality and storage potential of later picks in the harvest period. The benefits for the 21 DBH application include a delay in fruit maturation (delaying the harvest date for treated fruit) allowing for an increase in fruit size as well as improving fruit quality and storage potential (Rath, 2003). The advantages of ReTain® applied at 21-28 DBH on either a strip-pick or multiple pick fruit are well established (Valent BioSciences, 2001; Phan-Thien et al., in review). However, less data is available for the 7 DBH application. It is important to note that only multiple pick varieties benefit significantly from this use of ReTain®.

The data from Lenswood air storage shows the same benefits as those recommended on the new ReTain® product label. One of the key quality parameters for apples is flesh firmness as consumers prefer crisp apples (Harker et al., 2002). For the Lenswood fruit, fruit from the 7 DBH treatment were significantly firmer than the control fruit and slightly (although not statistically significantly) firmer than the 21 DBH treatment after 4 months in air storage at $0^{o}C$. The 7 DBH treatment also improved quality after 6 months in storage. After 6 months in storage, only the fruit harvested at M1 (SPI 2) and treated with ReTain® 7 DBH were significantly firmer than all other treatments. This is due to the fact that fruit harvested at an SPI of 2 are preclimacteric and therefore have a longer storage life (Little and Holmes, 2000). The recommended storage life in air at $0\text{-}1^{o}C$ for untreated Pink Lady™ apples is 4 months (Cripps et al., 1993). Treatment with ReTain® did extend the postharvest storage life of these apples.

For the Lenswood fruit stored in CA and harvested at the optimum maturity for CA (SPI of 2) treatment with ReTain® significantly reduced the rate of firmness loss. These fruit were harvested later and also showed a postharvest benefit from the treatment (Table 1). For the later harvested fruit the firmness was not significantly different to the control fruit despite harvest being delayed by 3 days (Figure 32).

In Shepparton, the benefit of the 7 DBH treatment for the air stored fruit was shown to be the extension of the harvest period by 7 days rather then extending the storage life of the fruit. After 2 months in storage, the fruit harvested at M1 (SPI = 2) and treated 7 DBH were firmer however, the differences were not significant later in storage (Figure 8). In this experiment, the 21 DBH treatment also gave the benefits cited by Valent Biosciences on the new product label. In both Shepparton and Lenswood, harvest of the M1 fruit was delayed by 7 days. For the M2 harvest the delay was 7 days in Shepparton and 3 days in Lenswood (Table 1).

In Shepparton there was both a benefit at harvest and a postharvest benefit in terms of reducing the loss of flesh firmness for fruit stored in CA (Figure 27). The benefit for the late harvested fruit stored in CA was only the delay in harvest time, as there was no significant difference in flesh firmness after 8 months in storage.

Some of the differences in the responses of the fruit from the 2 districts may be due to the seasonal and/or district climatic differences. Data from individual years would be required to develop a climate model rather than district averages. However, the district averages highlight some interesting differences. Shepparton is warmer (Figure 19) than Lenswood which could hasten fruit development, growth and maturity.

Fruit structure has also been correlated to seasonal weather conditions with cooler districts having denser fruit than warmer districts (Jobling, Sydney Postharvest Laboratory, pers. comm.). When the climate of the 2 districts was analysed it was found that they differed in mean daily minimum and maximum temperature, mean daily evaporation and mean monthly rainfall and these factors all interacted with the development of the apples, influencing their quality in storage. The times when climate plays an important role in apple development include 50 days after the trees reach full bloom and the period when the apples are reaching maturity. Fifty days after full bloom is the period of cell division and usually occurs between the months of September and early December. If this period is extended (by low temperatures) then the fruit tend to be denser (Jobling, Sydney Postharvest Laboratory, pers. comm.). This could be true for Lenswood as the fruit were much firmer at harvest (90 N, Figure 14) than the fruit from Shepparton (70 N, Figure 8). The period of fruit maturation occurs from March to April.

For this experiment, the period of fruit maturation began on the 16/04/2003 in Shepparton and on the 17/04/2003 in Lenswood. There was no difference in the timing of maturity for the 2 districts, although Lenswood was earlier in 2003 compared to other years (Frost, Agrisearch Services, pers. comm.). It seems that the district climate may explain the difference in initial firmness but the differences in the response to ReTain® is not so clearly related to simple climatic data.

One of the key physiological impacts of ReTain® is the reduction of ethylene production. This response has been reported to be ‘immediate’ and significant however, the impact of the reduction of ethylene production on fruit maturation is dependent on what stage of maturation that fruit is at when ReTain® is applied (Rath, 2003). The reduced response to ReTain® in Shepparton may be due to the fact that in a warmer climate the rate of fruit ripening may have been faster than at the cooler site of Lenswood (Figure 19). This indicates that the fruit may have been physiologically more active in Shepparton and replaced the inhibited ACC synthase enzymes before harvest which reduced the postharvest benefits of ReTain® in storage.

The level of ethylene production is correlated to the level of fruit softening (Jobling et al., 2003). The results from this experiment support this interaction. For consumers, flesh firmness is one of the key parameters used to determine the quality of apples out of storage and the extension of fruit firmness indicates that the quality of the fruit is maintained in storage and as a result this extends the marketable life of the fruit (Little and Holmes, 2000; Johnston et al., 2003). Harvested apples typically undergo an initial phase of slow softening followed by a phase of more rapid softening and a final phase of slow softening (Johnson et al., 2003). The pattern of ripening found from both districts supports these findings. Flesh firmness was found to be significantly higher in Lenswood than in Shepparton and this difference is likely due to the climatic differences between the 2 districts. As explained previously (Figure 2) the fruit from Shepparton were 62.1 N after 6 months storage in air compared to 76.7 N in Lenswood. The firmest fruit were fruit from Lenswood that were treated with ReTain® 7 DBH and harvested at M1. This treatment extended that marketable life of the fruit stored in air by 2 months.

The distribution of starch, indicated by the SPI, is one of the most useful determinates of harvest maturity and may be used as the sole determinate for harvest date (Little and Holmes, 2000). Fruit that is to be placed into long term storage, either air or controlled atmosphere, should have a SPI of 2 (M1) whereas fruit for the fresh fruit market may be harvested with a SPI of 4 (M2). The SPI measured over the course of the experiment indicated no significant difference in the starch distribution between Shepparton and Lenswood as both sets of fruit had begun to ripen after 2 months in storage, shown by the increasing ethylene levels (Figure 1). The treated fruit showed suppressed ethylene levels. Despite the low levels of ethylene, starch continued to be broken down.

It may be that starch degradation is initiated by ethylene rather than controlled by ethylene. Once the starch breakdown has been initiated it continues irrespective of the level of ethylene present.

Throughout the ripening process, the starch is broken down into sugars (Little and Holmes, 2000), the increase in SPI (Figure 3, Figure 28) indicates a reduction in starch over time which correlates to the increase in % TSS (Figure 4, Figure 30). The % TSS was not found to be significantly different for the 2 districts. For both districts the SPI increased significantly after 2 months in both air and CA storage and then continued to increase at a more gradual rate, reaching a maximum level after 6 months. During the first 2 months in storage, the majority of the starch was converted into sugars resulting in a significant increase in % TSS. After 2 months in air storage, % TSS was at a maximum level and reduced over the remaining time in storage as most of the starch had already been converted into sugars by this time and the fruit began using up the stored reserves of sugar. In CA the %TSS reached a maximum after 2 months but did not decrease as the low oxygen conditions in the atmosphere suppressed the rate of metabolism and hence the sugar reserves were not used up as rapidly as in air storage (Figure 30).

Colour is another important quality parameter, especially for blushed apple varieties such as the Pink Lady™ (Studdert, 2002). Background colour, used in combination with starch distribution, may be used to assess harvest maturity. The fruit from Lenswood stored in air showed a significantly higher background colour than the fruit from Shepparton after 2 and 6 months in storage (Figure 5) indicating that the fruit from Lenswood were ripening at a more rapid rate than the fruit from Shepparton. Despite being firmer, the maintenance of firmness in Lenswood could be due to the fruit being denser as discussed previously. It may also be due to the fact that the degradation of chlorophyll, which causes the yellowing of the background colour is another process initiated by ethylene but not controlled by it. In both districts, background colour increased significantly over time in air storage indicating that the fruit were ripening. In CA storage the changes in background colour were not as significant, again because the low oxygen in the atmosphere was slowing the metabolic ate of the fruit and hence the degradation of chlorophyll was also slowed (Figure 29 and 34). As fruit ripen the chlorophyll is broken down and the skin looks more yellow than green.

Skin greasiness is also a ripening process, as the wax composition on the fruit surface changes during storage and produces a “greasy” feel.. This is more of a problem for more mature fruit (SPI4) and also seems to be a possible problem in Victorian apples when Retain is applied 21 DBH and the fruit harvested at SPI4.

Blush colour was also assessed (Figures 20, 21, 22 and 23) and the fruit from Lenswood were significantly redder than fruit from Shepparton. The blush colour of apples may be affected by many field conditions (Little and Holmes, 2000), with climate providing the most significant effects. It is known that warm days followed by cool nights improves the production of the red pigment anthocyanin (Little and Holmes, 2000) however the climate data presented in the results indicated that Shepparton had a higher diurnal temperature range for all months of the year and consequently should have had more blush than the Lenswood fruit.

The higher maximum temperatures in Shepparton may act as a negative effect in terms of red blush development. Ripening and ethylene also effects red blush with higher ethylene also leading to an increase in the rate of anthocyanin accumulation (Faragher and Brohier, 1984). Treatment with ReTain® may suppress ethylene and colour development. Blush colour was not measured in this experiment however, a visual assessment shows no clear effect of ReTain® in either district.

There are five use-patterns recommended for the use of ReTain® on multiple pick apples: treatment of part blocks with ReTain® at 21-28DBH, treatment of all blocks with ReTain® at 21-28 DBH, treatment of all blocks with ReTain® at 7 DBH, treatment of part blocks with ReTain® at 21-28DBH and treatment of the remaining blocks with ReTain® at 7 DBH and treatment of all blocks with ReTain® at 21-28 DBH followed by the treatment of all blocks with ReTain® at 7 DBH (Rath, 2003). For this experiment, the use-pattern used was treatment with part blocks with ReTain® at 21 DBH and treatment of the remaining blocks with ReTain® at 7 DBH. The reported benefits of this use pattern include the advantage of harvest logistics management combined with harvesting high quality fruit in later harvests and being able to manage storage more effectively as all the fruit have been treated with ReTain® (Rath, 2003). The 21 DBH treatment allows for harvest management by delaying the onset of maturation while the 7 DBH treatment does not delay the first harvest but maintains even late picked fruit within export, supermarket or storage specifications (Rath, 2003).

In Lenswood, South Australia, half the block was sprayed on the 31/03/2003 (21 DBH) and harvested on the 23/04/2003 (M1, SPI 2) and 02/05/2003 (M2, SPI 4), while the other half of the block was sprayed on the 15/04/2003 (7 DBH) and harvested on the 17/04/2003 (M1, SPI 2) and 02/05/2003 (M2, SPI 4). After 4 months in air storage, the treated fruit all had the same quality of firmness despite the range of 16 days between harvest dates. The untreated fruit harvested between 17/04/2003 and 30/04/2003 were not as firm. The 30/04/2003 is too late to harvest fruit for long term storage therefore the harvest period was found to be smaller for the untreated fruit. Untreated fruit harvested at a SPI of 4 are really only suitable for the fresh market or only for short term storage, up to 2 months in air at 0^oC. By 6 months in air storage, 2 months longer than the air storage period recommended by Cripps, 2003, fruit treated with ReTain® at 7 DBH from both maturities were still firm (Figure 14).

In the CA storage trials the treated fruit harvested with an SPI of 2 were significantly firmer than the untreated control fruit. For the later harvested fruit there was a difference between the treated and control fruit after 6 months in CA but at 8 months there was no significant difference between the treatments for that harvest maturity (Figure 32). This extension of storage life for both air and CA stored fruit could be a significant commercial benefit to growers.

In Shepparton, Victoria, half the block was sprayed on the 25/03/2003 (21 DBH) and harvested on the 23/04/2003 (M1, SPI 2) and 05/05/2003 (M2, SPI 4), while the other half of the block was sprayed on the 09/04/2003 (7 DBH) and harvested on the 16/04/2003 (M1, SPI 2) and 01/05/2003 (M2, SPI 4). The UTC fruit were harvested up until the 29/04/2003. The change in flesh firmness was not as clear as in Lenswood in the air storage trial, only the M1 UTC fruit were significantly different after 4 months in air storage. This indicates that there was no significant difference between M2 UTC fruit and any of the treated fruit, except that the treated fruit were harvested 6 days later providing some flexibility for the grower over the management of the harvest. The IEC for M1 treated fruit however, was lower than the IEC for M2 treated fruit and the UTC fruit, indicating that there was a significant physiological treatment effect (Figure 7).

In the CA storage trial for the Shepparton fruit there was a significant effect of fruit maturity with the earlier harvested fruit (SPI = 2) being firmer than the late harvested fruit (SPI = 4) (Figure 27). For the early harvested fruit there was no significant difference between treatments up to 6 months in storage but after 8 months the treated fruit were firmer than the untreated control. For the late harvested fruit (SPI=4) there was a significant difference in the firmness of the treated fruit compared to the untreated control fruit after 6 months in CA storage (Figure 27). After 8 months in storage there is no significant difference between treatments for the late harvest fruit. For fruit being places in CA there was both a benefit at harvest and a storage benefit with the use of ReTain®.

Shelf life after storage is an important quality parameter for consumers. Treatment with ReTain® at either 7 DBH or 21 DBH significant reduces the rate of quality loss of Pink Lady™ apples stored in air and CA storage for 6 months and then stored at 20^oC for 14 days. The reduction in the rate of firmness loss after storage was most significant after air storage. This improvement in quality of air stored fruit would be greatly appreciated by apple consumers.

Volatile compounds are responsible for the development of aroma and significantly contribute to the overall sensory quality of apple fruit. Over 300 volatile compounds have been identified in apples, and the main groups are alcohols, aldehydes, carboxylic esters, ketones and ethers (Dimick and Hoskin, 1983). In this study, total volatile production was measured upon removal after six months storage at 0^oC and after a short-shelf life of 4 days at 20^oC. The measurement of volatiles is a lengthy process, and was therefore confined to one treatment combination, maturity 1 fruit from Lenswood. The results show that upon removal from storage, untreated air stored fruit had significantly more total volatiles than ReTain® treated fruit in air, or any CA stored fruit. This is reflected in the internal ethylene concentration of UTC fruit from Lenswood. The higher level of ethylene in air stored UTC fruit also had higher levels of volatile compounds. This observation has been well reported in a range of apple varieties and other fruits (Mattheis *et al*., 1991, Song and Bangerth, 1996, Golding *et al*., 1999). Interestingly CA storage suppressed both ethylene and volatiles production and produced similar levels of volatiles as ReTain® treated fruit in air stored fruit. CA has long been shown to suppress volatiles production, probably through reduced ethylene production (Mattheis *et al*., 1991).

Following a short shelf life period (4 days) at 20^oC, volatiles production in all air stored fruit was similar. This suggests that volatile production increased during the shelf life period, indicating the capacity to produce volatiles (aroma) was not permanently affected by ReTain® treatment. However more quantitative work is required to accurately describe this. The UTC fruit stored in CA had lower levels of volatile production than all the air stored treatments after 4 days at 20^oC. This illustrates the fact that CA storage suppresses volatile production as described earlier. The ReTain® treated fruit stored in CA had significantly lower levels than the control fruit indicating that there is a combined CA and ReTain® effect. The pattern of volatile production is similar to the levels of ethylene production for the same shelf life period (Figure 38). Our knowledge and understanding of the dependency of volatiles production on the ethylene climacteric is still evolving, but these results support the general observations of Mattheis *et al.* (1991).

Harvest management and the potential increase in fruit size due to the delay in harvest date provide considerable commercial advantages to apple growers. The main benefit of using ReTain® applied 21 DBH and 7 DBH in this experiment was the extension of the harvest window, this was apparent in both Shepparton, Victoria and in Lenswood, South Australia. An additional benefit was also seen in Lenswood where an extended storage life of 2 months beyond the recommended air storage life was observed when ReTain® was applied 7 DBH. This was also seen in the CA stored fruit. However fruit harvested at an SPI of 2 and stored in CA were of higher quality then the later harvested fruit after 8 months. An extended harvest window and an extension of the postharvest storage life of Pink Lady™ apples can have significant commercial benefits for apple growers. However, the application of ReTain® also adds to the production costs and growers must weigh up the cost benefit of using this product. This research does however show that the application of ReTain® on Pink Lady™ apples can be a useful tool for managing harvest and ensuring postharvest fruit quality after both air and CA storage.

Maturity at harvest is a key factor in the incidence of flesh browning in Pink Lady™ apples (see project AP02009). To increase the chance of successful outcomes about understanding fruit physiology in this project, fruit were sourced from two growing districts (Shepparton, Victoria and Lenswood, South Australia) and harvested at two different maturities. However, the incidence of flesh browning in apples from these regions in 2003, compared to fruit from other regions used in Flesh Browning apple project in 2003/2004, was too low to statistically compare treatments for flesh browning. However, there was a definite trend for Retain treated fruit to have less flesh browning than control fruit. This observation is to be reported in more detail in the Extension to the Pink Lady Apple Browning Project AP4008 (2004 – 2007).

Recommendations

To date, the recommended storage life in air for untreated Pink Lady™ apples is 4 months at 0-1ºC (Cripps *et al*., 1993). However, this research shows that the application of ReTain® on Pink Lady™ apples is a useful tool for managing harvest and extending postharvest fruit quality after both air and CA storage. ReTain® (soluble powder; 150 g aminoethoxyvinylglycine/kg, Valent Biosciences Corporation) was applied at a rate of 83 g/100 L together with the 0.1% MAXX organosilicone surfactant (Sumitomo Chemical Company Pty Ltd).

Application of ReTain® 7 days before harvest (DBH) at an early stage of maturity, as indicated by a starch pattern index (SPI) score of 2 on the ENZA 7 point starch chart, results in a reduction of internal ethylene concentrations (IEC) and improved firmness of Pink Lady™ apples during storage at 0ºC. The application of ReTain® 7 DBH at a later maturity (ENZA SPI=4) also results in lower IEC and improved firmness of Pink Lady™ apples, but the difference was not as large as at the earlier harvest. Harvest at an SPI score 4 (ENZA) is post-climacteric and more suited to sale on the fresh market.

Application of ReTain® to fruit 7 DBH at an SPI of 2 (ENZA) results in the extension of the storage life of Pink Lady™ apples to 6 months, due to the retention of flesh firmness. This effect is present in both air and CA (2% O_2 and 1% CO_2) stored fruit, although the effect was more obvious in the air-stored fruit. After 6 months storage at 0ºC and a simulated shelf life at 20ºC for 14 days, ReTain® treated air stored fruit were significantly firmer than the untreated air stored fruit. The difference between the treatments for the CA stored fruit was significant on removal from storage at 0ºC but not after 14 days at 20ºC.

Application of ReTain® at 21 DBH also assists to improve the firmness of Pink Lady™ apples during storage, however the results were not as great as occurred after ReTain® applied 7 DBH. ReTain® applied 21 DBH would more likely play an important role for harvest management, such as to potentially increase the fruit size by delaying the harvest date. Application times of 7 DBH and 21 DBH are proposed for the new ReTain® product label (Rath, 2003).

Based on this research, the best current recommendations for Retain® application and storage regime for Pink Lady™ apples are:

1. ReTain® applied at 7 days before harvest (DBH for fruit harvested at an SPI score of 2 (ENZA)), to Pink Lady apples followed by regular air storage 0ºC, resulted in the best quality as measured by firmness, colour, total soluble solids, fruit volatiles and low greasiness after 6 months storage.
2. ReTain® applied at 7 days before harvest (DBH for fruit harvested at an SPI score of 4 (ENZA)), to Pink Lady apples followed by CA storage at 0ºC, resulted in the best quality as measured by firmness, colour, total soluble solids, fruit volatiles and low greasiness after 10 months storage.

However, it is most important to ensure that all commercial usage of ReTain® should be according to current label recommendations.

A final recommendation for Retain® application and storage regime needs to be developed based on further work in the Extension to the Pink Lady Apple Browning Project AP4008 (2004 – 2007)

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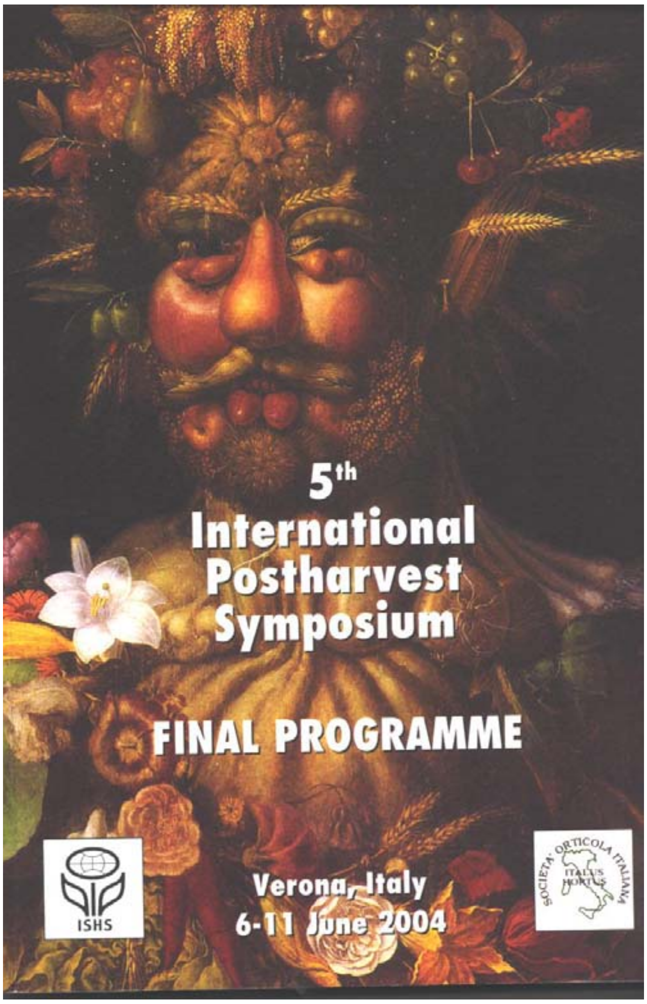
Conference Report



5th International Postharvest Symposium
Verona, Italy
June 6-11 2004

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NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Introduction

The 5th International Postharvest Symposium was held under the auspices of the International Society of Horticultural Science in Italy from June 6-11, 2004. The Postharvest Symposium is held every four years and is the leading postharvest and market access conference. I presented our collaborative research work with Pink Lady apples. The apple controlled atmosphere storage work was conducted at Gosford Horticultural Institute and provided important information for apple growers to improve orchard management and fruit quality. I was supported to attend this conference with funding from Horticulture Australia Ltd. (Project AP02031) and Valent BioSciences supplied a voluntary contribution. NSW Agriculture also supported my time in this project. This report will detail the major themes and key points to emerge from the conference and associated tours of berry and apple enterprises in the Trento region in northern Italy.

Postharvest research and development has been growing around the world due to the continued and growing importance of getting quality horticultural produce to market, especially onto export markets. Countries with major investments in postharvest and market access research and development include; Spain, New Zealand, the United States, Italy and Chile.

Conference Highlights

Over 600 delegates from 50 countries attended the 5th International Postharvest Symposium in Italy, June 6-11 2004. The conference was the largest postharvest conference ever and was divided into 13 sessions and 2 workshops:

Sessions

1. Ripening Physiology in Climacteric and Non-Climacteric Fruits
2. Handling, Packaging and Shipping Technology
3. Fresh Cut Produce
4. Senescence and Abscission
5. Postharvest Quality Management
6. Genetics and Molecular Genetics for Quality and Storability
7. Precooling and Storage Technology
8. Hormonal Regulation including Ethylene Biosynthesis and Action
9. Quality Aspects
10. Postharvest Pathology
11. Postharvest Pest Management
12. Non-destructive quality measurements
13. Postharvest in Developing Countries

Workshops

1. Genomics and Proteomics of Fruit Quality
2. New Strategies for Transport of Ornamentals and other Horticultural Products

Table 2 shows the distribution of presentations (oral and poster) at 5th International Postharvest Symposium. Apples were easily the most researched and discussed commodity with 74 presentations. This was in part due to the large amount of non-destructive quality measurement research and development currently being conducted, mainly in Europe. The next group of commodities included the large fruit crops; peaches, citrus and tomatoes, all with about 28 papers presented.

Table 2. Presentations at the 5th International Postharvest Symposium

	Oral	Poster	Total
Apple	25	49	74
Tomato	14	15	29
Peach/nectarine	16	12	28
Citrus	13	15	28
Cut flowers	13	15	28
Melon	4	7	11
Mango	2	8	10
Strawberry	3	5	8
Cherry	4	5	9
Pear	5	4	9
Plum	2	6	8
Herbs	3	4	7
Grapes	3	5	8
Kiwifruit	3	5	8
Vegetables (general)	2	6	8
Peppers	3	2	5
Onion	2	3	5
Lettuce	2	2	4
Carrot	2	2	4
Apricots	1	3	4

38 other crops (< 4 presentations per crop)

Araza, Artichoke, Asparagus, Aubergine, Broccoli, Cactus, Carumbola, Celery, Chicory, Cucumber, Currants, Custard apple, Date, Durian, Feijoa, Fresh cut, Guava, Longan, Mangosteen, Modelling (general), Mung beans, Mushroom, Nutraceuticals, Packaging, Papaya, Paw paw, Persimmon, Pineapple, Pomegranate, Potato, Quince, Sanitation, Soybean, Spinach, Sugar beet, Sweet potato, Yams

Genomics

A highlight of the plenary sessions was a summary of the current state of our understanding of climacteric fruit ripening by Jim Giovannoni at USDA-ARS / Cornell University; 'Genomics approaches to understanding ripening control and fruit quality in tomato'. He presented an elegant summary on their efforts toward characterising developmental, hormonal, and environmental (especially light) signal transduction systems that impact on quality and maturation of tomato fruit ripening. With the National Science Foundation and the Institute for Genome Research their research group is using gene expression profiling to gain further insights into fruit development and ripening. This fundamental research is essential to our understanding of fruit ripening and senescence and will be the basis of new potential varieties that will have enhanced quality and pest and disease resistance.

Genomics and proteomics are becoming integral to the development of our understanding of postharvest science. This was discussed at length in the workshop; 'Genomics and Proteomics of

Fruit Quality'. Interesting talks were presented on 'The metabolic engineering of tomato fruit carotenoids' and 'The molecular and genetic aspects of ripening and quantitative traits of peach and nectarine fruits'. The research work at Pietro Tonutti's group at the University of Padova (Italy) is elucidating some interesting molecular and genetic mechanisms in peach fruit ripening.

A highlight of this session was presented by researchers from The Wageningen, The Netherlands; 'Gains and loss of fruit flavour compounds produced by wild and cultivated strawberry species'. They showed the evolution of the diversity of strawberry fruit flavour was based on a change in enzyme localisation and alteration in gene expression profile. Through a change in subcellular localisation, enzymes encountered new substrates and produced novel metabolites characteristic of the polyploid strawberry cultivars of today. They also showed that an insertion mutation affected gene expression and caused the loss of other flavour compounds, typical of wild strawberries. Loss of a certain metabolite further influences the metabolic profile of the same plant species by initiating a metabolic chain reaction, in which substrates are no longer available for the production of downstream compounds (aroma). This presentation was particularly interesting as it directly relates to my previous aroma work (Golding *et al*., 1999). [Golding, J.B., Shearer, D., McGlasson, W.B. and Wyllie, S.G. (1999) Relationships between respiration, ethylene and aroma production in ripening banana. *Journal of Agricultural and Food Chemistry* 47, 1646-1651.]

Screening of Internal Breakdown

Another interesting and relevant talk was presented by Dr. Cameron Peace, a former Queenslander working at the University of California, Davis (USA). Their group have suggested that peach and nectarine susceptibility to internal breakdown has a genetic component. They evaluated 133 peach and nectarine varieties for their susceptibility to internal breakdown and showed that some varieties are more susceptible than others. They identified candidate genes encoding the cell wall degrading enzyme, endopolygalacturonase, and related this to mealiness. This may then become available in breeding programs for low susceptibility to internal breakdown symptoms. This is a practical and potentially very useful screening procedure in peach and nectarine breeding programs.

Carotenoids

Carotenoids are important human health promoting compounds which are found in very high concentrations in peaches. A research group in Italy looked at the levels and control of carotenoids during maturation and ripening in several peach varieties. They found peaches with morning sun exposure showed an increasing pattern of carotenoids during the postharvest period; whilst peaches with afternoon sun exposure tended to lose carotenoids. They also looked at the gene expression of carotenoid biosynthesis. If we can understand how these compounds are regulated, then we maybe able to optimise carotenoid content in peach fruit to improve human health.

Ripening physiology

MCP

A major component of the ripening and quality presentations was the use of 1-methylcyclopropene (MCP). MCP is a potent inhibitor of ethylene action in plants, and has the potential to significantly alter postharvest practices in a range of horticultural produce, such as apples. MCP is approved for use on apples in Argentina, Chile, Colombia, New Zealand, the United States, and has recently been approved for use on apples in Australia (April 2004). Since its commercialisation, there has been substantial research and commercial trials on MCP. Susan Lurie from the Volcani Centre in Israel presented a succinct summary of the effects of MCP on stonefruit from over five years research. They showed that apricots responded to MCP by slowing their softening, peel colour change and loss of titratable acidity. However ethylene production and respiration were not affected and the treatment with MCP enhanced internal flesh browning. Cherries are a non-climacteric fruit and treatment with MCP had no effect on their postharvest life.

Peaches and nectarines responded to MCP treatment in a similar manner to apricots by slower softening, colour change and loss of titratable acidity, with only a minor inhibition of ethylene production. However they report that fruit after storage which had received MCP treatment developed more flesh disorders, particularly bleeding, than untreated fruit.

Plums, both European and Japanese, responded positively to MCP. Where ethylene production was inhibited, as was softening, colour change and the loss of titratable acidity. They reported fewer storage disorders, such as internal browning and gel breakdown in MCP treated than non-treated fruit.

Postharvest Pest Management

A range of 'non-chemical' disinfestation techniques were discussed in this session.
The use of radio frequency (RF) heating to control insects in walnuts and cherries was presented by researchers from University of California, Davis. They showed that RF treatments appeared effective in controlling a serious quarantine pest (navel orange worm) in walnuts. But these treatments were less effective in controlling codling moth larvae in sweet cherry, where extensive fruit damage occurred.
Postharvest heat treatments (hot water treatment, short hot water rinsing and brushing and hot air treatment) were extensively discussed, and the use of these treatments in combination with agrochemicals was also presented. It was reported that low doses of the active ingredient are required for the suppression of pathogens when fungicide mixtures are applied as warm mixes due to; the synergistic action of heat, enhanced active ingredient uptake, better encapsulation and diffusion of active ingredient in the cuticular wax.

The use of ethyl formate (Vapormate™) was evaluated by researchers in New Zealand in a range of horticultural produce. Ethyl formate is a GRAS compound approved as a food additive by the US FDA. This compound shows particular promise as a niche methyl bromide replacement for disinfestation of fresh produce.

Nondestructive quality measurements

A range of non-destructive quality measures were discussed. The development of non-destructive measures of quality has been slowly evolving, with the main research and development occurring in Europe. Naturally the main crop of this research in Europe has been apple fruit.
The main techniques for the nondestrucutive measurement of quality include:

- Vibrational based techniques (based on the measurement of the vibrational spectrum after impact). Related to this is the laser puff firmness sensor and other impact devises.
- Optical techniques, such as near infrared (NIR)
- Electronic noses to measure fruit aroma
- Tomographic techniques to visualise the internal structure of the fruit. This includes laser confocal microscopy, magnetic resonance imaging and X-ray CT scan.

Near infrared (NIR) spectroscopy was a topical measure of quality measurement. NIR has been used on a variety of fruit mainly as a measure of sugar content but also for firmness. In addition NIR was reported as a tool for varietal discrimination and characterisation of the harvest stage in apples, detection of bruise on apples and forecasting chilling injury onset on citrus fruit.

A very wide range of non-destructive quality techniques were discussed which may potentially lead to an exiting era of nondestructive quality measurement in the future.

Postharvest in developing countries

A range of postharvest projects and systems from the developing world were discussed. An interesting case study from Ghana examined the postharvest handling and storage of white yams. Simple 'curing' technology could be adapted to significantly minimise decay and rots during storage. Simple technology and an understanding of the horticultural product were keys in designing and implementing postharvest technologies in rural communities with limited resources.

Quality Aspects

Apple aroma and consumer acceptability

Dr. Gemma Echeverría and the laboratory at UdL-IRTA Lleida, (Spain) presented three very interesting papers on Fuji apple aroma from storage and sensory acceptability. This research is of great interest to physiologists and the apple industry.

They looked at standard quality parameters and sensory characteristics (sensory acceptability and sensory acidity) of Fuji apples during and after storage 3, 5, and 7 months of cold storage in normal atmosphere (AIR: 21% O_2 + 0.03% CO_2) or under three different controlled atmospheres (CA), in which oxygen and carbon dioxide were held at 1% + 1% (ULO1), 2% + 2% (LO) or 1% + 3% (ULO_2), respectively. During post-storage ripening, apples were kept at 20ºC for 1, 5 and 10 days before analytical measurements were made.

Partial least squares regression models (PLS1) for sensory acceptability where the instrumental quality parameters were used as X-variables showed that the quality parameters most positively influencing acceptability were SSC, TA, firmness and background colour (Hue). The predicted sensory acidity versus the measured sensory acidity plot from the PLS1 model developed for sensory acidity revealed three groups defined by sensory acidity with a correlation coefficient of r= 0.94. Thus, ULO1-stored fruit had the highest sensory acidity score, ULO_2- and LO- stored fruit showed intermediate sensory acidity score, and finally AIR-stored apples had the lowest sensory acidity score. This has direct industry benefit and should be a model for other consumer acceptability projects.

In another paper, this research group measured volatile production, sensory acceptability and electronic nose responses in Fuji apples to evaluate the effects of different storage conditions, storage periods and shelf life days. Sensor responses registered by 21 different metal oxide sensors of an electronic nose (EN) were used to classify the apples using Principal Component Analysis (PCA). This PCA model containing data from all fruits (at harvest and after storage) showed that it was possible to identify fruits from harvest or storage; it was also possible to differentiate between AIR-fruits and CA-fruits. Principal components 1 (PC1) and 2 (PC2) respectively accounted for 63 and 30% of the total variability. On the other hand, a PCA involving volatile production, sensory acceptability and EN signals corresponding to all fruits allowed a better differentiation between fruits from 1%O_2 + 2%CO_2 and 3%O_2 + 2%CO_2 atmospheres, with an explained variance of 58 % (two first PCs). The use of electronic noses has been hampered by the sensitivity and selectivity, but this paper demonstrates their potential in postharvest horticulture.

A final research paper presented by this productive Spanish research group was examining 'Modifications in Biosynthesis of Aroma Volatile Compounds in 'Fuji' Apples after Controlled-Atmosphere Storage'. Although widely used to extend commercial availability of apples, controlled atmosphere (CA) storage may decrease volatile production in some apple cultivars, and hence be detrimental to fruit aroma. 'Fuji' apples were stored under air (21 kPa O_2 : 0.03 kPa CO_2) or under three different CA conditions (3 kPa O_2 : 2 kPa CO_2; 1 kPa O_2 : 1 kPa CO_2; or 1 kPa O_2 : 2 kPa CO_2) for 3, 5 or 7 months. Emission of volatile compounds and some related enzyme activities were assessed 4 days after removal from storage. Data were used for multivariate analysis of results. Storage caused modifications in aroma profile of 'Fuji' apples during subsequent shelf life. Principal Component Analysis (PCA) showed that both storage atmosphere and storage period accounted for differentiation among samples. Most of selected volatile compounds were associated to AIR-stored fruit, and their emission was higher after medium-term (5 months) storage. Partial Least-Square Regression (PLSR) models developed revealed that PDC activity explained part of discrimination between storage periods, whereas acetaldehyde content was partly responsible for differences between storage atmospheres. Therefore, both variables largely influenced volatile emission after storage. ADH activity also accounted for differentiation between storage atmospheres. Acetaldehyde is a substrate for ADH-catalysed reduction to ethanol, which can be subsequently acted upon by AAT to synthesise the corresponding ethyl esters, and therefore it is suggested to be an important precursor to ester biosynthesis after storage. AAT activity accounted for differences between storage periods, and was highest after medium-term storage, in agreement with highest emission of volatile compounds. This work is particularly interesting for industry and current HA consumer projects.

Preharvest nutrition
Gene Lester from ARS / USDA (Texas USA) gave a very interesting talk on 'Foliar applied Potassium: Effects on Cantaloupe quality, sugar content and related compounds'. He showed that Potassium (K) is important in sugar transport from leaf to fruit and unloading of sugars into the fruit, and that plant mineral analysis recommends about 4% K in petiole (indicating highly mobility). The availability of K in the soil is dependent on; K concentration, soil pH (pH8 or lower is best), moisture conditions, K buffering capacity etc, but plants also need healthy balance of other minerals (Ca, B, Zn, Mg, NO_3). K absorption by roots is dependent on soil type, transport, demand, root metabolites, uptake competition (Ca, Mg), root age, root growth stage. But productive plants need K prior to fruiting. He showed that the application of K-metalosate (24%K), applied weekly during fruit growth and maturation: increased SSC% (from 8 to 9%), where the concentration of K is proportional to sugar ($r^2 = 0.9$). Fructose concentration was higher in K treated fruit (also higher in sensory perception). It also resulted in an increase abscission by about 2-3 days (i.e. control fruit stay on vine longer), but the fruit were of the same weight. The weekly application of K also increased fruit firmness (control 13N V 18N). There was only a slight increase in ascorbate but β-carotene levels doubled with K nutrition. However he reported there no difference in flesh colour as it had reached saturation. This has significant implications to industry and this information has been forwarded to appropriate NSW DPI extension staff and industry.

Postharvest Calcium Treatments
Another interesting presentation was the postharvest treatments of calcium chloride for increasing peach firmness. Researchers from Poland and the USA showed that a postharvest calcium treatment (1% calcium chloride for 30 min) increased the firmness in peaches, compared to untreated fruit. The use of calcium is widespread in postharvest horticulture (eg apples) and was suggested to reduce bruising by increasing firmness. However the researchers did note that treated fruit had an undesirable taste which may commercially limit its use.

Hormonal Regulation including Ethylene Biosynthesis and Action
Ethylene
Harry Klee from the University of Florida gave an excellent talk on the control of ethylene responses during ripening. Using tomato fruit as models, they showed that ethylene receptors are

highly regulated. He presented compelling genetic and biochemical evidence that ethylene receptors act as negative receptors of down stream processes; in the absence of ethylene, receptors actively suppress expression of ethylene responsive genes. This is fundamental to our understanding of this important ripening hormone.

A research group at the University of Padova (Italy) are investigating the molecular basis to the differential response of apple and peach tissue to MCP. They examined the well known observation that following MCP application to apple fruit, the fruit remain firm and produce little ethylene for several days at 20C, whilst peach fruit firmness quickly decreased and ethylene evolution dramatically increased after a few hours from the end of the treatments. This work is fascinating and will help unlock some of the current mysteries of fruit sensitivity to ethylene.

Jasmonates

Methyl jasmonates are lipid derived compounds regarded as 'non-traditional' plant hormones which exert numerous effects on plant growth and development. There has been increased interest in jasmonates in recent years as they have been shown to be important mediators of wound and stressed induced signal transduction and induce expression of a variety of genes involved in plant defence. Several presentations confirmed the important role of jasmonates in fruit ripening eg tomato, strawberry etc. I believe that research with these compounds will continue to exhibit wide physiological functions in fruit and vegetable maturation and ripening which maybe commercially exploited.

Storage and Transport

An excellent talk was given by Marius Huysamer from the University of Stellenbosch (South Africa). He spoke on their experiences on the storage life of plums using controlled atmosphere shipping. Like Australia, South Afric is an important supplier of summerfruit to the northern hemisphere from November to March. South Africa exports about 62% of its fruit to Europe. The voyage by sea to Europe necessitates production of cultivars that are able to maintain acceptable eating quality for a period of at least 4 weeks from the time of harvest. They examined four cultivars of Japanese plums (*Prunus salicina* L.) with different storage regimes. They examined storage life under regular atmosphere (RA) conditions at -0.5C following either RA or controlled atmosphere (CA 5%O_2 and 10%CO_2) shipping, using either the commercial dual temperature (-0.5C, +7.5C, -0.5C) or a single high temperature (+7.5C) regime. They used the locally bred 'Sapphire', 'Songold' and 'Laetitia', as well as the well known 'Angeleno' plum. Storage life of the four plums could be extended for an additional two to three weeks under RA conditions at -0.5C without adverse effects on quality, provided the CA was applied during the shipping phase, either under dual or single high temperature regimes. Fruit firmness was best retained under CA (dual temperature) conditions. These fruit also had the lowest respiration rates, ethylene production rates and internal ethylene content. Skin colour development was better under single high than under dual temperature conditions, and also better under RA than CA shipping. However Marius confirmed that these results are cultivar specific, but show that CA can be beneficial for the out-turn of fruit onto overseas markets. However breakdown is an important consideration during storage and transport.

Horticulture Tours

Verona is located in the important Veneto region in the north of Italy. Horticultural production is worth more than Euros 850 million, and represents more than 17% of the regional agricultural gross income. Vegetable production is of major importance in the region contributing to about 67% of horticultural gross income. The major vegetable crops are radicchio, tomatoes, asparagus and potatoes. The fruit sector is led by apples, pears and kiwi fruit.

There are about 70 co-operatives operating in Veneto, and more than 90% of the 4,000 farmers belong to these organisations directly, or to one of the regional producers organisations. However despite this, there are still many small businesses which lack efficiency in the ever expanding and well organised distribution sector. The horticulture sector as a whole accepts there are many problems affecting the competitiveness of the horticultural system. They believe there is a prevalence of cultivars of little interest to the market, there is a lack of attention given to procedures of product differentiation and promotion, and little interest in the planning and logistical aspects of production. But their main concern in the high production costs, lack of machinery and available labour. These sound like similar concerns to Australian farmers, who do not have the luxury of EU and regional subsidies of up to 45%.

However Italian farmers and government do recognise the importance of research and development of new products and increasing the intrinsic and extrinsic quality of the produce. The regional government firmly believes in product promotion through the attainment of the European Union PDO and PGI designations, which have already been obtained for white asparagus from Cimadolmo, red radicchio of Teviso, variegated radicchio of Castelfranco, borlotti beans of Lamon, cherries of Marostica and chestnuts of South Zeno. Recognition is currently underway for red radicchio of Verona and Chioggia, salad greens of Lusia and white asparagus of Bassano.

In order to develop and improve the quality of other products, which are of great importance in Veneto but would have difficulties in attaining a European destination of origin, the regional government approved a series of specification for the horticultural sector which are available to producers who intend to request use of the regional brand name. It is now up to farmers, researchers and marketers to identify strategies for the re-organisation of the supply chain to add value to the horticulture sector in Italy.

Berries in Pergine Valsugana (Trento Province)

A mid conference tour was taken to Sant 'Orsola located in Pergine Valsugana. Sant Orsola is the most important Italian association of small fruits. This co-operative was founded in 1975 and includes more than 1,400 small growers, but only 50 full time growers. The average size of the grower's property is only 1,000m^2. There is little disease pressures as the crops are generally grown under plastic cover, although Powdery Mildew can be problem.

The packing line at the co-operative receives fruit all day, but growers receive a premium for morning deliveries. Postharvest disease is rarely a problem. Blueberries are stored for up to 100 days in CA (20%CO_2 and 5%O_2, 12%CO_2 for blueberries). The co-operative has 14 small CA rooms to extend the storage life.

A unique observation was the use of ice blankets / packs infield and export packs. These plastic bags / blankets contain about 1kg of water, and when frozen are placed in the box. They estimate it costs 20 cents per bag, and they are recycled.

Apples of the Trentino – Alto Adige (11-13, June 2004)

Trentino-Alto Adige is a mountain region in northern Italy, with high living standards. Trentino - Alto Adige is the most important apple growing area in Italy and produces top quality white and red wines from grapes grown at different altitudes.

San Michele all'Adige Research Institute

San Michele all'Adige Research Institute is located near Trento, and was originally a monastery in the 1100s, but was established as an agricultural school in 1874. It now has about 600 students (150 students live on campus), and has 80 teachers. It is run through the Provincial government and consists of about 85ha, of which 60% is in production. Apart from important applied research for the local industry, the Institute also has an extension service. Wine grape production is the major horticultural industry serviced by the institute, where the traditional pergola system is slowly being replaced by the newer trellising system common in Australia. The wine industry is a very important component of the local economy. This was reinforced at Mezzocorona where we visited the "Rotary" Co-operative winery producing one of the most famous Italian "spumante" (Italian equivalent of the French ‘champaign’).

Melinda Apple Consortium cold store A.V.N.

There are 17 co-operatives under the Melinda apple label with about 5,200 growers in the valley. There is no competition between growers in the region, as virtually all growers belong to the local co-operatives. The average farm size is 1-1.2ha, but this varies with different locations. Consequently land prices are extremely expensive. Generally Euro 30-35/m^2, but sometimes up to Euro 50/m^2 if the land has good apple varieties with irrigation (A$900,000/ha). Although the farmers receive significant subsidies (20%EU subsidy and 22%provincial subsidy), only 20% of growers are full time apple growers and 35% of growers have a second income. The co-operative buys in bulk chemicals for their farmers. In some circumstances, the co-operative even mixes the chemical at the correct rate for the farmer, and gives them exactly how much chemical is required (as the co-operative knows the acreage of orchard). The co-operative recognises that ‘Integrated Production’ is the only option for the growth of horticulture. The industry is quite intensive with about 15,000 workers, equivalent to garden workers which can essentially micromanage each orchard. The growers are randomly tested for chemical residues by the co-operative, but no problems have ever been detected. Traceability is a major issue for the co-operative, and have unique barcodes for each farmer / bin, but they are installing new software to have a unique code on each box.

Growers are paid on quality. Sample bins (1 in 4) are sampled for size, colour, defects, soluble solids content, red blush, firmness, and the farmer is paid accordingly.

EurepGAP is coming next year to the co-operative (2005), but they already have European supermarket certifications and believe the addition of EurepGAP will not be a difficult transition.

The co-operative also finances and supports itself and members, closing the money loop. Therefore all profits and finances are retained in the local region, supporting local businesses and communities. The co-operative also negotiates and buys new varieties and trees for members from nurseries. The co-operative believes their system works because all work is done properly from the finances to the growing and marketing. This is the strongest grower organisation in Italy, but the limited distribution creates difficulties. Even with their strong brand trademark, the co-operative believes it is still weak in the market.

Hail is considered a significant problem in the valley. They do not use guns to reduce hail, but rely on either; insurance, netting, or praying.

Postharvest

The co-operative do not use any postharvest fungicides. But can use DPA to control scald. They have noted that the plastic bins absorb DPA, and this must be taken into account when storing non-DPA dipped fruit. The co-operative is not keen on the use of returnable creates, as they have observed damage to the fruit. But will do what its customers (supermarkets) demand.

There are currently 16 different packing sheds in the 16 different co-operatives under the Melinda Consortium, but they wish to reduce and consolidate this to 4 packing shed with state of the art sorting and packing machinery in the next few years. The Melinda Apple Consortium cold store commercially utilises ultra low oxygen (ULO) and initial low oxygen stress (ILOS) applications. At the AVN packinghouse, they had 52 CA rooms and can pull down these rooms (600m^3) to 5% in about 8 hours. The rooms are tested for gas tightness every year. Each room has it own CO_2 scrubber. The co-operative receives about 1,100 tonnes of fruit per day. This is too much to process each day, and the fruit is stored straight into CA. Apples are pre-sorted for colour and size before CA storage with yellow coloured fruit sold in winter, and greener fruit sold later.

The 'Melinda' trademark is the best recognised apple in Italy and will be expanding to other co-operatives in other valleys. They market and advertise their own brands, including on television. Their best recognised brand is the red blushed Golden Delicious. This apple is highly appreciated in Italy and always brings a premium in the market. As expected the major variety is Golden Delicious (80%), with Canada (10%) and the rest accounting for 10% of production. However they are re-newing varieties with a decline in Golden Delicious, and increase in Gala (Galaxy mutants) and Fuji. The co-operative guarantee prices during the change in varieties. The production research is done in conjunction with the local San Michele all'Adige Research Institute.

Laimburg Agriculture and Forestry Research Centre

The Laimburg Agriculture and Forestry Research Centre has over 200 workers, of which 80 are researchers. South Tyrol area is the largest single area of apples in Europe, and also has extensive plantations of vines. Alpine horticulture (herbs, berries), vegetables (cabbages), ornamentals, and livestock (cows) are also important agricultural industries in the region. This region of Italy borders the Austria and is extremely wealthy. Approximately 15% of all people in South Tyrol are involved in agriculture and this translates into significant political influence. Hence significant amounts of agricultural research and development is conducted at the Laimburg Agriculture and Forestry Research Centre.

The research institute is a showpiece for agriculture and the local region. The postharvest facility rivals anything in the world. Dr Angelo Zanella manages the postharvest research at the Laimburg Agriculture and Forestry Research Centre and has excellent links with industry to assess and develop new storage technology for the local apple industry. The postharvest laboratory has an in-line NIR capable of 5 fruit/sec which assess internal disorders, SSC% and if calibrated correctly firmness. In addition they had an automated instrument to measure the weight, penetrometer firmness, SSC(%), TA and extractable juice on 30 apples at once! The postharvest laboratory has over 60 CA rooms / cabinets with ISOCELL CA for research. These rooms are regularly used in a range of storage trials that were presented at the conference in Verona (eg assessment of the chlorophyll fluorescence instruments during long term apple CA storage).

Farmers not only produce agricultural produce, but are also preservers of the land for locals and tourists. I think this is an important feature of the Italian / European agricultural identity and supports the financial subsidies and economic assistance agriculture receives from all levels of governments.

Other random notes from the Laimburg Agriculture and Forestry Research Centre:

- Frost protection is a major issue for apple growers in South Tyrol during flowering, and this is done with the use of sprinklers.
- Fireblight is a present and a concern of the local industry, but with stringent prevention and control regimes in place, the industry deals with any outbreak. Financial and orchard losses with fireblight can be enormous.

VARIETY TRENDS IN SOUTH TYROL

Varieties	**% of total crop production**								
	1960	***1970***	***1975***	***1980***	***1985***	***1987***	***1988***	***1989***	***1990***
Golden Delicious	1.9	26.2	35.1	51.3	47.6	51.3	45.2	51.8	47.3
Morgenduft	16.1	26.8	23.3	21	20.8	17.6	21.2	14.8	16.7
Red Delicious	1.1	9.5	8.8	8.9	10.4	12.1	13.4	10.7	11.6
Jonagold	-	-	-	0.1	1.2	2.3	3	4.9	5.3
Gloster	-	-	-	0.1	3.2	4.5	4.7	4.7	4.6
Jonathon	6.4	24.6	24.3	11.8	9.8	5.7	5	4	4.5
Granny Smith	-	-	0.6	1.8	2.7	2.5	2.8	3.4	3.9
Winesap	3	2.3	1.8	1.8	2.4	1.6	1.9	1.6	2.1
Elstar	-	-	-	-	0.1	0.5	0.8	1.7	1.6
Idared	-	-	-	-	0.6	0.8	0.9	1.2	1.3
Gravensteiner	14.3	4.4	5	2.7	0.7	0.7	0.4	0.4	0.3
Gala	-	-	-	-	-	-	-	0.1	0.2
Ozark Golden	-	-	-	-	-	-	-	0.2	0.2
Summerred	-	-	-	-	-	0.1	0.1	0.2	0.2
Jonagored	-	-	-	-	-	-	-	<0.1	0.1
Kalterer Bohmer	27.4	4	0.7	0.2	-	-	-	-	-
Champagner Reinette	19.1	1.2	0.2	-	-	-	-	-	-
Goldparmane	4.5	-	0.1	-	-	-	-	-	-
Kanada Reinette	3	-	<0.1	-	-	-	-	-	-
Wagner Reinette	1.2	-	<0.1	-	-	-	-	-	-
Brixner Platting	0.5	-	-	-	-	-	-	-	-
Tiroler Spitzlederer	0.5	-	<0.1	-	-	-	-	-	-
Goldnobel	0.4	-	-	-	-	-	-	-	-
Kosticher	0.1	-	-	-	-	-	-	-	-
Weiber Rosmarin	0.1	-	-	-	-	-	-	-	-
Weiber Winterkaivill	0.1	-	<0.1	-	-	-	-	-	-
Others	0.3	1	0.1	0.3	0.4	0.3	0.6	0.2	0.2
Total crop in tons	**221.728**	**300.000**	**440.975**	**485.318**	**600.000**	**698.000**	**553.500**	**510.400**	**688.200**

Cultivar	% of total crop production										Preview
	1991	*1992*	*1995*	*1996*	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	
Golden Delicious	52.3	43.7	47.3	44.0	48.9	48.5	50.0	48.5	49.8	48.9	↗
Red Delicious	9.8	13.3	11.9	11.8	11.0	10.0	11.9	11.7	11.5	11.2	↗
Gala	0.7	1.3	3.7	4.8	5.4	5.7	6.6	8.8	10.0	10.9	↗↗
Granny Smith	4.9	5.0	6.1	7.1	7.4	7.4	6.2	8.8	6.6	7.3	=
Braeburn	<0.1	<0.1	0.8	1.1	1.2	1.2	2.6	3.2	4.4	5.7	↗
Jonagold	4.8	6.5	8.5	7.7	5.1	8.1	6.9	5.7	5.4	4.5	↘↘
Morgenduft	13.3	15.3	10.6	12.3	10.8	9.7	6.8	6.1	4.4	4.1	↘↘
Fuji	-	-	0.5	0.2	0.2	0.3	0.7	0.8	1.2	2.0	↗↗
Winesap	1.5	2.3	1.8	2.0	1.7	1.2	1.8	1.4	1.4	1.4	=
Idared	1.3	1.2	1.6	1.6	1.5	1.6	1.9	1.5	1.7	1.2	=
Cripps Pink	-	-	-	-	-	-	-	0.1	0.4	1.2	↗
Elstar	2.4	2.8	2.4	2.4	2.0	2.1	2.0	1.4	1.3	0.8	↘
Gloster	3.9	4.2	2.2	2.5	2.4	1.8	0.6	0.5	0.3	0.2	↘↘
Jonathon	3.5	2.5	1.9	1.7	1.2	1.0	0.6	1.3	0.2	0.2	↘↘
Pinova	-	-	-	-	-	-	-	<0.1	0.1	0.1	↗
Sumerred	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	<0.1	↘
Ozark Golden	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	<0.1	↘↘
Gravensteiner	0.3	0.2	0.1	0.1	0.1	-	<0.1	<0.1	<0.1	<0.1	↘↘
Jonagored	0.2	0.3	-	-	-	-	1.0	0.9	1.0	-	↘
Andere / altri / others	0.6	1.0	0.1	0.4	0.3	0.2	0.2	0.2	0.2	0.2	↗
0.000 kg	**538.800**	**649.800**	**678.339**	**831.110**	**809.383**	**672.982**	**796.588**	**725.553**	**784.060**	**963.111**	

Source: Chamber of Commerce Bolzano

Increasing ↗
Strongly increasing ↗↗
Constant =
Decreasing ↘
Strongly decreasing ↘↘

The VOG Apple Group

The VOG Apple Group is also another grower own co-operative with about 500 grower 750ha. The production of the co-operative is about 35,000t per year. There are two production areas in the valley; 250m and 500-900m above sea level. Each area has its own environmental conditions which dictate variety and production methods. Golden Delicious is the main variety which makes up about 30% of all apples, closely followed by Gala (22-25%), Red Delicious (15%) and Granny Smith (10%). However the variety trends in the valley are against Golden Delicious, which is expected to decline to about 25% of total production. The directors of VOG believe Fuji has a big future in the valley. They believe that this variety will take up to 10-12% total production, but they recognise that this will be slow process as the market is not developed or educated on this new variety. VOG believe that Pink Lady apple production will increase from its current 2.5% production as the market develops. (Pink Lady production is second highest production outside France).

It is estimated that the production costs are 25 Euro cents per kg, and grower return is about 33 Euro cents per kg. Most of the orchards are small, and all work is done by the farmer, or their families, this reduces costs. However most farmers are not full time, some rent their houses for Bed and Breakfasts for the enormous tourist industry in the region.

Wine production is significantly more profitable than apple production, and like Australia vines are increasing in area.

Postharvest

The storage facilities and packing house of the VOG Apple Group are state of the art. They have a 44 lane sorter with 5 sizes and 4 colour grades. They use an AWETA system to sort for colour and defects. They employ up to 80 local workers on the line, and sometimes work 24 hours per day (but usually only 16 hours per day). They expect to have new packing lines every 8 years. They pack into 8 types of packaging, with 70-80% packaged, and the rest presized and sold in bins.

Under 'Integrated Production' (IP) rules, no postharvest fungicides can be used. Waxing is considered a postharvest treatment and is not used generally used in Europe. But some countries such as Greece, Portugal and Saudi Arabia, allow 'Stark' Red Delicious fruit waxing. Under IP if the fruit are to be sold after January 1, apple fruit can be postharvest dipped in DPA to prevent scald. The drench concentration is 1,000 – 2,000 ppm for Granny Smith, and half that for Pink Lady and Fuji. They store fruit for up to 11 months in plastic bins, and postharvest losses are 5-6% (in a good season).

The CA facilities at VOG were impressive. It takes about 4 days to fill a room, and takes about 48 hours to pull the temperature from incoming 28C to 1C. They are very respectful of airflow and respect ducts (bins up to 13 high). They use a range of step wise gas adjustment and temperatures for each variety, using a centralised control system. Ammonia / glycol is the preferred cooling system in all packing sheds visited. Although the cost can be up to 10% more expensive than a direct cooling system, they are more than happy with the quality and flexibility of the systems used.

Future Meetings

From the International Society for Horticultural Science, Quality and Postharvest Horticulture Commission business meeting during the conference, a number of items including the voting of the next ISHS Postharvest Symposium was conducted. From Santiago (Chile), Agadir (Morocco), Chaing Mai (Thailand) and Antalya (Turkey) as potential hosts for the 6th ISHS International Postharvest Symposium, Thailand won the day in a very close vote and will host the next Postharvest Symposium in 2008.

Future International Society for Horticultural Science Meetings
2004, 9-12 November, Postharvest Unlimited, Down Under, Sydney
2005, 29May- 2 June, Model IT, Leuven Belgium
2005, 5-10 July, CA Conference, East Lansing USA
2005, August 13-19, IHC Congress, Seoul Korea
2006, Quality in Chains, Bangkok Thailand
2008, 6th ISHS International Postharvest Symposium Chaing Mai Thailand

Other related meetings for the diary
2003 APEC Conference on Postharvest Systems, Thailand
2004 APEC Symposium on Quality Management in Postharvest systems, Thailand
2005 The Asia –Pacific Symposium on Quality Assuring and Safety of Fresh Produce
2005, 25-10 September, Australasian Postharvest Conference, New Zealand
2006, 18-22 June, Ethylene conference Pisa, Italy

Recommendations

Ensure that a postharvest component is included in all horticultural research and extension projects. Postharvest and market access links the grower to the retailer and consumer, and this can be where the greatest financial losses can occur. This *must* be addressed in all research, development and extension programs across all horticultural industries.

Research
Beyond Quality Maintenance
Postharvest research must continue to think beyond the maintenance of product quality. Other important parameters such as aroma, flavour, and 'healthiness' are often ignored in determining and optimising shelf life. This needs to be addressed with specific basic and industry funded research, development and extension. For example demonstrate and evaluate pre- and postharvest treatments on the human health benefits of fruits and vegetables. Relevant research and extension that significantly assist Australian horticulture to capitalise on the nutritional aspects of fruit and vegetables include,

- The effects of reduced light intensity on lycopene production in hydroponic tomatoes
- Effects of storage on antioxidant capacity in apples
- Preharvest factors affecting phenolic content of apples

Food Safety
Continue to support food safety initiatives, such as the development and implementation of HACCP-based systems.

Collaboration
Encourage co-operation and collaboration between both national and international research and extension institutes. More collaboration should occur between private, state Departments of Agriculture and overseas research institutes, such as Washington State University. Exchange and development with world class horticulture laboratories and scientists will enhance the potential of Australian horticulture.

Encourage liaison and assist the International Society for Horticultural Science, Food and Agriculture Organisation and large development agencies and institutes to improve the productivity and profitability of small farmers and their communities in developing countries.

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Appendix 1

Full paper presented at 5th International Postharvest Symposium

ReTain™ Maintains Pink Lady™ Fruit Quality during Long Term Storage

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Keywords: *Malus* x *domestica*, storage, CA, ethylene, firmness, AVG, shelf life, fruit quality

Abstract

Pink Lady™ apples were harvested from two growing regions (South Australia and Victoria) and at two harvest maturities (early and late). Fruit were treated with aminoethoxyvinylglycine (AVG, ReTain™) at two different times in the orchard (7 and 21 days before harvest). Fruit were harvested according to maturity and stored at 0°C in controlled atmosphere (CA) storage (2% O_2 and 1% CO_2) or regular air storage. Fruit quality (internal ethylene concentration, firmness, background colour, starch, soluble solids content) were assessed at two monthly intervals for up 10 months storage. Fruit were subject to a two week shelf life at 20°C after six months storage. All air stored fruit were removed at six months storage. The ReTain™ treatment significantly reduced ethylene production across all treatment and storage regimes, compared to the untreated control. Ethylene production was also lower in the ReTain™ treatments during the 14 days shelf life at 20°C after six months storage. Flesh firmness (measured by penetrometer) was generally higher in the fruit stored in CA and in the ReTain™ treated fruit. This effect was maintained during the entire storage period. The results show that the preharvest application of ReTain® on Pink Lady™ apples can be a useful tool for managing harvest and ensuring postharvest fruit quality after both air and CA storage.

INTRODUCTION

The timing of the apple harvest is extremely critical, as the physiological age of apples at harvest determines their storage life and quality. It is often difficult to harvest the entire crop at the right time for optimal quality and storage. Delaying harvest can improve the amount of red blush for Pink Lady™ apples which can lead to greater economic returns. However a delay in harvest will mean a reduction in the storage potential of the fruit. For long term storage, fruit need to be harvested before they begin producing ethylene. This compromise is a difficult practical and economic issue for all apple growers.

Aminoethoxyvinylglycine hydrochloride (AVG) is a plant growth regulator commercially registered as ReTain® Plant Growth Regulator. AVG competitively inhibits 1-aminocyclopropane-1-carboxylic acid (ACC) production and consequently inhibits ethylene production (Dussi et al, 2002; Green, 2003). The preharvest application of ReTain® offers the potential for delaying the onset of fruit ripening. The aim of this study was to assess the postharvest effect of preharvest applications of ReTain® on quality characteristics (internal ethylene concentration (IEC), firmness, starch pattern index (SPI), percentage soluble solids content (SSC %), background colour and skin greasiness) of Pink Lady™ apples stored in air for 6 months and in controlled atmosphere (CA, 2% O_2 and 1% CO_2) for 10 months at 0^oC. The experiments also evaluated the sensitivity to application time, harvest maturity and growing district.

MATERIALS AND METHODS

Pink Lady™ apples were harvested from two important commercial growing districts: Shepparton, Victoria and Lenswood, South Australia. ReTain® (soluble powder; 150g Aminoethoxyvinylglycine / kg, Valent Biosciences Corporation) was applied at a rate of 83 g/100 L together with 0.1% MAXX organosilicone surfactant (Sumitomo Chemical Company Pty Ltd). For both districts, ReTain® was applied to part blocks at 21 days before harvest (DBH) and to the remaining blocks at 7 DBH (25/03/2003 and 09/04/2003 in Shepparton, Victoria and 31/03/2003 and 15/04/2003 in Lenswood, South Australia) a third block was left as an untreated control (UTC). The application date was determined using district historical commercial harvest data as well as a combination of quality factors including fruit size, colour, firmness and the SPI of samples of fruit. Each treatment was harvested at two maturities; Maturity 1 (M1) at a SPI of 2 and Maturity 2 (M2) with a SPI of 4, as measured on the ENZA 7 (0-6) point starch chart. The harvest dates for all treatments of the 2 districts are given in Table 1.

For both the air and CA storage regimes, 80 fruit from each treatment for the air storage and 120 fruit for the CA storage were randomly selected and divided into replicates of 20 fruit. For each harvest date, a sample of 20 fruit was assessed on arrival and the rest of the fruit were placed in storage at either 0^oC in air for 6 months or in controlled atmosphere storage (flow through 2% oxygen and 1% carbon dioxide) at 0^oC for 10 months. Samples of 20 fruit were removed from storage and assessed at 2, 4 and 6 months after harvest in the air trial and after 2, 4, 6, 8 and 10 months in the CA study. The samples were left for 3 hours to warm to room temperature (21^oC) after storage before evaluation.

At each removal time, samples of 20 fruit from each treatment were assessed for flesh firmness, %SSC, SPI), background colour and skin greasiness; for the assessment of IEC, samples of 10 fruit were used (Jobling and McGlasson, 1995). After 6 months air and CA storage, additional fruit of M1 from South Australia were assessed for 2 weeks shelf life at 20^oC. Quality assessments were conducted at 4, 7 and 14 days at 20^oC.

All data were analysed using general linear models with means comparisons by KLsd (5% level of significance) using SimStat (Provalis Ver. 2.5).

RESULTS AND DISCUSSION

One of the key physiological effects of ReTain® is the reduction of ethylene production by competitively inhibiting 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity which subsequently reduces ethylene production (Dussi et al., 2002; Green, 2003). The reduced level of ethylene production was observed in fruit from both districts and in both storage atmospheres (Figure 1a and b). This response has been reported to be ‘immediate’ (within 24 hours) and significant however, the impact of the reduced ethylene production on fruit maturation is dependent on what stage of maturation that fruit is at when ReTain® is applied (Rath pers comm. 2003). The reduced response to ReTain® in Shepparton may be due to the fact that in a warmer climate the rate of fruit ripening may have been faster than at the cooler site of Lenswood.

The level of ethylene production is often related to the level of fruit softening (Jobling et al., 2003). The results from this experiment support this interaction (Figure 2a and b). Flesh firmness is one of the key parameters consumer use to determine the quality of apples. The extension of fruit firmness indicates that the quality of the fruit is maintained in storage, and as a result extends the marketable life of the fruit. Flesh firmness was found to be significantly higher in Lenswood than in Shepparton and this difference is likely due to the climatic differences between the two growing districts. The firmest fruit were those from Lenswood treated with ReTain® 7 DBH and harvested at the first harvest maturity (M1). This treatment extended that marketable life of the fruit stored in air by up to two months (Figure 2).

Shelf life after storage is an important quality parameter for consumers. Preharvest treatment with ReTain® at either 7 DBH or 21 DBH significantly reduced the rate of quality loss of Pink Lady™ apples stored in air and CA storage for 6 months and then stored at 20°C for up to 14 days (Figure 4). The reduction in the rate of firmness loss as a result of treatment with ReTain® after storage was most significant after air storage. Indeed the untreated control air stored fruit did not have enough shelf life for the two week assessment and were discarded after one week at 20°C. The levels of internal ethylene were significantly higher in untreated control fruit from both air and CA storage, even after 14 days at 20°C (Figure 3), showing the effects of ReTain® were maintained even after 6 months storage at 0°C and 2 weeks at 20°C.

CONCLUSIONS

The main benefit to growers of using ReTain® applied at either 21 DBH or 7 DBH was the extension of the harvest window through controlling fruit maturation. This was apparent in both Shepparton, Victoria and in Lenswood, South Australia, but a further benefit was also seen in Lenswood where an extended storage life of two months beyond the recommended air storage life was observed when ReTain® was applied 7 DBH. This was also seen in the CA stored fruit. However fruit harvested at an SPI of 2 and stored in CA were of higher quality than the later harvested fruit after 8 months. An extended harvest window and an extension of the postharvest storage life of Pink Lady™ apples may have significant commercial benefits for both domestic and export apple producers. This research shows that the application of ReTain® on Pink Lady™ apples can be a useful tool for managing harvest and ensuring postharvest fruit quality after both air and CA storage.

ACKNOWLEDGEMENTS

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Tables

Table 2 Dates of application of ReTain® and the harvest dates for the two maturities at Shepparton, Victoria and Lenswood, South Australia (2003).

Treatment	Shepparton Victoria	Lenswood South Australia
Application of ReTain® applied 21 DBH	25 March	31 March
Application of ReTain® applied 7 DBH	09 April	15 April
Harvest of M1, UTC	16 April	17 April
Harvest of M1, ReTain® applied 7 DBH	16April	17 April
Harvest of M1, ReTain® applied 21 DBH	23 April	23 April
Harvest of M2, UTC	29 April	30 April
Harvest of M2, ReTain® applied 7 DBH	01 May	02 May
Harvest of M2, ReTain® applied 21 DBH	05 May	02 May

Figures

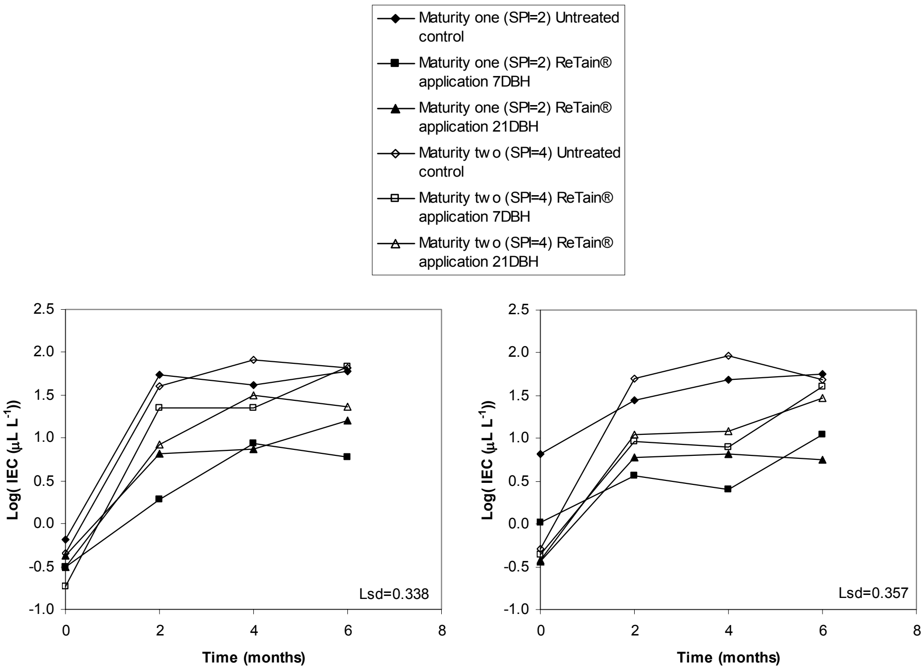

Figure 1a. Mean IEC of Pink Lady™ apples grown in Shepparton, Victoria (Left) and Lenswood, South Australia (Right) during a postharvest storage period in air at 0°C for 6 months. Values represent means of 10 replicates. Least significant difference (5%) shown.

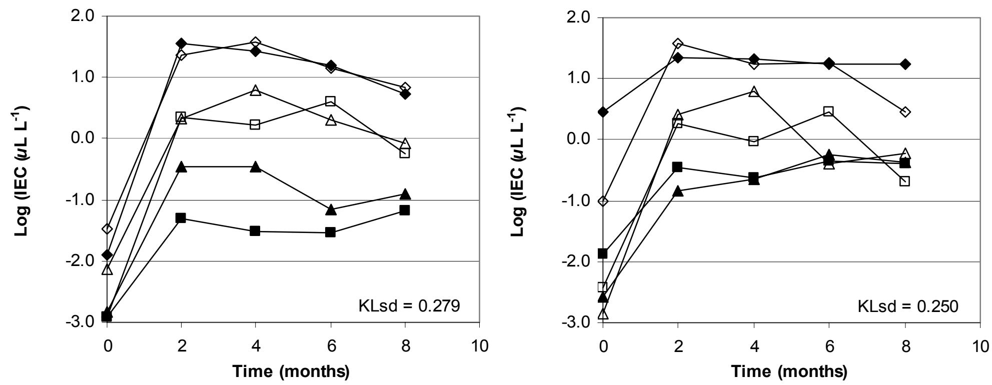

Figure 1b. Mean IEC of Pink Lady™ apples grown in Shepparton, Victoria (Left) and Lenswood, South Australia (Right) during a postharvest storage period in controlled atmosphere at 0°C for 8 months. Values represent means of 20 replicates. Least significant difference (5%) shown.

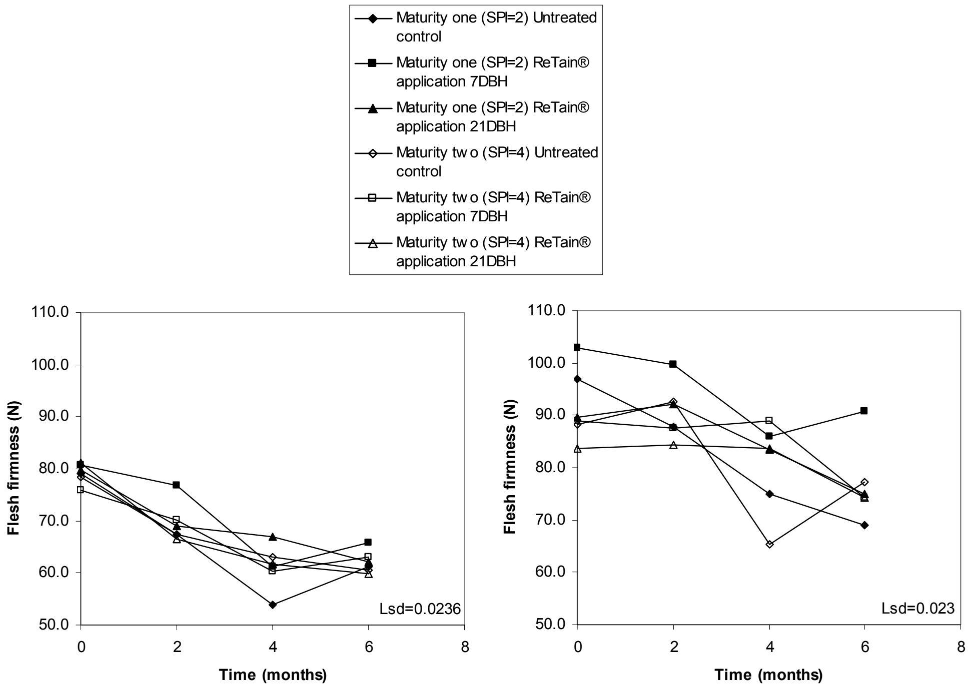

Figure 2a. Mean flesh firmness of Pink Lady™ apples grown in Shepparton, Victoria (Left) and Lenswood, South Australia (Right) during a postharvest storage period in air at 0°C for 6 months. Values represent means of 20 replicates. Least significant difference (5%) shown.

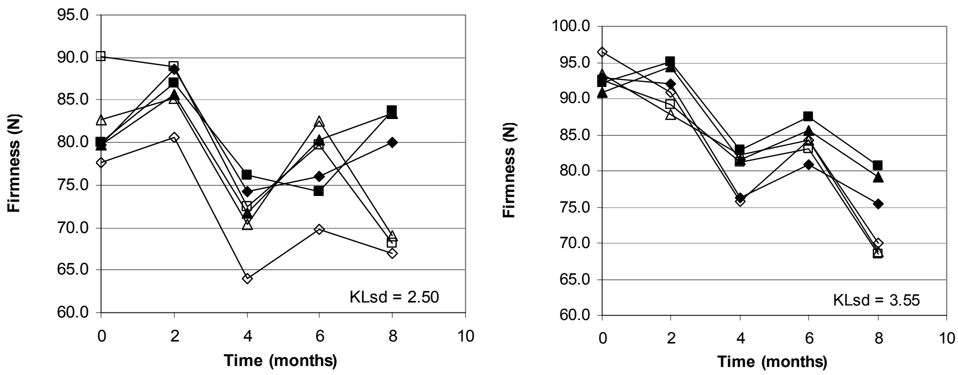

Figure 2b. Mean flesh firmness of Pink Lady™ apples grown in Shepparton, Victoria and Lenswood, South Australia (Right) during a postharvest storage period in controlled atmosphere at 0°C for 8 months. Values represent means of 40 replicates. Least significant difference (5%) shown.

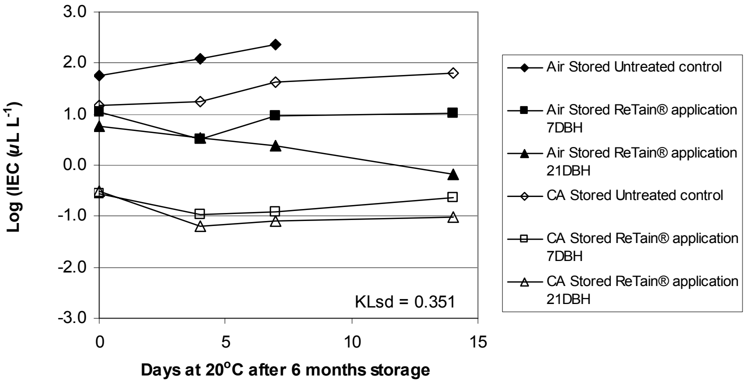

Figure 3. Changes in internal ethylene levels of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A. after 6 months storage and 14 days at 20°C. Values represent means of 10 replicates. Least significant difference (5%) shown

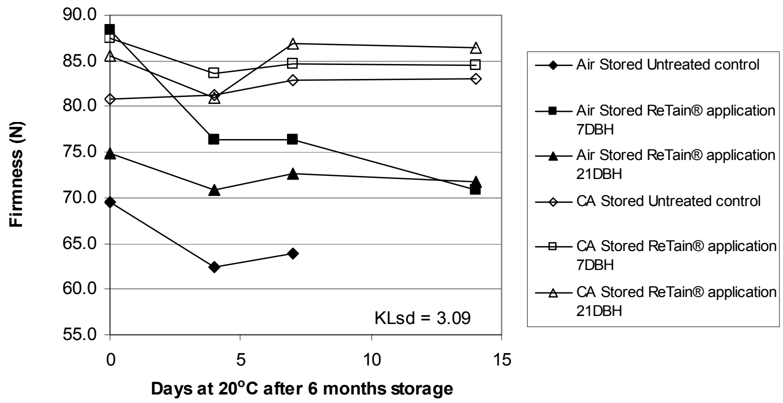

Figure 4. Changes in the flesh firmness of Pink Lady apples™ from the first harvest of fruit from Lenswood, S.A. after 6 months storage and 14 days at 20°C. Values represent means of 40 replicates. Least significant difference (5%) shown.