RIPENING RESPONSES OF 'FORELLE' PEARS

By

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DECLARATION

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

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Elke M. Martin **Date**

SUMMARY

'Forelle' is one of three blushed pear cultivars produced in South Africa. A mandatory minimum cold storage duration of 12 weeks at -0.5°C to ensure even ripening, prevents 'Forelle' from being marketed earlier. Since earlier marketing can result in premium prices (in excess of 50% more per carton) research in recent years has been directed at reducing this 12 week cold storage period. Intermittent warming treatments, controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals, and ethylene treatments, have been tested as alternatives to the 12 week cold requirement. However, none of these treatments delivered a better internal quality in terms of mealiness and astringency.

Fruit harvested from the Warm Bokkeveld and Theewaterskloof areas at commercial maturity were stored at -0.5°C for up to 21 and 22 weeks, respectively, to understand the changes in ripening and mealiness of 'Forelle' pears after cold storage (Paper 1). Samples were removed every third week, placed at 15°C, and maturity factors, total ACC concentration, ethylene production and respiration rates monitored every third day for 12 days. Fruit from the Warm Bokkeveld and the Theewaterskloof areas ripened after 6 and 7 weeks at -0.5°C, respectively. However, after 6 weeks of cold storage followed by 6 days at 15°C, all fruit harvested in the Warm Bokkeveld, and 70 % of the fruit harvested in the Theewaterskloof area, were mealy. With extended storage at -0.5°C (> 15 weeks) the incidence of mealiness declined in fruit from both areas, but never disappeared, when evaluated at 15°C over 12 days.

Since harvest maturity affects the incidence of mealiness in other pear cultivars, the effect of harvest maturity on 'Forelle' pears with regard to mealiness development was examined (Paper 2). Fruit were harvested from the Ceres area, in weeks 8 (pre-optimum), 10 (optimum), 12 and 14 (both post optimum). Maturity indices, juice content, mealiness, total ACC content, ethylene production and internal ethylene were monitored at harvest, after 6 weeks of storage at -0.5°C and again after 7 days at 15°C. Fruit harvested 2 weeks before commercial harvest (week 8) had the highest total ACC concentration, ethylene production and the potential to ripen, but also developed the highest incidence of mealiness (80%). However, fruit of all harvest maturities (except where contamination with 1-MCP occurred) were mealy. It would

appear that factor(s), other than harvest maturity, play a more important role in the initiation of mealiness in 'Forelle' pears.

Although ethylene has been shown to shorten the cold requirement of 'Forelle', there are conflicting reports as to its effectiveness in reducing mealiness. Consequently, the aim of the third paper was to evaluate the effect of ethylene on ripening and mealiness of 'Forelle' pears. Fruit harvested from the Elgin area at commercial maturity were stored for 3 weeks at -0.5°C, treated with ethylene (100 μ $L.L^{-1}$, 24h, 20°C) and held at 20°C for a further 2 days (without ethylene). Control fruit were held at 20°C for 3 days. Fruit were returned to -0.5°C for a further 3 weeks. After a subsequent 3 days at 20°C, flesh firmness was 4.6 kg in treated fruit compared to 6.1 kg for control fruit. At this point all fruit treated with ethylene were mealy. Control fruit all exhibited mealiness after a further 3 weeks at -0.5°C followed by 7 days at 15°C. Ethylene treatment advanced fruit maturity, but did not prevent or alleviate mealiness.

Mealiness is a textural disorder recognized by a dry soft pulp. This has previously been recorded in 'd'Anjou' pears, as a result of storing the fruit for too long at -1.1°C, but has also been the result of a chilling injury in fruit like nectarines, kiwi and persimmon. The role of storage temperature on ripening, and specifically mealiness, of 'Forelle' was thus investigated (Paper 4). Fruit harvested from the Elgin area at commercial maturity were stored at -0.5°C, 4.0°C and 7.5°C for 0, 3 and 6 weeks. Samples were removed every third week, placed at 15°C, and maturity indices, extractable juice content, mealiness, total ACC content, internal ethylene concentration and ethylene production were monitored on removal and after 7 days. Flesh firmness of the 4°C stored fruit was 0.5 kg lower than fruit stored at -0.5°C, on removal from storage. Fruit stored at 4°C and 7.5°C ripened with little to no mealiness (0 and 8% respectively) in contrast to fruit stored at -0.5°C (70% mealy). Total ACC accumulation and ethylene production were higher for fruit stored at 4°C and 7.5°C than fruit stored at -0.5°C. Storage temperature appears to play a role in the development of mealiness.

Although storage temperatures influenced mealiness development, this research should be repeated before this can recommended as a commercial treatment. The

underlying mechanism of action in the development of mealiness should be investigated.

By examining fruit grown in different climatical areas, harvested at different maturities, treated with exogenous ethylene and stored at different temperatures, this research has helped to a better understanding of the role of factors affecting ripening and development of mealiness in 'Forelle'.

OPSOMMING

'Forelle' is een van drie blos peer kultivars wat in Suid Afrika geproduseer word. 'n Minimum van 12 weke by -0.5°C opberging, is deur wetgewing vasgestel, om eweredige rypheid van 'Forelle' te verseker. Vroeër bemarking van 'Forelle' kan daartoe lei dat vrugte premium pryse, van 50% meer per karton behaal. Aangesien die vasgestelde koue periode verhoed dat 'Forelle' vroeër bemark kan word, het onlangse navorsing gefokus op vermindering van die vasgestelde opberging van 12 weke by -0.5°C.

Afwisselende verwarmings behandelings gedurende koue opberging, beheerde atmosfeer (BA) opberging in kombinasie met gewone atmosfeer (GA) opbergings intervalle, en etileen behandelings, is getoets as alternatiewe vir die 12 weke opbergings vereiste by -0.5°C. Geen van die bogenoemde behandelings kon egter 'n beter interne kwaliteit in terme van meleringheid en frankheid as 'n alternatief verseker nie.

Die doel van die eerste proef was om die invloed van koue opberging by -0.5°C op rypwording en melerigheid van 'Forelle' te bepaal. Vrugte is in die Warm Bokkeveld and Theewaterskloof areas geoes tydens kommersiële oesrypheid, waarna hulle vir 21 en 22 weke, respektiewelik, by -0.5°C opgeberg is. Monsters is elke derde week vanaf die -0.5°C koue opberging geneem en by 15°C geplaas, waarna rypheids indeksering, totale ACC konsentrasie, etileen produksie and respirasie tempos elke derde dag vir 12 dae gemonitor is. Vrugte wat in die Warm Bokkeveld en Theewaterskloof areas geoes is, het rypgeword by 15°C, na 6 and 7 weke by -0.5°C, respektiewelik. Alle vrugte vanaf die Warm Bokkeveld en 70% vrugte vanaf die Theewaterskloof area, het

egter na die 6 en 7 weke by -0.5°C, respektiewelik, en 6 dae by 15°C, melerigheid ontwikkel. Vrugte wat vir langer as 15 weke by -0.5°C opgeberg is, het in albei areas 'n afname in melerigheid getoon, maar die defek het nooit heeltemal verdwyn tydens evaluasie by 15°C nie.

Oes rypheid affekteer die ontwikkeling van melerigheid in ander peer kultivars. Die tweede proef was dus gefokus op die invloed van oes rypheid op 'Forelle' se ontwikkeling van melerigheid. Vrugte is in die Warm Bokkeveld area geoes in week 8 (pre-optimum), 10 (optimum), 12 and 14 (albei post-optimum). Rypheids indekse, uitdrukbare sapinhoud, meleringheid, totale ACC konsentrasie, interne etileen vlakke en etileen produksie is gemonitor, na opberging vir 6 weke by -0.5°C en 7 dae by 15°C. Vrugte wat 2 weke voor kommersiële rypheid geoes is (week 8) het die hoogste totale ACC konsentrasie en etileen produksie gehad, en het reeds die potensiaal gehad om ryp te word. Die hoogste vlakke van melerigheid (80%) is ook in vrugte wat tydens hierdie oes verkry is, waargeneem. Vrugte wat op alle tye geoes is, het melerigheid ontwikkel, behalwe waar kontaminasie met 1-MCP plaasgevind het. Dit wil voorkom asof 'n ander faktor 'n belangriker rol in die ontwikkeling van melerigheid in 'Forelle' speel as oes rypheid.

Etileen is bewys om die vereiste koue opberging vir 'Forelle' te kan verkort, maar daar is konflik oor die effektiwiteit van etileen op die verlaging van melerigheid in 'Forelle'. Die doel van die derde proef was dus om die effek van eksterne etileen behandeling op rypheid en melerigheid van 'Forelle' te toets. Vrugte wat in die Elgin area by kommersiële oesrypheid geoes is, is vir 3 weke gestoor by -0.5°C, met etileen behandel (100 μ L.L^{-1}, 24h, 20°C) en vir 'n verdere 2 dae by 20°C gehou (sonder etileen). Kontrole vrugte is vir 3 dae by 20°C gehou. Daarna is vrugte weer vir 'n verdere 3 weke by -0.5°C opgeberg. Na 'n verdere 3 dae by 20°C, was vlees fermheid van etileen behandelde vrugte 4.6 kg in vergelyking met 6.1 kg vir die kontrole vrugte. Alle vrugte wat met etileen behandel is, was melerig op hierdie stadium. Die kontrole vrugte het ook almal melerig geword, maar slegs na 'n verdere 3 weke by -0.5°C en 7 dae by 15°C. Etileen behandeling het dus die rypheid van 'Forelle' bevorder, maar het nie melerigheid voorkom nie.

Melerigheid is 'n tekstuur probleem, wat gekenmerk word aan sagte droë pulp. Hierdie tekstuur probleem is voorheen waargeneem in 'd'Anjou' pere wat te lank by -1.1°C opgeberg is, maar is ook kenmerkend by vrugte soos nektariens, kiwi en persimmon wat aan koue skade ly. Die gevolg is dat die effek van opbergings temperatuur op 'Forelle' rypwording en melerigheid getoets moes word. Vrugte is geoes van die Elgin area tydens kommersiële oesrypheid en gestoor by -0.5°C, 4.0°C en 7.5°C vir 0, 3 en 6 weke. Vrug monsters is geneem, na verwydering van bogenoemde stoor temperature en na 7 dae by 15°C, waarby rypheids indekse, uitdrukbare sapinhoud, meleringheid, totale ACC konsentrasie, interne etileen vlakke en etileen produksie gemonitor is. Vlees fermheid van vrugte wat vir 6 weke by 4°C opgeberg was, was 0.5 kg laer as vrugte wat by -0.5°C opgeberg was. Vrugte wat by 4°C and 7.5°C vir 6 weke gestoor is en by 15°C ryp geword het, het min tot geen melerigheid ontwikkel (0 and 8%, respektiewelik) nie. Vrugte wat by -0.5°C opgeberg was het egter hoë vlakke van melerigheid bereik (70% melerig). Totale ACC akkumulasie en etileen produksie was hoër vir vrugte wat by 4°C en 7.5°C gestoor was, teenoor vrugte wat gestoor was by -0.5°C. Dit wil voorkom asof na-oes opbergings temperature wel 'n rol speel in die ontwikkeling van melerigheid.

Alhoewel dit voorkom asof na-oes temperature wel 'n rol speel in die ontwikkeling van melerigheid in 'Forelle' pere, is meer basiese navorsing nodig om die meganisme van werking in melerigheid ontwikkeling te verstaan. Na-oes temperatuur as 'n faktor wat melerigheid kan beïnvloed, moet oor 'n reeks van seisoene nagevors word. Laasgenoemde is van uiterste belang aangesien die invloed van seisoenale variasies op melerigheid nog nie gekwantifiseer is nie.

Die navorsing was gefokus daarop om vas te stel watter faktore 'n fisiologiese rol speel in rypwording en melerigheid van 'Forelle' pere. Deurdat vrugte van twee areas met verskillende klimate, vrugte met verskye oesryphede, ekterne etileen behandeling, en vrugte van verskillende opbergings temperature ondersoek is, het hierdie navorsing gehelp om 'n beter begrip te vorm van die rol van hierdie faktore op die rypwording en die ontwikkeling van melerigheid in 'Forelle'.

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GENERAL INTRODUCTION

'Forelle' is one of three blushed pear cultivars produced in South Africa. The blushed pear market begins with 'Rosemarie', followed by 'Flamingo' and then 'Forelle'. Discontinuity in the availability of the blushed pears occurs between 'Flamingo' and 'Forelle'. A mandatory minimum cold storage requirement of 12 weeks at -0.5°C prevents 'Forelle' from being marketed earlier (De Vries and Hurndall, 1993). There is a strong financial incentive to reduce the 12 week cold storage requirement of 'Forelle' since earlier marketing can result in premium prices. This incentive has directed research in recent years.

Intermittent warming treatments (De Vries and Hurndall, 1993), controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals (De Vries and Hurndall, 1993; De Vries and Hurndall, 1994; De Vries and Moelich, 1995), and ethylene treatments (Du Toit *et al.*, 2001), have all been tested as alternatives to the 12 week cold treatment. However, none of these treatments resulted in fruit of consistently acceptable internal quality. Ethylene treatment successfully reduced the storage period, when judged by colour and firmness, but fruit were still mealy and astringent. The only improvement in internal quality of 'Forelle' in terms of texture and flavour, was with extended cold storage (16-25 weeks) in controlled atmosphere conditions (De Vries and Hurndall, 1993).

Pears do not ripen normally until they have been exposed to a critical period of cold storage (Hansen, 1961; Sfakiotakis and Dilley, 1974; Mellenthin and Wang, 1976; Puig *et al.*, 1996, De Vries, 2001). Cold storage prior to ripening of pears causes an accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor to ethylene (Wang *et al.*, 1985), to the point where the ripening resistance is reduced and expression of autocatalytic ethylene results in normal ripening (Lelièvre *et al.*, 1997). In contrast to the cold requirement for ripening, inactivation of ACC-oxidase and suppression of ethylene in nectarines by chilling temperatures was recently shown to be related to woolliness in nectarines (Zhou *et al.*, 2000). Chen *et al.* (1983) described fruit stored at -1.1°C for longer than 5 months and ripened at 20°C as having a mealy texture compared to fruit stored for only four months and ripened normally. The role

of cold storage on 'Forelle' ripening and textural development needs to be established.

Mealiness in 'd'Anjou' pears was described where fruit were harvested overmature (Chen and Mellenthin, 1981; Murayama *et al.*, 1998) or stored for too long at high temperatures (Hansen and Mellenthin, 1979). Consequently, mealiness was classified as a senescence related disorder (Murayama *et al.*, 1998). Although optimum harvest maturities have been established for 'Forelle', and later harvested fruit tend to have a higher incidence of mealiness, this is not always the case for every season, and needs clarification.

Hansen (1961) associated a 53-70% incidence of mealiness with growing seasons with high total heat units. Mellenthin and Wang (1976), also found that fruit grown in high daily-hour temperatures during the six weeks before harvest, failed to ripen uniformly and were susceptible to certain physiological disorders, including mealiness. The growing areas in the Western Cape have major climatic differences. The Warm Bokkeveld and Stellenbosch areas are known to have much higher temperatures above 30°C, than the Elgin area. The time before harvest and harvesting dates coincide with the time of the year in which the maximum yearly temperatures are reached. It is not clear how these maximum temperatures influence ripening of 'Forelle', but the first mentioned areas are known to have a higher incidence of mealiness.

This study was thus aimed at profiling ripening of 'Forelle' during extended cold storage at -0.5°C. To determine which factors affect ripening and development of mealiness, and to gain a better physiological understanding of this specific pear, the effect of harvest maturity, ethylene treatment, and storage temperatures on ripening of 'Forelle' pears were considered. Each of these factors are discussed in the following papers, while chilling injury is reviewed in the first chapter. A complete literature review on ripening of pears will form part of the PhD thesis, which is a continuation of this research project.

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CHAPTER 1: LITERATURE REVIEW: CHILLING INJURY

1.1 Introduction

Chilling injury was reported by Sacks in 1865 and Moelich in 1896 (Lyons, 1973; Wang, 1982) and can be described as a physiological disorder that occurs in some plant parts as a result of low temperatures, above freezing temperature (Parkin *et al.*, 1989). The extent of the injury is usually a function of the temperature extreme, the duration of exposure, plant species, and morphological and physiological condition of the plant material at the time of exposure (Lyons *et al.*, 1979). To avoid chilling injuries, threshold temperatures should be avoided. The dilemma is that low temperature is the most effective way of prolonging post-harvest life of fresh commodities (Bramlage, 1982), either by slowing the metabolic processes that lead to senescence, reducing decay, or by controlling damaging insects (Couey, 1982).

Despite evolutionary chilling resistance and genetic adaptations developed by most temperate zone plants, some are injured by chilling temperatures (Lyons, 1973). Examples of chilling-sensitive temperate-zone crops include apples, asparagus, cranberries, nectarines, peaches and plums (Hardenburg *et al.*, 1986). Bramlage (1982) suggests the following explanations for the occurrence of chilling injuries in temperate-zone plants:

- Fruit are grown at the limits of their ecological adaptation.
- Fruit carry rudimentary chilling sensitivity, which is expressed when they receive another stress condition (like senescence) in addition to the cold stress itself.
- Detached organs are stored at temperatures below those at which the whole plant is adapted to survive.
- Sudden changes in climate that affect the otherwise normal development of the plant in a given location.
- During storage, normal metabolism is slowed, making symptoms of senescence, such as chilling injury symptoms, more noticeable.
- In the case of deciduous fruit trees that have a cold hardiness to withstand winter temperatures, their fruits can develop chilling injuries when stored at temperatures below which they will tolerate according to their genetic potential.

1.2 Symptoms

Morris (1982) listed a few general symptoms of chilling injury:

- Surface lesions – pitting, large sunken areas, and discolouration
- Water-soaking of tissues
- Internal discolouration (browning) of pulp, vascular strands, and seeds
- Breakdown of tissues; e.g., in peaches
- Failure of fruit to ripen, e.g., in tomatoes and bananas
- Accelerated rate of senescence
- Increased susceptibility to decay
- Reduced storage life or shelf life due to one or more of the above responses
- Compositional changes, especially in relation to flavour and taste.

Chilling symptoms are variable for different commodities and will be discussed individually.

2. CURRENT THEORIES

Despite many papers published in this field, there is no agreement on the cause or the mechanism of action of chilling injury, specifically when primary events triggering low temperature damage are discussed. Postharvest stresses, including chilling injury often lead to similar physiological patterns of deterioration. This suggests that tissue deterioration occurs by a universal mechanism for both stress-induced and senescence injuries (Palta, 1990). The proposed mechanism in reaction to stress is as follows: The biophysical changes in membrane lipids and enzymatic and non-enzymatic lipid peroxidation leads to altered membrane properties and results in defects such as ion leakage and cellular decompartmentation which leads to accelerated death (Fig. 1) (Marangoni *et al.*, 1996).

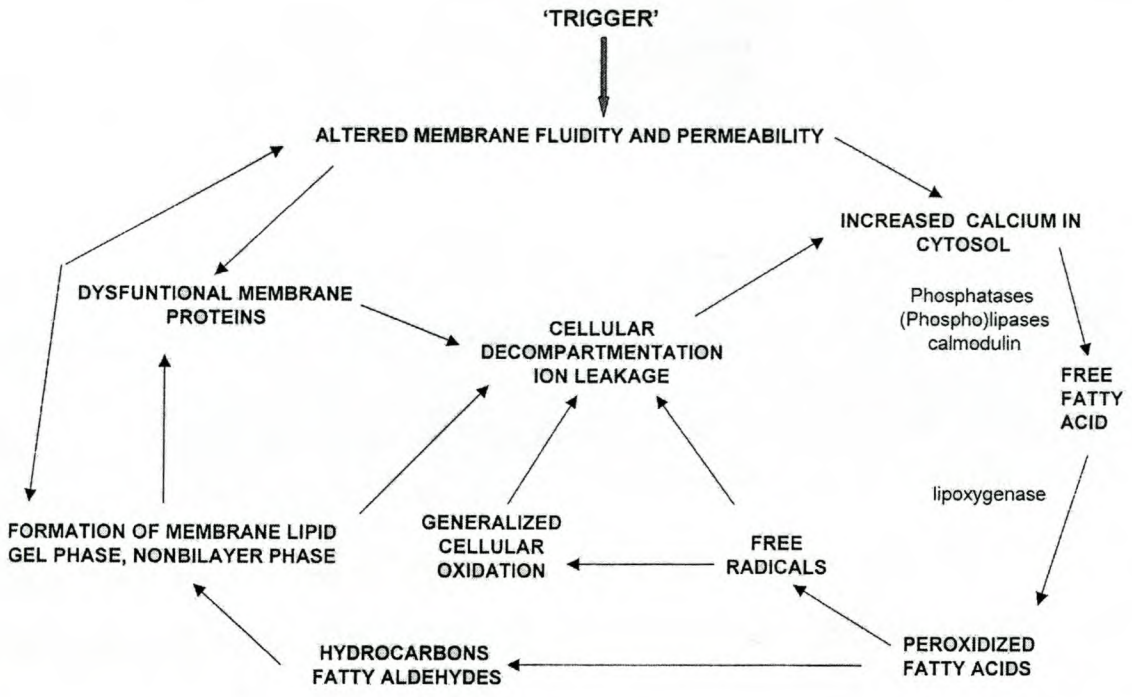

Fig. 1 Generalized scheme to account for membrane deterioration in plant tissue due to senescence or postharvest stresses (Marangoni *et al.*, 1996).

Wang (1982), discussed the major physiological responses as a result of chilling injury, in their approximate order of appearance (Table 1).

Table 1 Physiological responses to chilling injury (Wang, 1982).

Rapid response
Phase change in membranes and proteins.
Changes in membrane organisation.
Cessation of protoplasmic streaming.
Depolymerisation of microtubules.
Increased solute leakage (K^+ and amino acids).
Slower response
Increased membrane permeability.
Accumulation of toxic substances.
Failure of essential reactions.
Stimulation of respiration and ethylene production.
Interference with energy production (reduced ATP levels).

Slower response (continued)
Changes in cellular structure.
Changes in protein structure and enzyme activity.
Synthesis or activation of enzymes.

Although this series of events is confirmed in many cases, the mechanisms can be variable for different organelles, tissues and type of injuries involved. To understand mechanisms in membrane deterioration and the stresses that influence them, scientists are focusing on the 'trigger' of these reactions (Marangoni *et al.*, 1996).

2.1 Membranes

Cellular membranes are semi-permeable, dynamic barriers, and are thus very selective playing an essential role in the regulation of physiological processes. Experimental evidence continues to support membrane damage as the key event leading to a cascade of biochemical reactions resulting in tissue deterioration and economic loss. Lyons (1973) proposed the first theory regarding the nature of chilling injury, which was termed the bulk membrane lipid phase transition theory. The extent of fatty acid unsaturation determined the phase-transition temperature associated with changes in enzyme activity. Numerous studies failed to confirm a direct correlation between fatty acid composition and chilling sensitivity (Patterson *et al.*, 1978).

Lyons *et al.* (1979) refined his theory, suggesting that lipid composition and physical properties of bound membrane protein change at these chilling temperatures. He also suggested that the primary event occurs at the critical temperature, causing injury because of 'secondary responses'. Shewfelt and Erickson (1991) now hypothesise that the primary event in modification of the membrane structure involves conformation changes of critical protein, changes in cytoskeleton structure and changes in concentration of cytosolic calcium. Although it has been found that the lipid domains that undergo transitions are only 2-5% of the total lipids present in chloroplasts and mitochondria (Raison and Orr, 1986), these small physical changes can lead to metabolic imbalances in the tissue resulting in cell death. Cell death is thus reached when a time temperature threshold causes irreversible damage whereby recovery is inhibited (Parkin *et al.*, 1989).

The clearest evidence of membrane deterioration during chilling injury is from ultrastructural studies. Plastid, and to a lesser extent, mitochondrial membrane were rapidly disturbed following chilling temperatures. Ilker *et al.* (1979) found that the plasmalemma appeared to be most resistant to ultrastructural perturbations brought about by chilling temperature exposure, while irreversibility of chilling injury was ascribed to the loss of tonoplast integrity (Niki *et al.*, 1978).

2.2 Membrane lipids

Membranes consist of fluid bilayers of phospholipids containing embedded proteins and sterols. The lipid bilayer is a general permeability barrier because most water-soluble (polar) molecules cannot readily cross its nonpolar interior. All membrane molecules are able to diffuse freely within the plane of the membrane, permitting membranes to change shape and membrane molecules to rearrange rapidly (Singer and Nicolson, 1972).

Phospholipids contain a saturated fatty acid (C16:0), a long chain unsaturated fatty acid such as C18:1, 18:2 or 18:3, and a phosphate-containing sterol group. The steroid skeleton is rigidly planar and causes an increase in microviscosity, whereas the aliphatic sterol chain is mobile and results in greater fluidity (Leshem, 1992). Sterols seem to act as stabilizers of membrane fluidity allowing normal membrane function to be maintained over wide ranges of temperature (Leshem, 1992).

In addition to sterols that favour the fluidity of the membranes, unsaturated fatty-acid containing lipids are more fluid than saturated lipids. Mechanisms which allow maximum fluidity to compensate for low temperature include shortening the fatty acid tails, increasing the number of double bonds, and increasing the size or charge of the head groups. Roughan (1985) proposed that the proportion of disaturated-phosphotidylglycerol species correlated with the relative chill sensitivity among several species of plants (50-60% for chilling sensitive (CS) and <25% for chilling resistant (CR) species). Orr and Raison (1987) studied thermal properties of phosphotidylglycerol and Sulphoquinovosyldiacylglycerol (SQDG) and concluded that CS is related to both of these saturated polar lipid classes. Opposing the theory, Kendrick and Bishop (1986) suggested that lipid composition is simply a reflection of genetic origin.

Temperature also affects the solubility of O_2, CO_2 and ethylene in the cell. The solubility of O_2 increases with reduced temperature. Oxygen concentration is rate limiting for the synthesis of unsaturated fatty acids and will favour the synthesis of saturated fatty-acids under these low temperature conditions. This will in turn have an effect on the fluidity of the membrane (Scandalios, 1993; Makhlouf *et al.*, 1990).

2.3 Integral protein and enzymes

Proteins define the specificity of each membrane system, by having a specific function. All integral proteins are amphipathic molecules, i.e. they have portions which are hydrophilic and exposed in the fluid phase surrounding the membrane and a hydrophobic region in contact with the lipid molecules themselves (Robertson, 1959). The primary role of most integral membrane proteins is transport (e.g. Ca^{+2}-ATPase and H^{+}-ATPase pumps), signalling, anchoring the cytoskeletal elements to cell wall molecules, and assembly of cellulose fibrils from cytosolic substrates (Leshem, 1992).

Functional membranes are fluid since phospholipids can move freely in lateral dimensions. However, the presence of proteins and sterols, decrease and increase membrane fluidity, respectively (Marangoni *et al.*, 1996). The membrane-bound enzyme ATPase does not function when lipids are in the solid state (Grisham and Barnett, 1973). Lyons (1973) related this to conformation changes caused by the compression of membrane-bound protein molecules. This, in combination with a reduced energy supply due to suppressed mitochondrial respiration under chilling conditions, disrupts the normal energy balance of the cell. Consequently, protoplasmic streaming is inhibited. This prevents uptake of ions into cells and may even cause leakage of ions out of cells (Levitt, 1980).

Heat shock pre-treatments for short periods can acclimatize fruit to low temperature storage, in various plant tissue. Conditioning to low temperatures in watermelons at 26°C for 4 days before storage at 0 or 7°C reduced chilling injury (Picha, 1986). A water treatment at 53°C reduced chilling injury in long term storage of Valencia oranges (Wild and Hood, 1989). Reduction in chilling injury by high temperature treatment conditioning has only recently been related to the formation of heat shock proteins (hsp) (Lafuente *et al.*, 1991; Saltveit, 1991). Plant tissues produce two major type of hsp: high molecular weight (70 to 95 kDa) and

low molecular weight (15 to 30 kDa). The temperature shift required to induce hsp synthesis varies depending on the plant species. However, the response can also be specific for certain cultivars, such as in tomato, where 'Rutgers' showed no benefit through heat treatment (Whitaker, 1994), whereas other cultivars were reported to benefit from it (Lurie, and Sabehat, 1997).

Potatoes have been used to show the physical changes in cold sensitive protein (enzymes). Phosphofructokinase (PFK), a key enzyme in glycolysis, exists in active tetrameric form. This enzyme dissociates into inactive dimers at low temperatures, and causes sweetening of cold sensitive potatoes (Doucette and Pritchard, 1993).

Chemical reactions that are enzyme-catalyzed are also temperature dependant. Certain enzymes have thresholds for activation or synthesis near the chilling temperature. An example is Caffeoyl-CoA:quinic acid 0-caffeoyltransferase (CQT) activity which has a threshold temperature between 10°C and 12°C in tomato and 2°C and 5 °C in potatoes (Liebermann *et al.*, 1958). The inactivation of phenylalanine ammonia-lyase (PAL) in russet spotting of lettuce, with low temperature is another example. In addition, low temperature has an effect on pectolytic enzymes of nectarines (Ben-Arie and Sonego, 1980) and plums (Taylor *et al.*, 1994). The hypothesis is that polygalacturonase (PG) activity is inhibited by low temperature, but pectinesterase (PE) not. The different responses of PG and PE to chilling temperature causes partial or imbalanced pectin degradation and woolliness development (Buescher and Furmanski, 1978; Lurie *et al.*, 1994).

2.4 Senescent breakdown and oxidative stress

Peroxidation of fatty acids resulting in free radical formation has been described as one of the major deteriorative processes of membranes (Stanley, 1991). Unsaturated fatty acids are prone to attack by lipoxygenase (Driollard *et al.*, 1993), and increased free radical production has been observed in tissues injured by chilling or freezing temperatures, as well as dehydrated or senescent tissues (Shewfelt and Erickson, 1991; Sharom *et al.*, 1994). Whether lipid peroxidation is the main mechanism by which injury is transduced remains to be proven (Marangoni *et al.*, 1996).

To prove that peroxidation is a primary event for plasma membrane degradation, the lipoxygenase enzyme must be isolated in a pure form in the plasma membrane to induce disorders *in vivo*, and the peroxidation products must alter the membrane function (e.g. specifically of proteins) (Shewfelt and Erickson, 1991). Phospholipid hydrolyses, fatty acid peroxidation, and breakdown to hydrocarbons would induce the formation of gel-phase lipids that lead to gel-phase formation (Shewfelt *et al.*, 1994). The oxidation hypothesis in chilling injury is supported in that antioxidant enzymes such as superoxide dismutase and catalase (Spychalla and Desborough, 1990), glutathione reductase (Hausladen and Alscher, 1994), superoxide dismutase and dehydroascorbate peroxidase (Sen Gupta *et al.*, 1993), as well as antioxidants such as α -tocopherol (Spychalla and Desborough, 1990) seem to be involved in the prevention of chill-induced oxidation of lipids.

Palma *et al.* (1995) observed an increase in the saturation index of tomato microsomal membrane lipids after a week of chilling. This value decreased in week two of the chilling and increased when fruit were placed at 22°C. It seems that an initial loss of saturation, followed by acclimation during chilling and an induction of accelerated senescence follows removal from the chilling stress. With the onset of senescence, the chemical composition of membrane lipids change, resulting in lipid phase separations within the bilayer. The separation of lipid phases appears to be attributable to an accumulation of lipid metabolites and protein catabolites in the bilayer that are formed during turnover and metabolism of membrane lipids. Lipid-protein (deteriosomes) particles form in the membrane and serve as a secretion body for catabolites from the membrane to the cytosol and within organelles (Yao *et al.*, 1991). Thompson *et al.* (1997) proved that accumulation of lipid metabolites of senescing membranes coincide with impairment of lipid-protein particle formation. It remains to be proven that this mechanism is impaired by chilling and other stresses.

Compartmentalization of Ca^{+2} is extremely important, since membrane associated phospholipase activity is activated by Ca^{+2} (Paliyath *et al.*, 1987). Chilling injury can render the endoplasmic reticulum leaky (specifically regions free from integral membrane proteins), and unable to compartmentalize Ca^{+2}, resulting in phospholipid catabolism.

3. TEXTURAL DISORDERS CAUSED BY CHILLING INJURY

3.1 Mealiness in pears

Mealiness has not previously been related to chilling injury. The only relationship between mealiness and low temperature storage has been reported by Chen *et al.* (1983). They observed that 'd'Anjou' fruit stored for longer than five months at -1.1°C ripened with a mealy, dry texture, whereas fruit stored for two to four months ripened at 20°C with a juicy texture.

3.2 Woolliness in peach and nectarine

Woolliness is the main symptom of chilling injury in nectarines and peaches *(Prunus persica* L.*)* when stored below 8°C for 2 weeks or longer (Pressey *et al.*, 1971; Lill *et al.*, 1989). Symptoms are variable among peach varieties but include; flesh browning, a dry coarse texture resembling corn meal (mealiness), wool (woolliness), or leather (leatheriness), failure to ripen and to develop normal flesh colour, translucency of the flesh, and lack of the characteristic aroma (Mitchell and Kader, 1989). Early season cultivars are known to be less susceptible to this disorder (Mitchell and Kader, 1989).

Chilling injury results in a loss of membrane integrity. Fluids passing through these membranes bind to pectic substances with high molecular mass in the middle lamella resulting in low levels of extractable juice (Von Mollendorff *et al.* 1992). Ben-Arie and Lavee (1971) reported that PE activity decreased and PG increased, during storage of nectarines at 8°C, whereas PE activities stayed the same and PG decreased in fruit stored at 0°C. The development of woolliness coincided with these changed enzyme activities (Ben-Arie and Sonego, 1980). The ratio between PG/PE can thus determine whether woolliness will develop or not (Zhou *et al.*, 2000a). Chilling injury can thus be seen as an incomplete cell wall degradation, which influences the middle lamella (pectin degradation) but not the cell wall (King *et al.*, 1989).

Von Mollendorff and De Villiers (1988) found that the respiration rate of 'Peregrine' peaches decreased sharply at the onset of woolliness. This occurred when fruit were moved from 2°C

to 10°C. They noted that the rate of increase in ethylene production was less for fruit that developed woolliness.

Ben-Arie and Sonego (1980) reported that delayed storage (20°C, 48h) postponed the onset of woolliness in 'Somerset' peaches, and that intermittent warming (25°C, 24h, after 10 and 20 days at 0°C) prevented woolliness. Both had higher PG and lower PE activity than control fruit stored at 0°C. Zhou *et al.* (2000a) confirmed this, but found no difference in the mRNA and the protein content of PG and PE between delayed stored and the control cold stored 'Flavortop' nectarines. This suggests that the PG enzyme synthesis was not affected by cold storage but that enzyme activity was affected at these low temperatures.

Controlled atmosphere (3kPa O_2, 10kPa CO_2,) also prevented woolliness but repressed both the mRNA and activity of PG during storage. This recovered during ripening (Zhou *et al.* 2000a). It is clear that the two storage processes prevent woolliness by different mechanisms.

Brovelli *et al.* (1998) noted that peach genotypes with melting flesh were notably more susceptible to the development of mealiness than non melting flesh types. The latter are firmer phenotypes with a higher resistance to mechanical damage and have a lower capacity for pectin degradation (Lester *et al.*, 1996). The low capacity for pectin degradation is the result of the non melting flesh cultivars not possessing endo-PG (Fishman *et al.*, 1993). At a histological level, chilling brought about a dissolution of the middle lamella and an expansion of the intercellular spaces in melting flesh mesocarp tissue but did not affect non melting flesh fruit.

Woolliness has been anatomically observed as the separation of the mesocarp cells leading to increased intercellular spaces which are filled with pectic substances (Von Mollendorf *et al.*, 1992; Harker and Sutherland, 1993). No changes in the cellulosic component of the cell wall of woolly fruit could be found (Luza *et al.*, 1992). Sonego *et al.* (1995) noted the appearance of small and numerous air inclusions in chilling-injured nectarines. Brovelli *et al.* (1998) confirmed this by looking at cell separation patterns for chilled and non-chilled ripened peaches. The mesocarp cells of non-chilled ripe fruit were released individually, while chilled cells were released in clumps. Therefore, individual cell separation was reduced, resulting in large air inclusions.

Two types of chilling injuries, viz woolliness and leatheriness, occur in peaches and nectarines. Both have low extractable juice but the same total water content as in juicy fruit. Woolliness and leatheriness symptoms are thus not the cause of dehydration, and the main difference in these two symptoms is a difference in firmness, the latter staying firm during the ripening process (Harker *et al.*, 1997a). Fruit firmness is determined by a combination of internal turgidity and integrity of the cell wall (Harker *et al.*, 1997b). Structural changes in peach cell walls become apparent during early softening stages due to dissolution of the middle lamella and disintegration of the cell wall fibrillar material (Ben-Arie and Sonego, 1980).

In an ultrastructural study, Luza *et al.* (1992) showed that cell walls of leathery peach become three times thicker than in fruit ripened after harvest or in woolly fruit. This implies that some cell wall synthesis may have occurred in these tissues, and an increase in Calcofluor flourescence was evidence for new cellulose deposition. The cell walls were also bent, showing a collapse in cell shape, and a disintegration of the tonoplast and disorganization of the cytoplasm. Although these changes did not occur in woolly tissues, the plasma membrane drew away from the cell wall, and the intercellular spaces were brightly fluorescent after Coriphosphine staining, indicating high amounts of pectic substances, in both leathery and woolly fruit. Woolly tissue was associated with dissolution of the middle lamella, causing cell wall separation, beginning at the intercellular spaces and moving along adjacent cell walls, forming a continuous extracellular matrix. The cell wall had an increasing fibrillar appearance, but no cell wall degradation occurred. This was apparent just after the plasma membrane developed an irregular contour and after plasmolyses (Luza *et al*, 1992).

That the mechanism of action of chilling injury differs between mealy and leathery fruit is confirmed by Ju *et al.,* (2000), who found no differences in 1-aminocyclopropane-1-carboxylic acid (ACC) content, 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) activity, and electrolyte leakage between juicy and woolly 'Huangjin' peaches. However, leathery fruit contained less ACC, had lower ACO activity, and showed a higher percentage of electrolyte leakage than juicy and mealy fruit. Although the extractable juice content, and gel forming capacity of leathery and woolly fruit are the same, the PG and galactosidase (GAL) content differed. Woolly fruit had higher levels of PG and GAL activity than in leathery fruit, but the same activity as in juicy fruit. Although Von Mollendorf and De Villiers (1988) had similar results, this seems to contradict other publications (Ben-Arie and Sonego,

1980; Buescher and Furmanski, 1978, Zhou *et al.*, 1999, 2000a, b; Obenland and Carroll, 2000), which state that chilling injured fruit have a lower PG activity during ripening. The description of the woolliness disorder with low firmness and low juice content is similar in all papers and it is thus not clear why these different results were obtained.

'Flavortop' nectarines stored for 24h at 20°C, and then 30 days at 0°C or treated with 1-Methylcyclopropene (1-MCP) (0.1 $\mu L \cdot L^{-1}$, 24h at 20°C and then 30 days at 0°C) developed woolliness, while ethylene treated fruit (15 $\mu L \cdot L^{-1}$, 24h at 20°C and then 30 days at 0°C) did not (Dong *et al.*, 2000). Firmness was not mentioned in the paper and so it is possible that 1-MCP treated fruit were leathery rather than woolly. The difference between control and ethylene treated fruit emphasises the importance of ethylene in the development of good texture. It can be concluded that the suppression of ethylene-dependent reactions also plays a role in the development of woolliness in nectarines.

3.3 Graininess in Kiwifruit

Chilling injury in kiwifruit is characterised by a grainy appearance of the outer pericarp, followed by water soaking. This change in texture is also associated with an extreme loss in firmness (Lallu, 1997).

Bauchot *et al.* (1999) reported a 30% increase in cell wall polysaccharide, and a 70% increase in galactosyl content in affected fruit, compared to unaffected fruit stored under the same conditions. Redgwell and Fischer (2002) attribute these changes to *de novo* cell wall synthesis, but also suggest that the difference in cell wall polysaccharide, and galactosyl levels between affected and unaffected fruit could also arise from an anomaly in cell wall metabolism during cold storage (i.e. inhibition of pectin solubilization and ß-galactosidase).

3.4 Mealiness in persimmon

Chilling injury, accompanied by mealiness, occurs in persimmon when stored for more than 4 weeks at 0°C (Mac Rae, 1987). Chilling injury in more severe cases causes browning of the flesh and skin, and the formation of a firm gel in the flesh (Grant *et al.*, 1992). Other

symptoms include reduction in flesh firmness, off-flavours, and increased ethylene production on removal from low temperature storage (Woolf *et al.,* 1997).

Grant *et al.* (1992) noted that the solubilised pectic polymers in the cell wall of chilling-injured fruit, had a higher molecular mass and contained more neutral sugars than in fruit which ripened normally. Data indicated that there was an increase in insoluble wall material.

Woolf *et al.* (1997) showed that heat treatment before cold storage alleviated chilling injury and induced a decrease in the viscosity of the cell wall and pectin fraction.

It is not known whether the formation of insoluble wall material in persimmon is related to PG/PE activation mechanisms, as in plums (Taylor *et al.*, 1994) or nectarines (Ben Arie and Sonego, 1980). Although solubilization of pectins occurs in persimmon, endo-PG has not been detected (Cutillas-Iturralde *et al.,* 1993). Redgwell and Fischer (2002), concluded that the levels of endo-PG are undetectable by *in vitro* assay, or that a different pectin depolymerising enzyme might be affected by low temperature.

3.5 Internal browning and gel breakdown in plums

Internal browning of plums, occurs in the mesocarp under the skin, and changes from a light to a dark brown depending on the severity (Dodd, 1984; Taylor, 1996). Leaky membranes, a consequence of chilling damage, allows polyphenol oxidase (PPO), the enzyme that catalyses the oxidation of phenolics, to come into contact with the membrane bound phenolics, and the result is browning (Taylor *et al.,* 1993a). Since immature fruit have high concentrations of phenolic compounds, the incidence of internal browning is higher in less mature fruit (Kotzé *et al.*, 1989). Internal browning occurs when water soluble pectin viscosity is highest, which explains lack of juice associated with the disorder. The development of this disorder in 'Songold' plums could be successfully prevented by storing fruit for 10 days at -0.5°C followed by 18 days at 7.2°C (dual-temperature), instead of 28 days at -0.5°C (single temperature) (Hartmann *et al.*, 1988).

Gel breakdown refers to the gelatinous breakdown or translucency of the plum mesocarp surrounding the stone of the fruit (Gant, 1992). It is also characterised by low extractable juice, associated with high viscosity of water soluble pectin (Taylor *et al.,* 1993b). Ultrastructural studies indicated cell wall thickening in affected inner mesocarp, similar to

woolliness in nectarines, which were caused by formation of gel complexes in intercellular spaces. Gel breakdown develops earlier in fruit stored at dual temperatures rather than the conventional 28 days at -0.5°C. However, after fruit stored at -0.5°C ripened, gel breakdown levels were higher than in fruit stored at dual temperature (Taylor *et al.*, 1994). Although temperature was shown to have an influence on the severity in the development of gel breakdown, thickening of the cell wall has also been recorded at harvest (Taylor *et al.*, 1993b). Post optimum harvested fruit developed more gel breakdown than optimal harvested fruit (Taylor *et al.*, 1995). Taylor *et al.* (1995) described two possible mechanisms, namely: gel complexing of the pectins and chilling damage on pectolytic enzymes.

Highly methoxylated pectins bind water to form gel complexes if sugar levels and pH are favourable (Doesburg, 1965). Since higher soluble solids determine the gel strength, lower sugar levels in optimal harvested fruit may inhibit formation of pectin gels. It was also reported that the fruit harvested at optimum maturity had a better membrane integrity than those harvested more mature. The latter were prone to develop gel complexes since cell fluids were more freely available at an earlier stage in storage to bind to high methoxyl pectins (Taylor *et al.*, 1995).

Another factor to be considered is the pectolytic enzymes, PG and PE. These enzymes work synergistically. PE de-esterifies methylated pectic substances and enables PG to hydrolyse the reaction product, which forms a hydrolysed soluble pectin (Whitaker, 1972). In nectarines that ripened without woolliness, PE activity decreased and PG increased, during storage at 8°C. When fruit were stored at -0.5°C, PE activities stayed the same and PG decreased in fruit stored at 0°C (Ben-Arie and Lavee, 1971). In nectarines, the ratio between PG/PE can thus determine whether woolliness develops or not (Zhou *et al.*, 2000b). In plums, Taylor *et al.* (1994) found that PG activity was higher for fruit stored at -0.5°C for 10 days followed by 18 days at 7.5°C, than for fruit stored for 28 days at -0.5°C. The latter storage regime resulted in an increase in PE which added to the high viscosity of protopectin.

Taylor (1996) reported that the factors influencing the development in gel breakdown related to membrane integrity, and that the aim should be to maintain membranes pre-harvest and post-harvest. In an attempt to maintain membrane integrity, 'Celebration' plums were harvested less mature, and this, combined with dual temperature storage, improved the internal quality with regard to gel breakdown (Taylor and De Kock, 1995). Although both disorders are described as chilling injuries, it appears that temperature plays a more important

role in the initiation and development of internal browning. Gel breakdown is not only a chilling injury, as it has been found at harvest in ripe fruit (3.5 kg) (Taylor *et al.*, 1994). The mechanism of low temperatures causing an imbalance in the pectolytic enzymes which in turn cause high viscosity of pectin and low extractable juice is, however, very similar to chilling injury in nectarines.

3.6 Mealiness in tomato

Tomato fruit are very susceptible to chilling injury (<10°C) at the mature-green stage when they are harvested and transported. Chilling injured tomatoes are characterised by increased rates of respiration and ethylene production, slow abnormal ripening, and increased disease susceptibility (Saltveit and Cabrera, 1987). Fruit tolerance to chilling injury varies with cultivar, harvest date, and degree of ripeness (Dodds *et al.*, 1991). Increased rates of electrolyte leakage occur from chilled tissue, and have been used to measure the increased permeability of plasmalemma membranes following chilling injury (King and Ludford, 1983). However, this method has been inconsistent in tomato cultivars that are chilling sensitive (Côté *et al.*, 1993). The ripe fruit are also chilling sensitive (<0°C) but do not exhibit as many chilling related disorders. Like nectarines, tomato pectin also consists of galacturonan interspersed with rhamnose to which side chains of arabinan and galactan, are attached. In mealy fruit these side chains are not removed from the pectic polymer (Carrington *et al.*, 1993). Suppression of PG mRNA has been noted in chilling injured tomato (Watkins *et al.*, 1990). The enzyme reaction is thus different to that in nectarines, where only PG activity is inactivated by temperature.

Heat shock protein induction (42°C for 36 and 48h) in mature green tomato is one factor influencing tolerance of chilling injury and is used commercially to protect tomato against chilling injury (Lurie and Sabehat, 1997).

4. OTHER TEXTURAL DISORDERS

Mealiness in apples

Tasting panels perceived the apple mealiness disorder as non-crisp, non-juicy, soft, granular and floury (Barreiro *et al.*, 1998). Although nectarines, persimmons and kiwis are non-juicy, soft and granular when affected by low temperature breakdown (Ben-Arie and Lavee, 1971; Bauchot *et al.*, 1999; Woolf *et al.*, 1997), apple mealiness is classified as an overmaturity

disorder caused by the dissolution of the middle lamella (Harker and Hallet, 1992; Harker *et al.*1997a).

Ben-Arie *et al.* (1979) used transmission electron microscopy to visualise the dissolution of the middle lamella in mealy apples. They concluded that if the cell wall is stronger than the middle lamella, the tissue gives way between the cells, and the cell contents will not be released during mastication. If the cell wall is weaker than the middle lamella, rupturing will occur through the cells and the liquid content will be released. Harker and Hallet (1992) applied tensile test to mealy and juicy tissue and confirmed this hypothesis, and found that mealy fruit had air rather than juice in intercellular spaces.

De Smedt *et al.* (1998) used microscopic observations to examine mealy and non-mealy tissue of 'Cox's Pippin', 'Boskoop' and 'Jonagold' apples. Mealy tissue in all cultivars showed that cells were separated, whereas in non-mealy tissues, cells were interconnected. The cells separated in mealy tissues due to dissolution of the middle lamella. Differences in cell structure between susceptible cultivar 'Boskoop' and 'Cox's Pippin' apples and less susceptible cultivar like 'Jonagold', were calculated by cell area, perimeter and roundness. It was possible to discriminate fresh and mealy 'Cox's Pippin' and 'Boskoop' apples, but not 'Jonagold', because these parameters were the same for fresh and stored fruit. This confirmed that 'Jonagold' apples are less susceptible to development of mealiness.

All apples are prone to textural breakdown due to overmaturity. Any mechanism which retards the maturation process, e.g. correct harvest maturity, controlled atmosphere storage, low temperature storage and application of 1-MCP, will retard breakdown of the middle lamella. This will in turn help to maintain textural apple quality.

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CHAPTER 2: PAPER 1

RIPENING AND MEALINESS OF 'FORELLE' PEARS

ABSTRACT

'Forelle' pears usually require at least 12 weeks of cold storage to ripen evenly but this cold period does not prevent the development of a mealy texture on ripening. The aim of this paper was to establish the role of cold storage on ripening, ethylene production and mealiness of 'Forelle' pears. Fruit harvested from the Warm Bokkeveld and Theewaterskloof areas at commercial maturity were stored at -0.5°C for up to 21 and 22 weeks, respectively. Samples were removed every third week, placed at 15°C, and maturity factors, total ACC content, ethylene production and respiration rates monitored every third day for 12 days. Ground colour, flesh firmness, total soluble solids and titratable acidity indicated that fruit ripened after 6 and 7 weeks for the Warm Bokkeveld and the Theewaterskloof area, respectively at -0.5°C. However, after 6 weeks of cold storage at -0.5°C followed by 6 days at 15°C, all fruit harvested in the Warm Bokkeveld, and ± 80 % of the fruit harvested in the Theewaterskloof area, were mealy. With extended storage at -0.5°C (> 15 weeks) the incidence of mealiness declined in fruit from both areas, but never disappeared completely, when evaluated at 15°C over 12 days. Mealiness was also related to low extractable juice content. Six weeks of storage at -0.5°C was sufficient for ACC accumulation and a peak in ethylene production.

Keywords: (*Pyrus communis* L.), ethylene, 1-aminocyclopropane-1-carboxylic acid, extractable juice.

1. INTRODUCTION

'Forelle' is one of three blushed pears produced in South Africa and has a high market value. Mealiness and astringency are internal quality problems of 'Forelle'. In addition to poor internal quality, 'Forelle' is harvested late in the bicolour pear season and does not ripen before it has been stored for 12 weeks at -0.5°C (De Vries and Hurndall, 1993). Although internal quality has been a problem in the past, recent research has been directed by the financial incentive to reduce the 12 week cold storage period of 'Forelle'. Intermittent warming treatments (De Vries and Hurndall, 1993), controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals (De Vries and Hurndall, 1994; De Vries and Moelich, 1995), and ethylene treatments (Du Toit *et al.*, 2001), have all been tested as a means to

reduce this storage period. Du Toit *et al.* (2001) reported that ethylene treatment successfully reduced the storage period, but mealiness was not described. The development of mealiness during storage still needs to be described and understood.
Many factors can affect texture of pears, the most important being ripening. 1-Aminocyclopropane-1-carboxylic acid (ACC) is the precursor of ethylene. The ACC concentration in climacteric fruit such as pears, is low during the pre-climacteric stage, increases dramatically during the climacteric and decreases in the post-climacteric stage (Yang and Hoffman, 1984). Most winter pears require 6 to 12 weeks of cold storage to induce ethylene synthesis. The ethylene climacteric of the fruit is, in turn, needed for fruit to ripen evenly, achieve good colour, texture and flavour (Lelièvre, *et al.,* 1997; Agar *et al.*, 1999). Ripening resistance in winter pears that have not been cold stored sufficiently, is generally related to delayed synthesis of ACC (Van Eeden *et al.*, 1997).

In contrast to a cold requirement, Chen *et al.* (1983) described mealiness in 'd'Anjou' pears as a result of being stored for too long at low temperatures. Fruit stored at -1.1°C for longer than 5 months, ripened mealy at 20°C, whereas fruit stored at -1.1°C for only four months and transferred to 20°C, ripened normally.

Woolliness, the main symptom of chilling injury in peach *(Prunus persica* L.*),* is characterised by low levels of extractable juice and high insoluble pectins (Ben-Arie and Lavee, 1971; Pressey *et al.*, 1971). This disorder is comparable to mealiness in pears (Chen *et al.*, 1983; Wang *et al.*, 1985). Chilling injury results in a loss of membrane integrity. Fluids passing through these membranes bind to pectic substances in the middle lamella resulting in low levels of extractable juice (Von Mollendorf and De Villiers., 1988).

Water soluble pectin content of fruit increases during normal ripening concomitant with an increase of polygalacturonase (PG) activity and a decrease in the activity of pectin esterase (PE) (Ben-Arie and Lavee, 1971; Buescher and Furmanski, 1978). PE acts to remove the methyl group in the protopectin from the C-6 position of galacturonic acid. PG hydrolyses the α(1-4) link between adjacent demethylated galacturonic acid residues, to form the water soluble pectin. Solubilization of

protopectins is thus synergistic with PE generating sites for PG action (Buescher and Furmanski, 1978). PE activity and the inhibition of PG activity due to prolonged storage at 0°C, leads to a demethylated galacturonic acid, without depolymerization (Ben-Arie and Sonego, 1980). The demethylated pectin polymer binds free water liberated through leaky membranes, causing the water insoluble form of pectin which results in woolliness in nectarines (Von Mollendorf *et al.*, 1988).

In more recent studies it has been found that inactivation of ACC-oxidase (ethylene forming enzyme) during chilling temperatures resulted in the inhibition of ethylene production of nectarines (Zhou *et al.*, 2000), and that the severity of woolliness was closely related to the inhibition of ethylene production in these fruit (Dong *et al.*, 2001; Zhou *et al.*, 2001).

The low temperature requirement in 'Forelle' for accumulation of ACC, the precursor of ethylene, plays an important role in ripening and may affect the development of mealiness. In contrast to the long cold requirement for ripening of 'Forelle', extended cold storage causes woolliness in peaches which is a clear indication of a chilling injury. Consequently, the aim of this study was to determine the role of the duration of cold storage and endogenous ethylene on 'Forelle' pear ripening, and in particular mealiness.

2. MATERIALS AND METHODS

2.1. Fruit source

'Forelle' pears *(Pyrus communis* L.*)* were sourced in the Warm Bokkeveld and Theewaterskloof areas, in the Western Cape, SA. Fruit from the Warm Bokkeveld were harvested at commercial maturity and had an average flesh firmness of 6.8 kg and total soluble solids (TSS) of 15%. Ground colour, measured with a Unifruco colour chart for pears, was 2.0 (where 0.5 = dark green, 5 = deep yellow). Fruit from the Theewaterskloof area had an average flesh firmness of 6.7 kg, ground colour index of 2.0 and TSS of 13.4% at harvest. Fruit were stored for 4 weeks at -0.5°C, before commencing the experiment. At this stage, these fruit had an average flesh firmness of 5.4 kg, ground colour chart index of 3.5 and TSS of 13.4%.

2.2 Experimental layout

Fruit from the Warm Bokkeveld were stored for 0, 3, 6, 9, 12, 15, 18 and 21 weeks at -0.5°C and fruit from the Theewaterskloof area were stored for 4, 7, 10, 13, 16, 19 and 22 weeks at -0.5°C. Fruit removed after these storage times at -0.5°C were placed at 15°C, and maturity factors, total ACC concentration, ethylene production and respiration rates monitored every third day for 12 days. Forty fruit were used for maturity indices on each sampling date, consisting of 5 replicates of 8 fruit per replicate. Every 3 weeks, on removal from -0.5°C, a sample of 50 fruit were used to determine ethylene production during the 12 days at 15°C. The ethylene production sample consisted of 5 replicates with 10 fruit per replicate.

2.3. Maturity indices

Ground colour changes from green to yellow, were measured as hue angle (h°) using a Nippon Denshoku Model HR-3000 colorimeter (Tokyo, Japan) and a Unifruco colour chart for pears (where 0.5 = dark green, 5 = deep yellow). Flesh firmness (kg) was measured using a penetrometer (Southrade fruit pressure tester, model FT 327, Alphonsine, Italy) fitted with an 8 mm probe. TSS % of a pooled juice sample of each replicate was measured by a hand held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Titratable acidity (TA) was calculated as percentage malic acid, by titrating the pooled juice sample with 0.1 N NaOH to an end point pH of 8.2, using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland).

2.4. Mealiness

Mealiness was determined on each fruit subjectively. Fruit were cut equatorially and squeezed. Fruit with expressed juice on the compacted equatorial surface were classified as non-mealy, whereas those with a dry cut surface were considered mealy.

2.5. Extractable juice

Extractable juice (EJ) ($mL \cdot cm^{-3}$) was measured with a Chylofel (COPA -Technologie S.A., St. Etienne du Gress, France) which best estimates release of juice on chewing. The instrument consists of a spring mounted base, which is mobile along the vertical axis. The peeled fruit is placed on this base. When fruit is pressed onto the instrument, a fixed, 25 mm-long cylindro-conical nozzle with a stop ring mounted on the lower

part of the shaft is inserted into the fruit and explores a constant volume of 3 cm^3. The droplets of juice released by the fruit are collected in a graduated beaker placed under the base plate. The Chylofel was used on opposite cheeks, and juice of the fruit in each of the 5 replicates was pooled.

2.6. Ethylene production and respiration rates

Ethylene production was measured every three weeks beginning at the start of the experiment. At each date, fruit were removed from storage and transferred to 15°C. Ethylene production was measured after 0, 3, 6, 9 and 12 days at 15°C. Fruit measured after 0 days at 15°C were allowed 12 h to reach 15°C before readings were taken. Problems with the supply of air to the flowboard prevented measurement of ethylene and respiration rates for the first two storage dates at -0.5°C, for fruit from both areas. Five replicates of 10 fruit each were placed into 5 L containers at 15°C and connected to a flow through system on air (± 95% RH). Flow boards were fitted with needle valves and set at a flow rate of 600 $mL \cdot min^{-1}$ using a bubble meter. Three ethylene samples per replicate were measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian Instrument Group, Palo Alto, California, USA). Respiration rate was measured as a function of the differences of CO_2 concentration at the container inlet and outlet with an infrared gas analyzer (Model S-151; Qubit Systems Inc., Kingston, Ontario), on the same sampling date.

2.7. ACC (1-aminocyclopropane-1-carboxylic acid)

Peeled fruit disks from each replicate were frozen in liquid nitrogen and stored at -80°C for ACC quantification. The frozen disks were chopped with an onion chopper, and then ground further by hand with a mortar and a pestle while adding liquid nitrogen. Fifteen mL of an 80% ethanol solution was added to 3 g of ground frozen pulp and homogenized by Ultra Turrax (model pp/10, Janke and Kunkel Ika, Staufn, Germany), for 30 seconds. Samples stood for an hour, whereafter they were centrifuged at 500 g_n for 10 minutes. An aliquot (5 mL) of the supernatant was concentrated in vacuo with a savant (Speedvac concentrator, SVC, 200H, Farmdale, N.Y.), and ACC analysed according to the method of Lizada and Yang (1979). ACC data are presented for the date on removal from -0.5°C.

2.8. Data analysis

Data were analyzed using the General Linear Means procedure (GLM). *P*-values illustrate the significant differences in the figures presented. Significant differences between parameters were determined using Fisher's protected least significant difference test with a 95% confidence interval. The STEPDISC (Stepwise Discriminant Analysis) procedure was used for separating all variables (firmness (kg), TSS (%), EJ (ml), TA (%)) likely to be related to mealiness. The CANDISC (Canonical Discriminant Analysis) procedure was used to test the effectiveness of extractable juice content as a measure of mealiness, for the Warm Bokkeveld and Theewaterskloof areas. The percentage mealiness in a replicate has been categorized as follows for the CANDISC procedure (0% – 35% = 0, 35% – 70% = 1 and 70% – 100% = 2). All procedures mentioned were used in the SAS (Statistical Analysis System) programme (SAS Institute Inc., 1990).

3. RESULTS

3.1 *Warm Bokkeveld area*

3.1.1 *Colour index and hue angle*

A colour progression from green to yellow was noted during cold storage (Fig. 1 and Fig. 2). Acceptable ground colour development (4.5 units colour index) only occurred after 6 weeks cold storage at -0.5°C followed by 12 days at 15°C.

3.1.2 *Flesh firmness*

Flesh firmness decreased over time (Fig. 3), dropping by 1.8 kg over 21 weeks when stored at -0.5°C. After 6 weeks at -0.5°C and 12 days at 15°C fruit firmness dropped to below 3 kg.

3.1.3 *Mealiness*

Mealiness disorder reached a maximum after 6 weeks at -0.5°C and 9 days at 15°C (Fig. 4). The mealiness disorder was only noted from day 6 of the shelf-life test, regardless of the cold storage duration at -0.5°C. Fruit not subjected to a cold treatment (week 0) did not develop significant mealiness and didn't ripen within the shelf-life period. After 3 weeks cold storage at -0.5°C, fruit exhibited increasing levels of mealiness from 6 days up to and including 12 days at 15°C. Between 6 and 15 weeks cold storage 'Forelle' had unacceptable levels of mealiness, regardless of

shelf-life period from day 6. After 18 weeks of cold storage, mealiness levels declined below 35% during the shelf-life test, but were still unacceptable from a commercial perspective (Fig. 5).

3.1.4 *Extractable juice and mealiness*

Fruit that had not been stored at -0.5°C had a high EJ content (Fig. 6A) but were not ripe at this point. After 3 weeks in cold storage and 6 days at 15°C, EJ declined gradually with an increase in mealiness (Fig. 6B). After 18 weeks at -0.5°C the relationship between EJ and mealiness over time at 15°C, was less clear. This was due to over ripeness of fruit, and indicated the end of the cold storage capacity of 'Forelle'. With the stepwise discriminant analysis using mealiness and variables flesh firmness (kg), TSS (%), EJ (ml) and TA (%), 3 variables were selected as being related with mealiness: EJ, firmness, and TA, in order of decreasing importance. With a canonical discriminant analysis using only the variable that carried most weight, namely extractable juice, it was possible to distinguish between the categories of mealiness (Fig. 7).

3.1.5 *TSS and TA*

Soluble solids increased from 15% to 20% over the 21 week storage period at -0.5°C (Fig 8). During the shelf-life simulation period at 15°C, an additional increase in total soluble solids (%) was noted up to week 12, indicating further ripening during this time. This increase in soluble solid levels corresponded to a decrease in titratable acidity (Fig. 9).

3.1.6 *Ethylene production and total ACC concentration*

Ethylene production measured at 15°C, for fruit harvested in the Warm Bokkeveld, started increasing after 12 weeks of storage at -0.5°C (Fig. 10). Fruit stored for 12 weeks had an inexplicably high ethylene production on removal and also on day 12 at 15°C. The storage at -0.5°C for various weeks did not show an increased ethylene production on a specific day at 15°C during ripening. The only fruit with a visible autocatalytic increase in ethylene production during days at 15°C were 'Forelle' pears stored for 21 weeks. This ethylene peak (± 60 $\mu L \cdot kg^{-1} \cdot h^{-1}$) was measured on days 6 and 9.

To determine ethylene production over an extended storage period at -0.5°C, ethylene production on removal from -0.5°C was plotted separately from the shelf-life simulation. The maximum ethylene production rate (60 μ $L.kg^{-1}.h^{-1}$) in 'Forelle' was reached after 12 weeks at -0.5°C. Total ACC concentration reached a maximum at the corresponding point of ethylene production rate (Fig. 11).

3.1.7 *Respiration rate*

Respiration rates varied considerably both on removal from cold storage and during the shelf-life simulation. No clear respiratory climacteric was evident (Fig. 12). Respiration rates were 10-15 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ on removal from 12, 15 and 21 weeks of storage at -0.5°C. After 3 days at 15°C, respiration increased to 20-25 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$. The respiration rates remained within these levels during the 12 days at 15°C for fruit stored for 12 and 15 weeks at -0.5°C, but increased to above 30 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ for fruit stored for 12 weeks at -0.5°C. Fruit stored for 9 weeks at -0.5°C had respiration rates above 20 $mg \cdot kg^{-1} \cdot h^{-1}$ but these decreased after 6 days at 15°C. Respiration rates for fruit stored for 18 weeks at -0.5°C, remained between 25 and 30 $mg \cdot kg^{-1} \cdot h^{-1}$ for the 12 days at 15°C.

3.2. *Theewaterskloof area*

3.2.1. *Colour index and hue angle*

A gradual ground colour change from green to yellow was noted during storage at -0.5°C (Fig. 13). This change was more rapid when fruit were ripened at 15°C. Hue angle also indicated changes from dark green (111.1) to light green (105.3) during storage at -0.5°C. Fruit were a deep yellow (hue angle = 101.9°- 95.04°) after fruit were stored at -0.5°C for longer than 13 weeks and ripened for 12 days at 15°C (Fig. 14).

3.2.2. *Flesh firmness*

A decrease in firmness was noticed only after 16 weeks at -0.5°C, and was ±1 kg lower after 22 weeks at -0.5°C (Fig. 15). Fruit stored for longer than 7 weeks at -0.5°C and ripened for 6 days at 15°C had an edible flesh firmness of <3 kg. Fruit ripened for 12 days at 15°C after being stored for longer than 7 weeks, were overripe.

3.2.3. *Mealiness*

Mealiness reached a maximum of ± 80% after 7 weeks of storage at -0.5°C, when measured on day 6 and 9 during ripening at 15°C (Fig. 16). By day 12 at 15°C mealiness levels had decreased to 64%. Fruit stored for 13 and 19 weeks at -0.5°C experienced a decline in mealiness to 2.5% on day 12 at 15°C. Fruit stored for longer than 19 weeks showed mealiness incidence below 25% during ripening (Fig. 17).

3.2.4 *Extractable juice and mealiness*

Fruit stored for 4 weeks at -0.5°C only ripened after 12 days at 15°C. This was reflected by a decrease in EJ between day 9 and 12 (Fig. 18A). On day 12 there was a decrease in EJ associated with the development of mealiness (10%). After 7, 10, 13 and 16 weeks in cold storage at -0.5°C, there was an inverse relationship between EJ and the development of mealiness (Fig. 18B, C, D, E). With longer storage, EJ did not decline as mealiness increased, and showed EJ values which related to fruit which ripened normaly. With the stepwise discriminant analysis using the variables flesh firmness (kg), TSS (%), EJ (ml) and TA (%), EJ was still selected as the variable best related to mealiness. Using only EJ as a variable in the canonical discriminant analysis, it was possible to distinguish between the categories of mealiness (Fig. 19).

3.2.5 *TSS and TA*

Total soluble solids (%) increased from ±13% to ±16% during the cold storage period at -0.5°C (Fig. 20). Titratable acidity values for fruit from Theewaterskloof area were lower (0.08% - 0.21 %) than in fruit from Warm Bokkeveld (0.18% – 0.34%), and tended to decrease during storage (Fig. 21).

3.2.6 *Ethylene production and total ACC concentration*

Ethylene production at 15°C increased with increased cold storage time up to 16 weeks at -0.5°C (Fig. 22). On removal from 19 weeks of storage at -0.5°C, ethylene production was measured at 74 $\mu L \cdot kg^{-1} \cdot h^{-1}$ decreasing to 30 $\mu L \cdot kg^{-1} \cdot h^{-1}$ after 3 days at 15°C, and remaining within that range for the duration of the following 9 days at 15°C. The ethylene production for fruit stored at -0.5°C for 22 weeks also remained within 25 and 38 $\mu L \cdot kg^{-1} \cdot h^{-1}$.

Ethylene production measured on removal from -0.5°C, peaked after 19 weeks of cold storage (Fig. 23). The total ACC concentration measured on removal from 10 weeks at -0.5°C was 5.3 $nmol \cdot g^{-1}$ (data not shown). This concentration increased to 46.8 $nmol \cdot g^{-1}$ after 13 weeks at -0.5°C. After 16 weeks at -0.5°C the ACC concentration remained at 40.4 $nmol \cdot g^{-1}$, but increased again to 54.6 $nmol \cdot g^{-1}$ after 19 weeks at -0.5°C.

3.2.8 *Respiration*

At the start of shelf-life simulation (15°C) respiration rates for fruit from all storage times (-0.5°C) were ±12 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, except for fruit stored for 16 weeks which had a higher respiration rate (20.6 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) (Fig. 24). The highest CO_2 production during shelf-life simulation was measured for fruit stored for 22 weeks at -0.5°C.

4. DISCUSSION AND CONCLUSIONS

Fruit from the Theewaterskloof area, stored for up to 22 weeks at -0.5°C, had the same trend in mealiness as fruit harvested from the Warm Bokkeveld (Figs. 18 and 6 respectively). Fruit from Theewaterskloof never reached 100% mealiness, as was the case for the Warm Bokkeveld. The maximum mealiness incidence for fruit from the Warm Bokkeveld and Theewaterskloof area was measured after 6 and 7 weeks, respectively, and started declining after 12 and 15 weeks, respectively. The mealiness never disappeared in fruit from either area but reached minimum incidences of 12-18%. Commercial mealiness data from the Warm Bokkeveld in the 1999 season (Crouch, 2000), was much lower (maximum 76%), and a decline was apparent after 3 weeks of cold storage, which is not the case for both areas in the 2000 season. Clear seasonal fluctuations in mealiness are apparent, but cannot be explained without considering all variable factors involved in previous seasons.

Extractable juice for fruit from both areas seemed to be a distinguishing measurement for the classes of mealiness according to the CANDISC graphs (Fig. 7 & 19). This relationship was also noted in woolliness of peach (*Prunus persica* L.) (Ju *et al.*, 2000). However, in 'Forelle' this relationship was less clear after 12 days of ripening (Fig. 6 and 18). At this stage fruit were overripe and the pectin gels may have released

the bound water at this time. Mealiness also followed the same trend, especially in fruit that were stored for long periods at -0.5°C (Fig. 4 and 16).

A decrease in EJ is normal during the latter stages of fruit ripening (Chen *et al.,* 1983). This is a result of an attraction between the released juice and the ripened buttery tissue. Although this results in a lower extractable juice content, the fruit does not have a "dry mouth feel" or texture. However, a low EJ may also be an indication of mealiness. In this case the tissue appears dry or mealy on mastication (Harker *et. al.,* 1997). This may be similar to woolliness of nectarines and peaches where juice binds to the pectins, forming a gel. Zhou *et al.* (1999) described an increase in expressible juice with the decrease in viscosity during normal ripening and decrease in expressible juice with increasing viscosity in woolly nectarines. The juice content change over time should thus be compared to mealy and non-mealy fruit. This will separate factors influencing EJ during normal ripening from changes due to mealiness.

Minimum ethylene production measured for 'Forelle' ranged between 25-32 $\mu L \cdot kg^{-1} \cdot h^{-1}$. This concentration was still relatively high compared to other winter pears; e.g. 'd'Anjou' pears have been reported to peak in ethylene production at these concentrations (Chen *et al.,* 1983; Chen *et al.,* 1997; Gerasopoulos and Richardson, 1997).

Clear differences in the development of mealiness, and ethylene production, were noted for 'Forelle' harvested from the two areas, viz Warm Bokkeveld and Theewaterskloof. These values should not be compared or used as definitive values, since it is not known how seasonal fluctuations in climate influences the incidence in mealiness in each area. The trend of mealiness over time or during prolonged storage at -0.5°C was very similar for fruit from both areas; the longer the storage period at -0.5°C the less incidence of mealiness in 'Forelle' pears. Even though it is not known what threshold ethylene concentration 'Forelle' needs for certain ripening processes, the concentration seemed to be high enough for ripening after 10 weeks at -0.5°C; in comparison with other winter pears. This emphasizes the need to investigate all other factors possibly involved in the development of mealiness.

The mandatory 12 week cold storage period at -0.5°C recommended by De Vries and Hurndall (1993), was sufficient for ripening, but was not enough to reduce mealiness levels below 35% for fruit harvested in both the Warm Bokkeveld and Theewaterskloof areas. Mealiness levels only declined below 35%, after 18 and 19 weeks at -0.5°C for the 2 areas, respectively. This data reflects only one season. As mealiness levels fluctuate seasonally and geographically, a better understanding of these influences should be gained before changing the existing recommendation.

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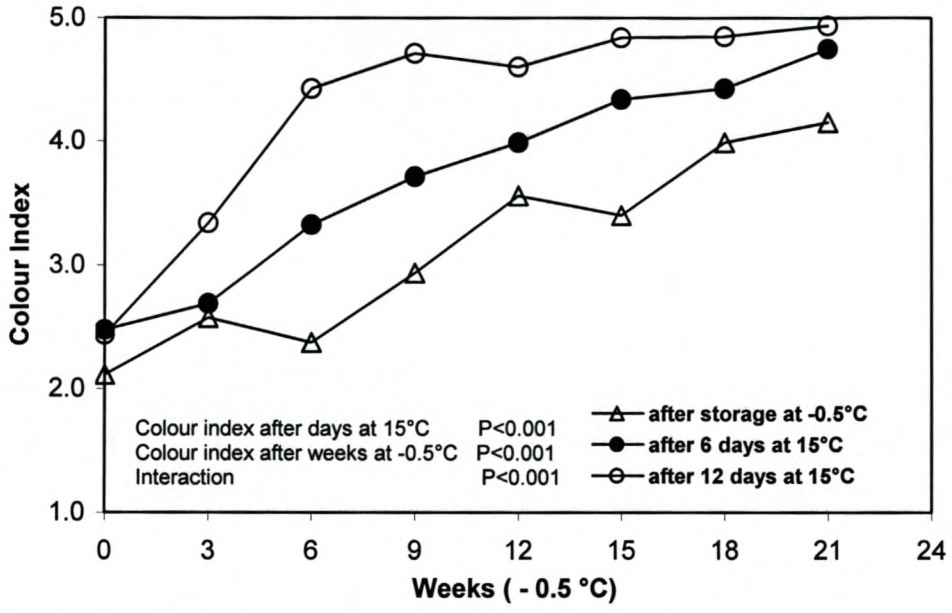

Fig. 1. Ground colour index (Unifruco colour chart) of 'Forelle' pears sourced from the Warm Bokkeveld, stored at -0.5°C for up to 21 weeks and transferred to 15°C for 12 days ($LSD_{5\%}$= 0.24)

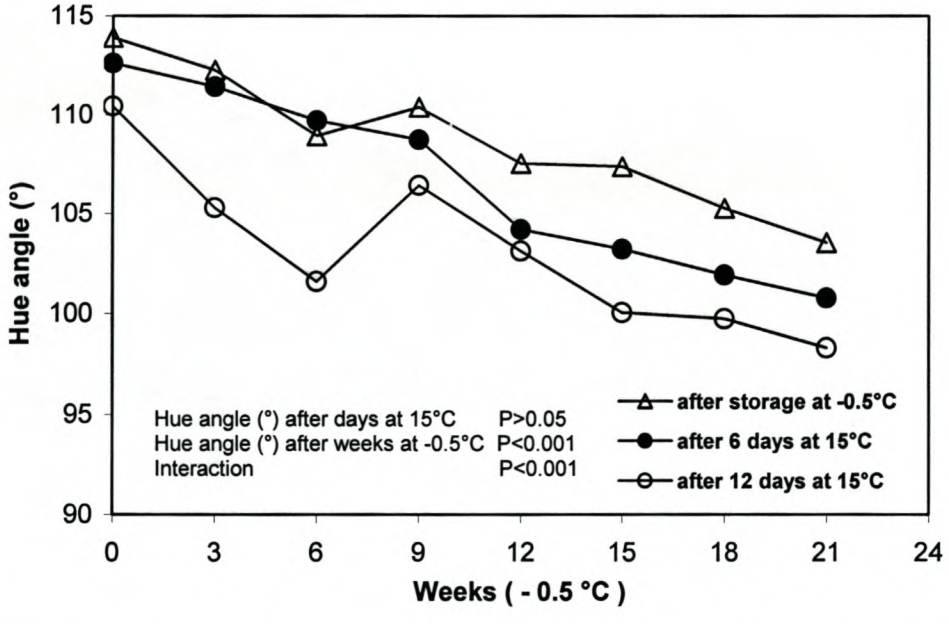

Fig. 2. Hue angle (°) of 'Forelle' pears harvested in the Warm Bokkeveld, stored at -0.5°C for up to 21 weeks and transferred to 15°C for 12 days ($LSD_{5\%}$= 2.65).

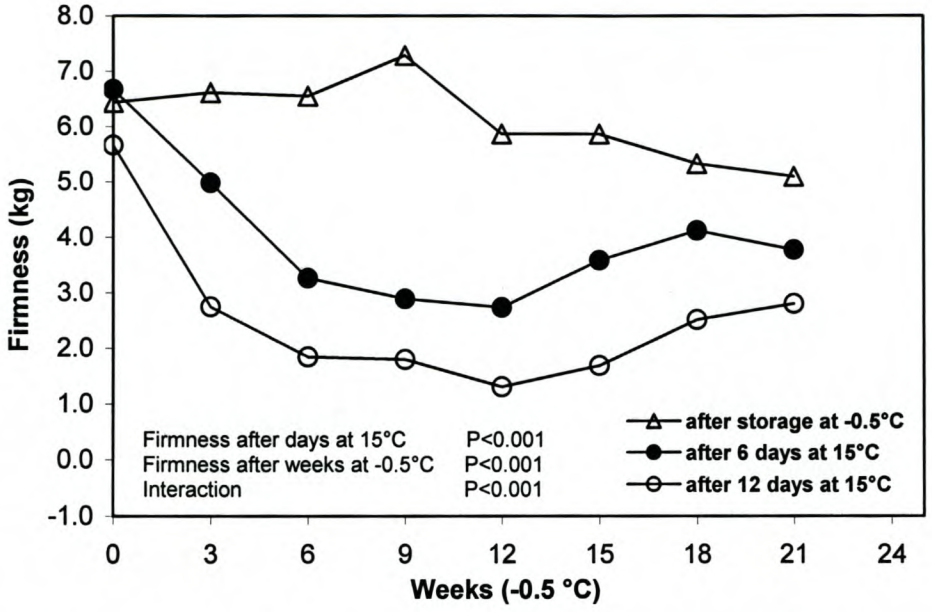

Fig. 3. Firmness (kg) of 'Forelle' pears harvested in the Warm Bokkeveld, stored at -0.5°C for up to 21 weeks and transferred to 15°C for 12 days (LSD $_{5\%}$ = 0.39 kg)

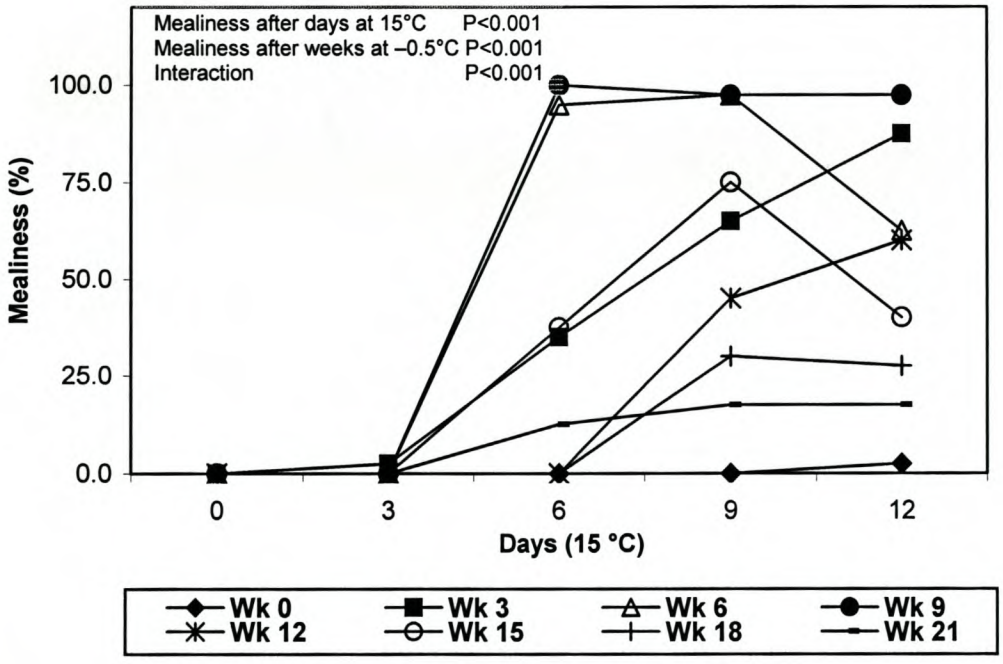

Fig. 4. 'Forelle' pear mealiness in the 2000 season of fruit harvested from the Warm Bokkeveld area, after weeks of storage at -0.5°C and days at 15°C (Logit transformed data).

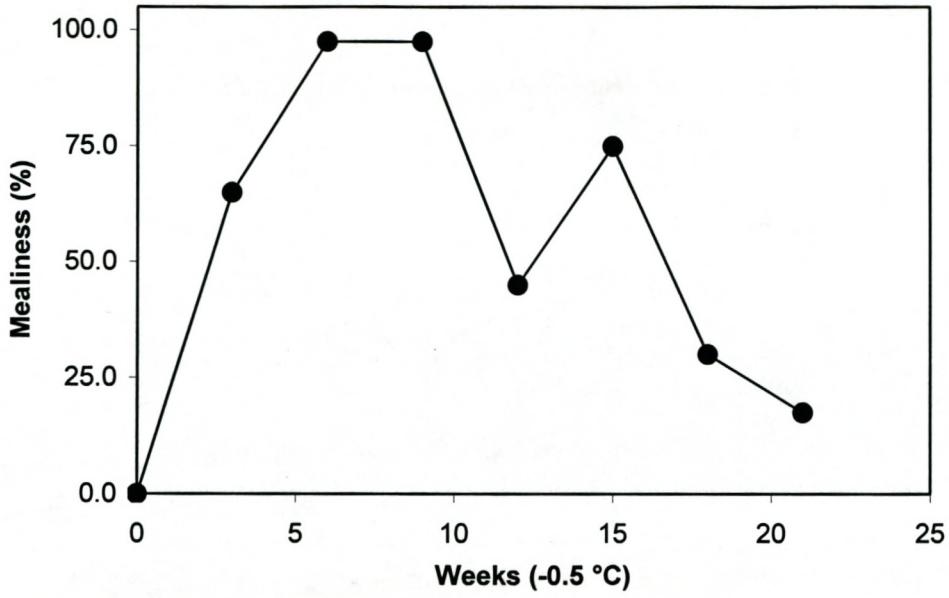

Fig. 5. Mealiness of 'Forelle' pears in the 2000 season, stored at -0.5°C for up to 21 weeks, and sampled on day 9 at 15°C (Logit transformed data).

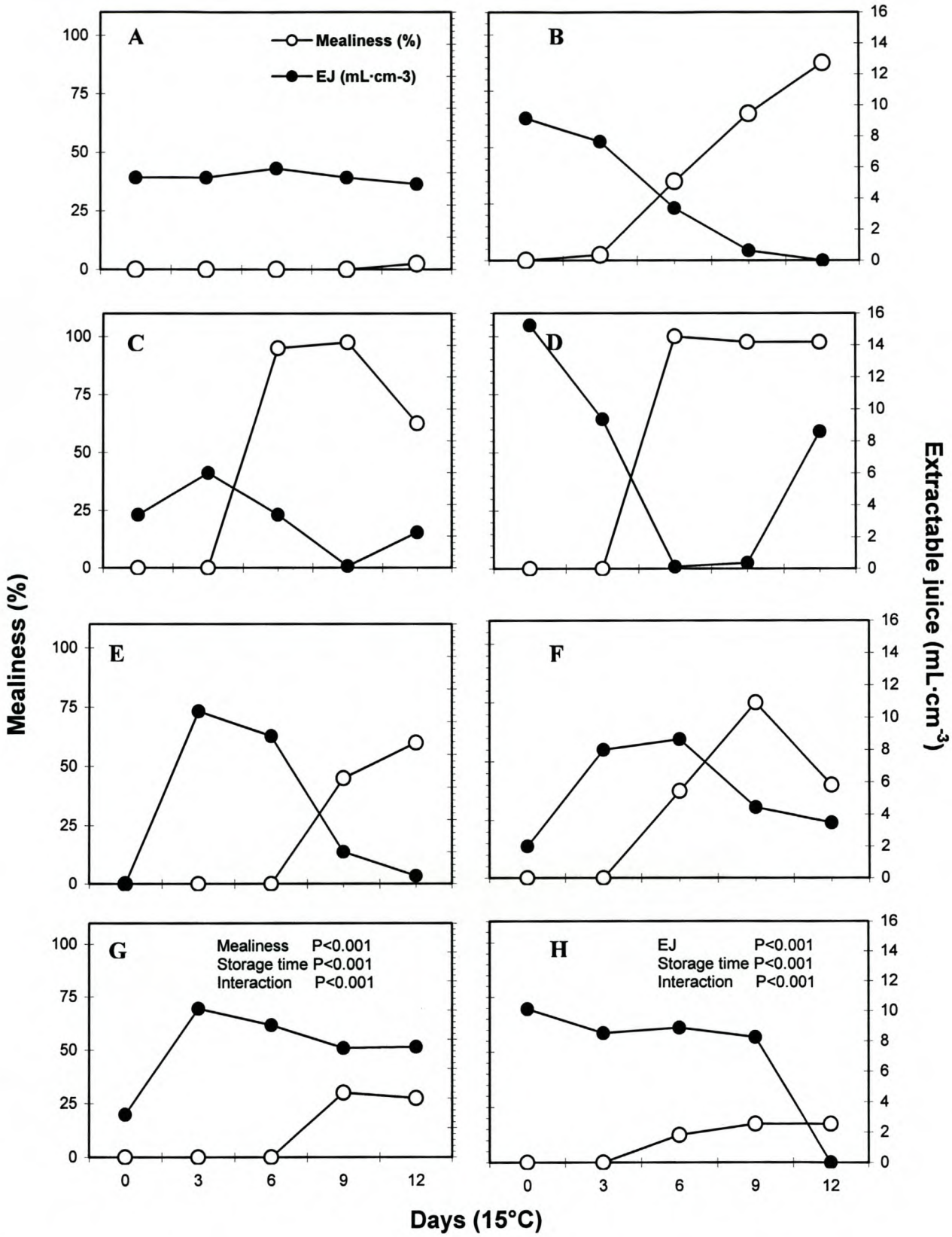

Fig. 6. Mealiness (%) (○) (Logit transformed data) and EJ ($mL \cdot cm^{-3}$) (●) ($LSD_{5\%}$= 0.6 $mL \cdot cm^{-3}$) of 'Forelle' pears from the Warm Bokkeveld area during 12 days of ripening at 15°C after 0 (**A**), 3 (**B**), 6 (**C**), 9 (**D**), 12 (**E**), 15 (**F**), 18 (**G**) and 21 (**H**) weeks of storage at -0.5°C.

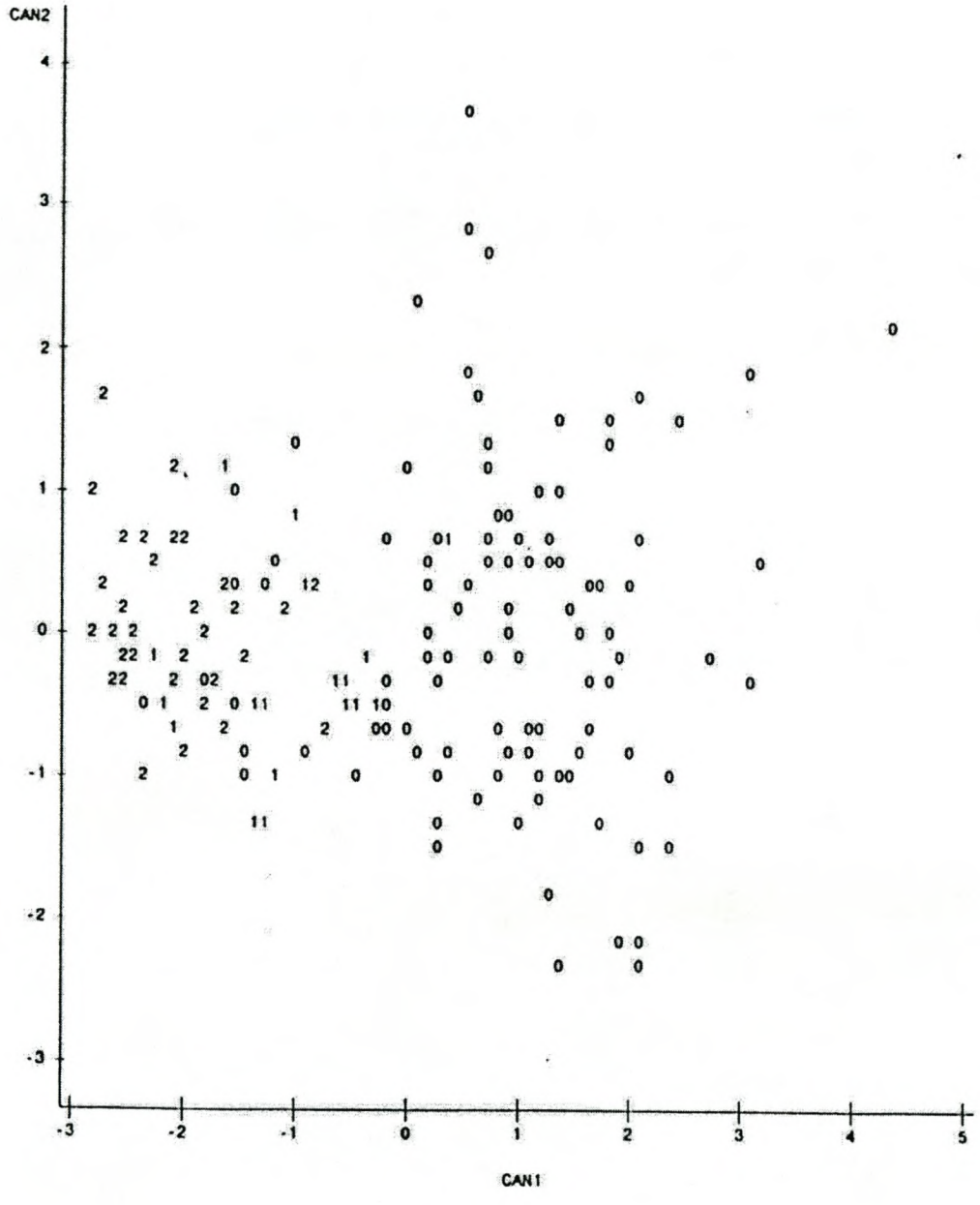

Fig. 7. Discrimination between 'Forelle' pears from the Warm Bokkeveld area that are 0 - 35 % (**0**), 35 - 70 % (**1**) and 70 – 100 % (**2**) mealy, with extractable juice content as variable, chosen in a stepwise discriminant analysis, and processed with the canonical discriminant analysis procedure.

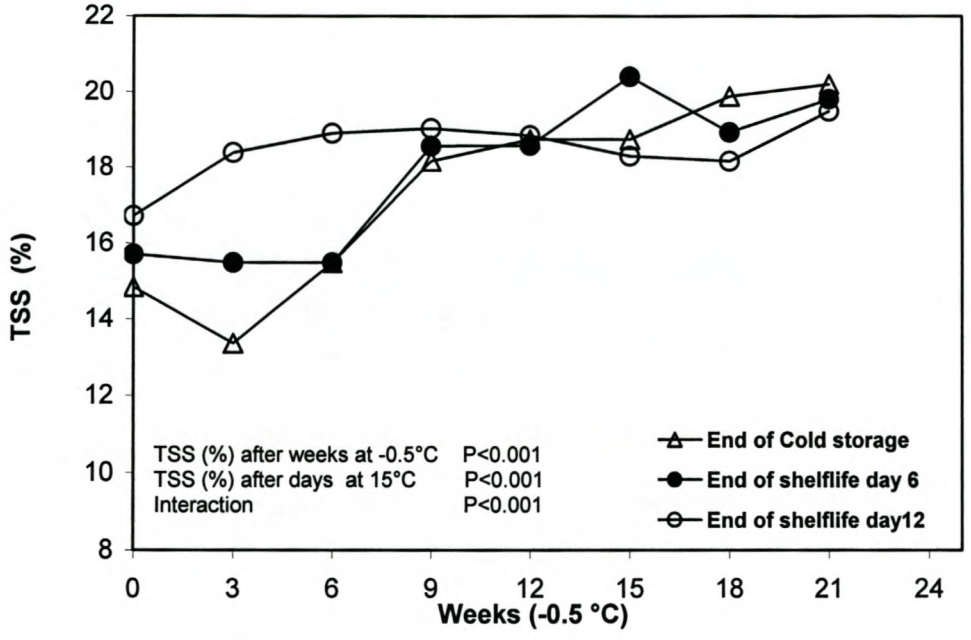

Fig. 8. TSS (%) of 'Forelle' pears harvested in the Warm Bokkeveld area, stored at -0.5°C for up to 21 weeks and transferred to 15°C for 12 days ($LSD_{5\%}$= 0.89%).

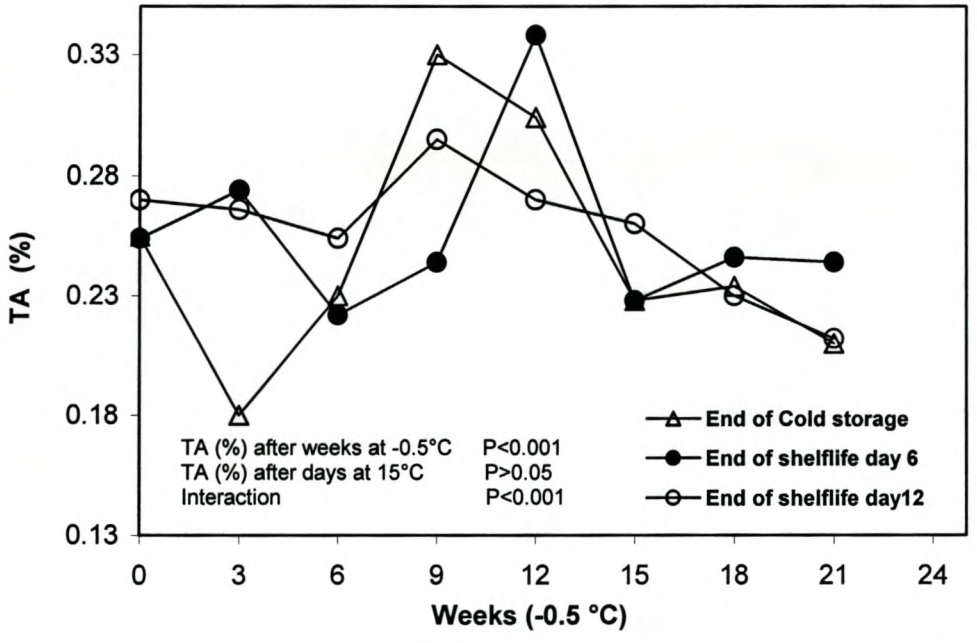

Fig. 9. TA (%) of 'Forelle' pears harvested in the Warm Bokkeveld area and stored at -0.5°C for up to 21 weeks ($LSD_{5\%}$ = 0.04%).

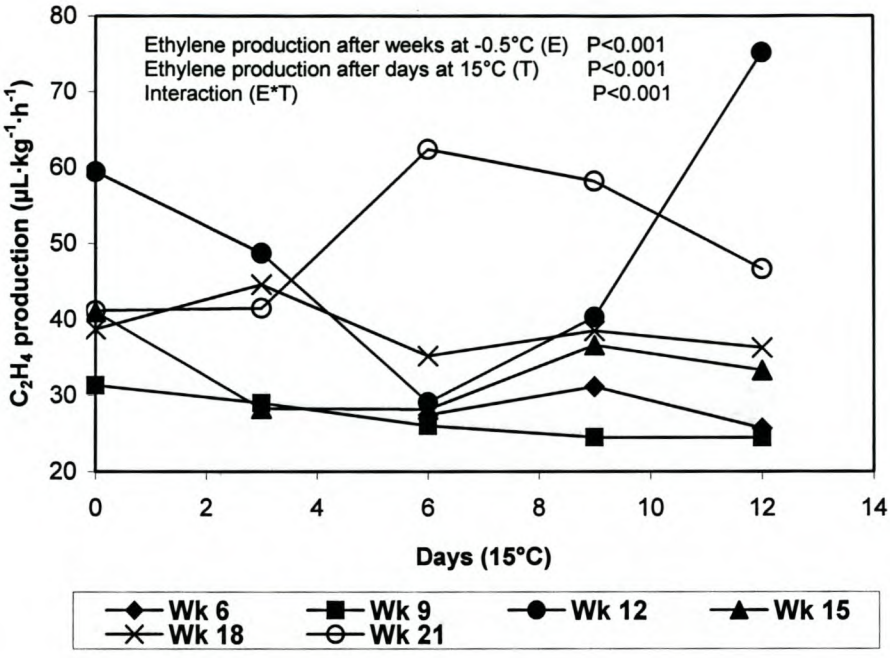

Fig. 10. Ethylene production ($\mu L \cdot kg^{-1} \cdot h^{-1}$) of 'Forelle' pears, harvested in the Warm Bokkeveld area, on days at 15°C, after weeks (Wk) of storage at -0.5°C ($LSD_{5\%}$ =14.7).

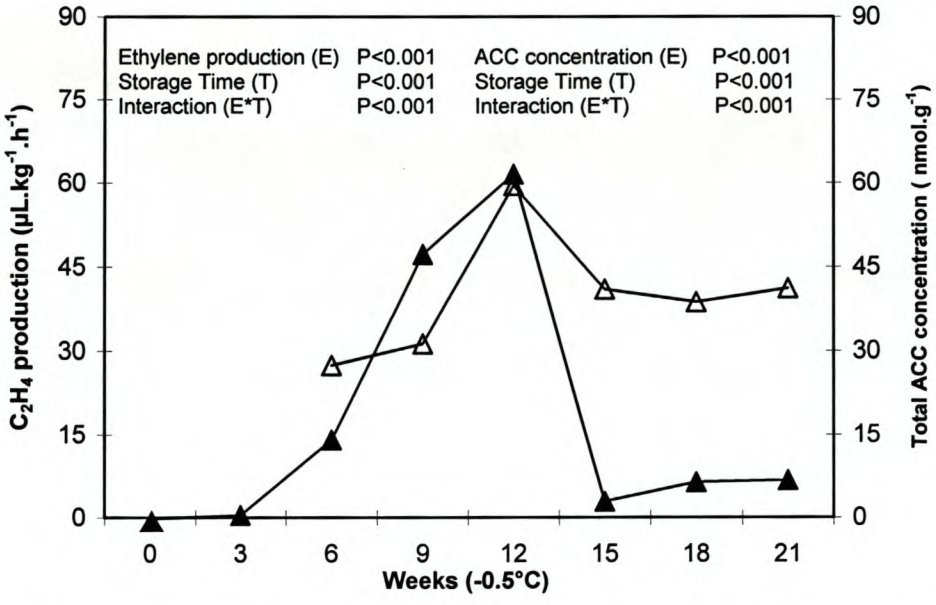

Fig. 11. ACC concentration (▲), of 'Forelle' pears harvested in the Warm Bokkeveld, after storage at -0.5°C ($LSD_{5\%}$ =17.2) and ethylene production (△) on day 0 after 12 h from transferral to 15°C ($LSD_{5\%}$ =14.7).

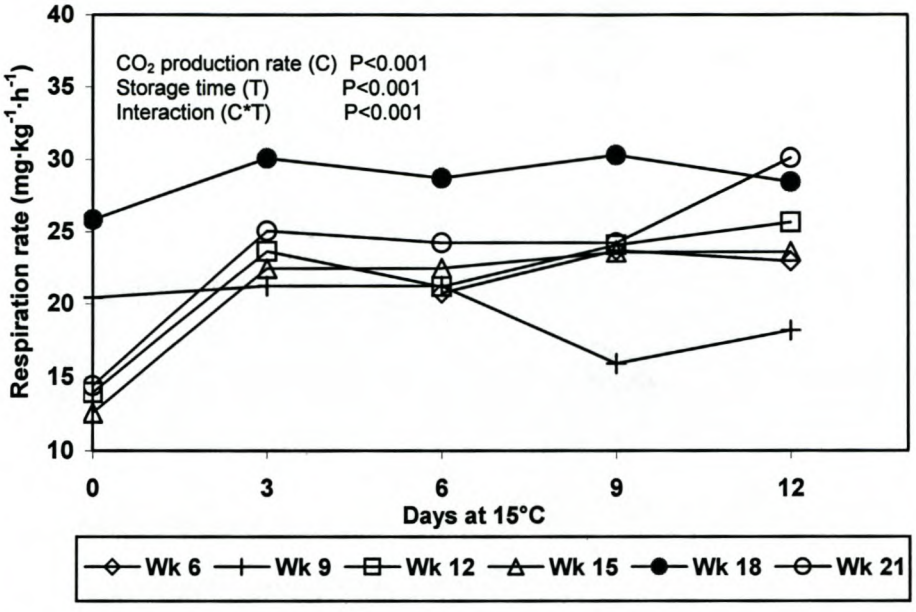

Fig. 12. Respiration rate (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) of 'Forelle' pears, harvested in the Warm Bokkeveld area, on days at 15°C after weeks (Wk) of cold storage at -0.5°C ($LSD_{5\%}$ = 7.3).

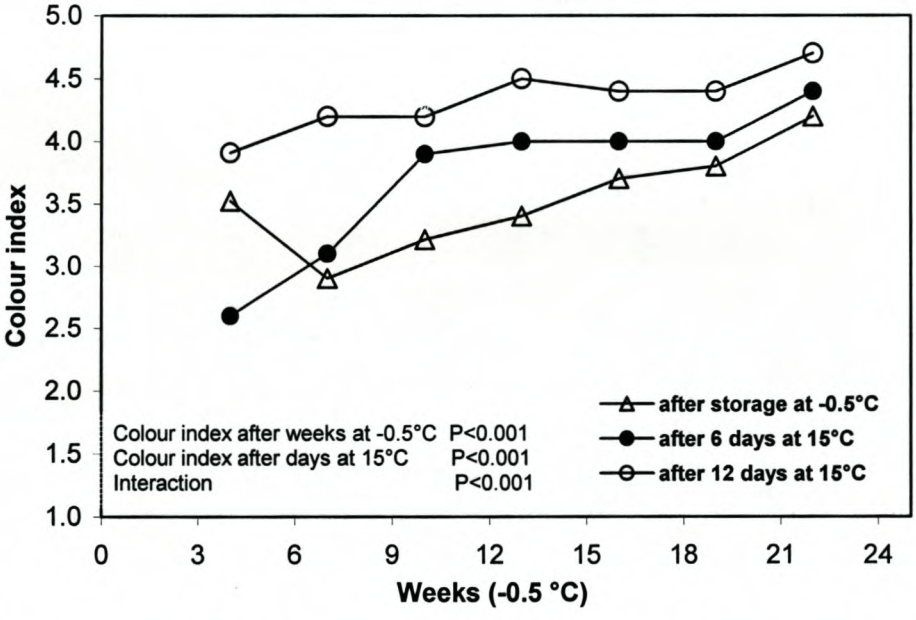

Fig. 13. Ground colour index (Unifruco colour chart) of 'Forelle' pears harvested in Theewaterskloof area, stored at -0.5°C for up to 22 weeks and transferred to 15°C for 12 days ($LSD_{5\%}$= 0.41).

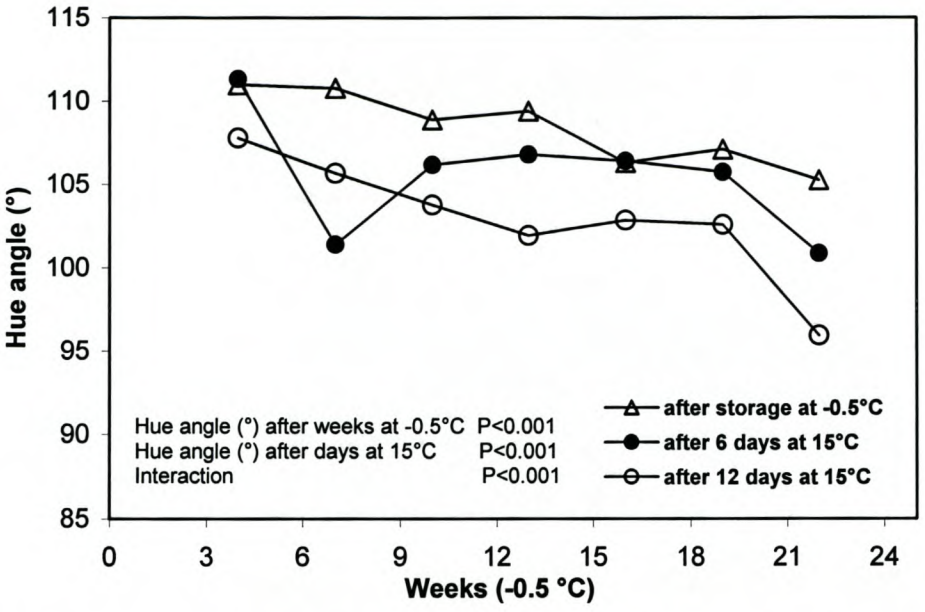

Fig. 14. Hue angle (°) of 'Forelle' pears harvested in the Theewaterskloof area, stored at -0.5°C for up to 22 weeks and transferred to 15°C for 12 days ($LSD_{5\%}$= 2.66°).

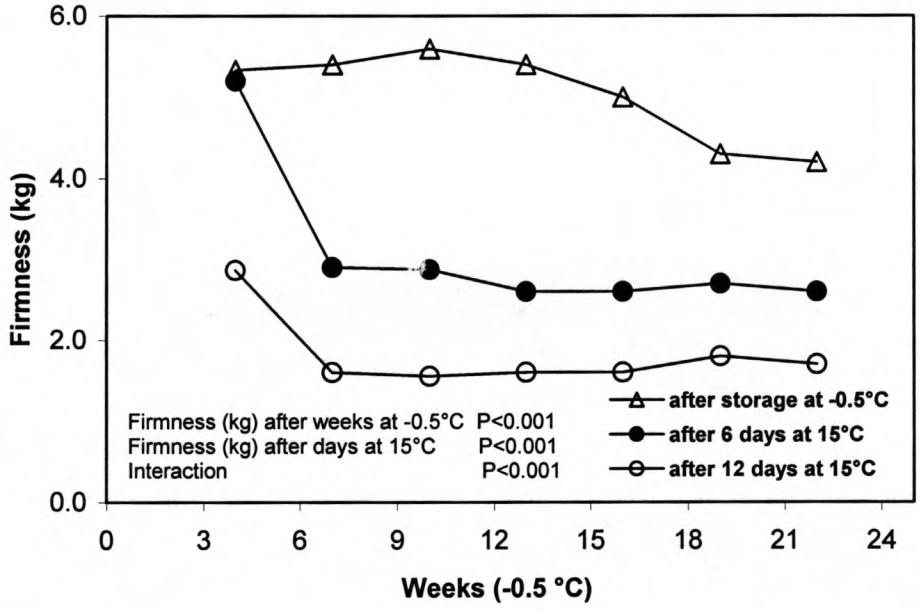

Fig. 15. Firmness (kg) of 'Forelle' pears harvested in the Theewaterskloof area, stored at -0.5°C for up to 22 weeks, and transferred to 15°C for 12 days ($LSD_{5\%}$= 0.39 kg).

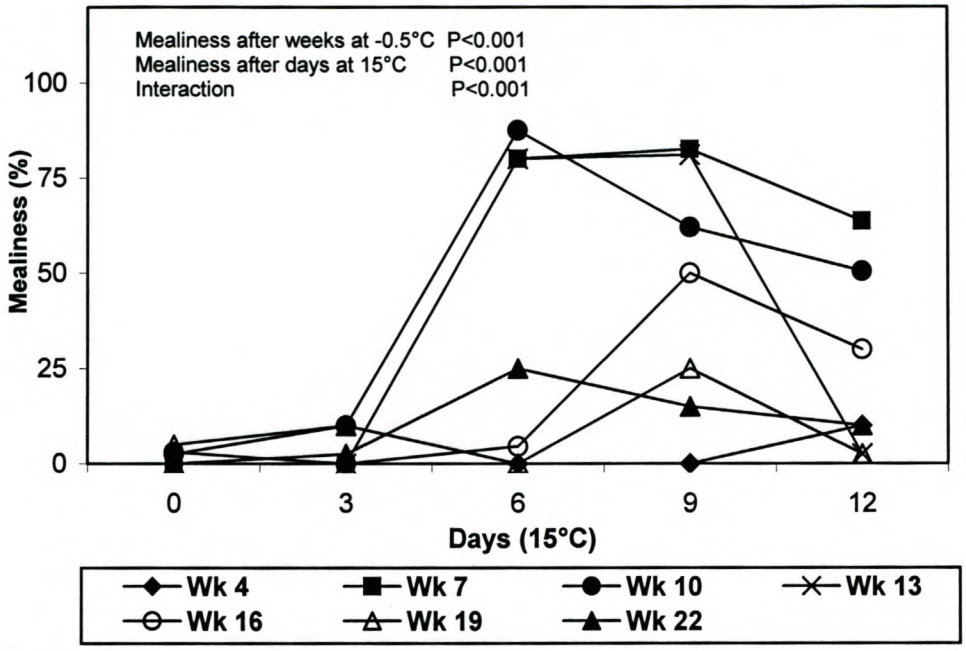

Fig. 16. 'Forelle' pear mealiness in the 2000 season of fruit from the Theewaterskloof area, after weeks of storage at -0.5°C and transferral to 15°C for 12 days (Logit transformed data).

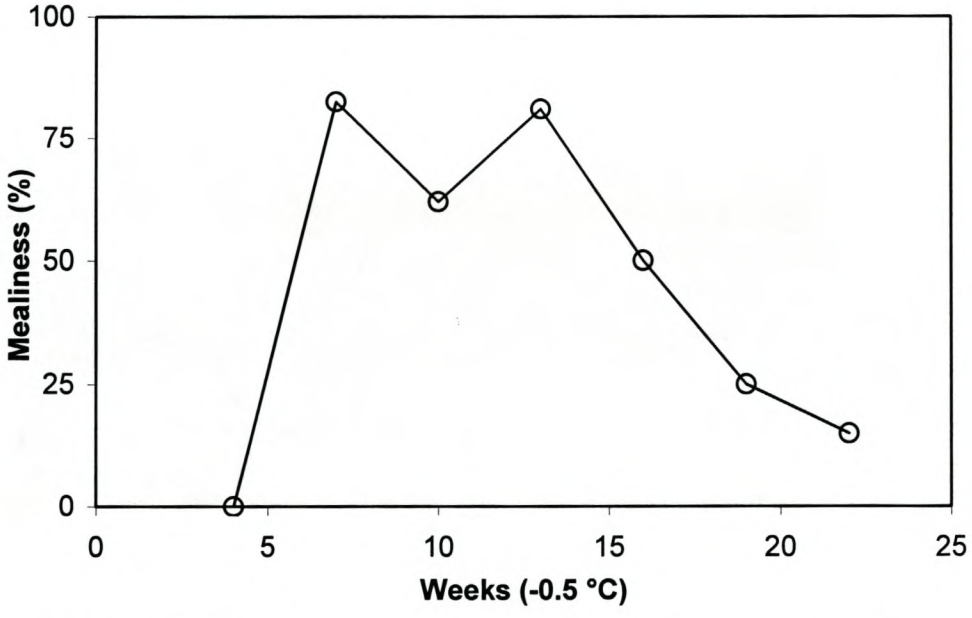

Fig. 17. Mealiness of 'Forelle' pears harvested from the Theewaterskloof area, stored for up to 22 weeks at -0.5°C, and sampled on day 9 at 15°C (Logit transformed data).

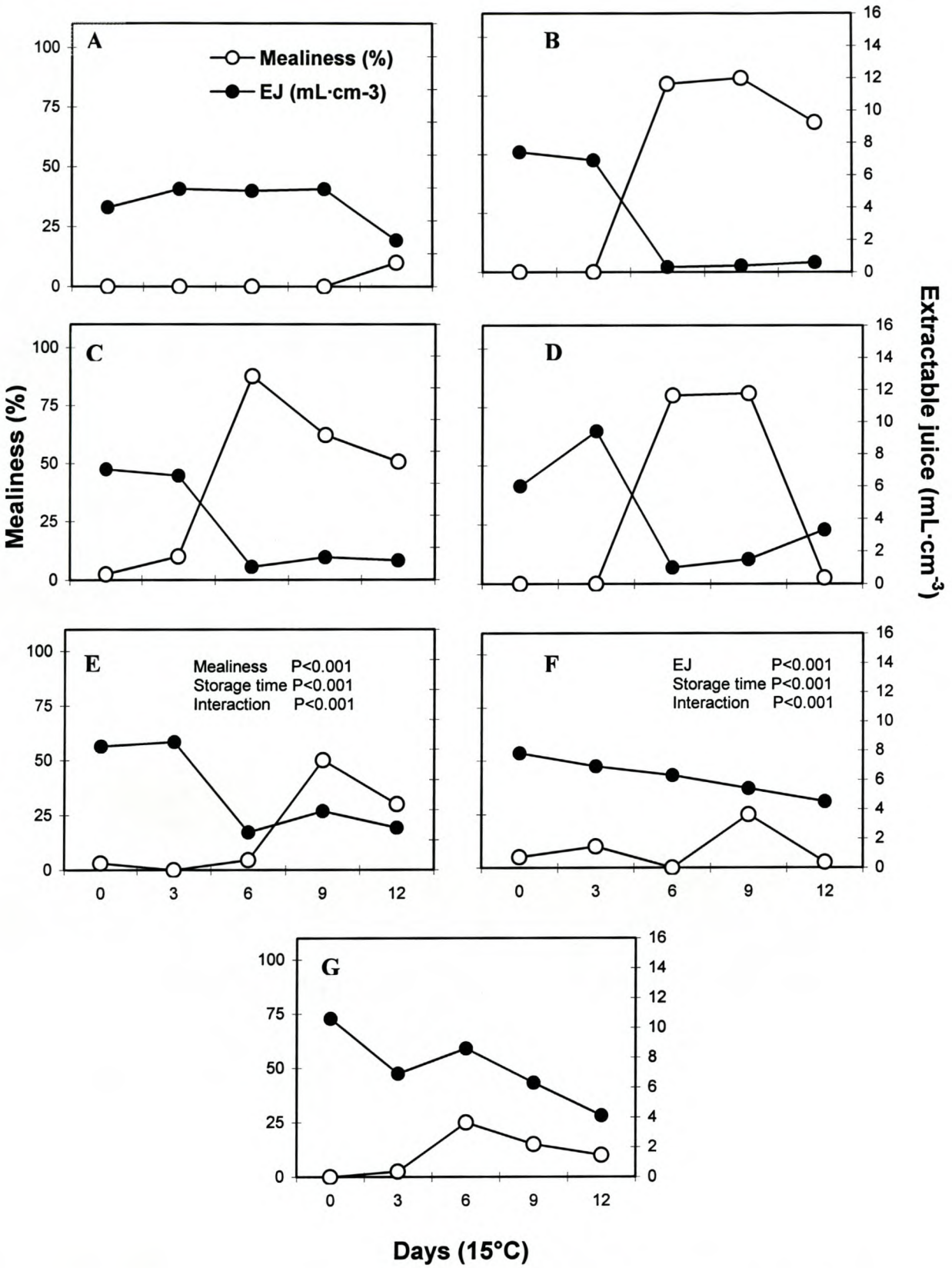

Fig. 18. Mealiness (%) (○) and EJ (mL·cm^{-3}) (●) of 'Forelle' pears from the Theewaterskloof area during 12 days of ripening at 15°C after 4 (**A**), 7 (**B**), 10 (**C**), 13 (**D**), 16 (**E**), 19 (**F**) and 22 (**G**) weeks of storage at -0.5°C.

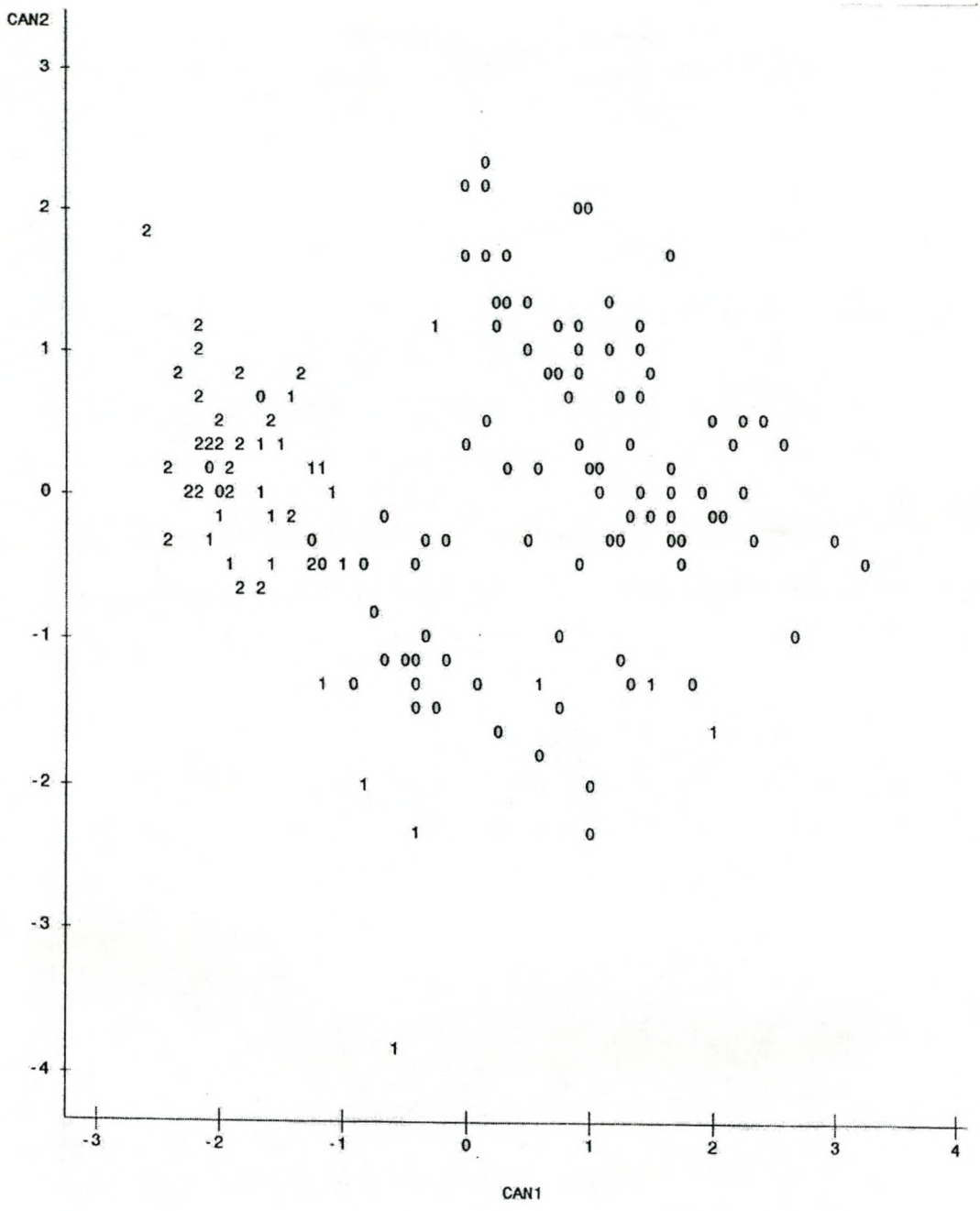

Fig. 19. Discrimination between 'Forelle' pears from the Theewaterskloof area that are 0 - 35 % (**0**), 35 - 70 % (**1**) and 70 – 100 % (**2**) mealy, with extractable juice content as variable, chosen in a stepwise discriminant analysis, and processed with the canonical discriminant analysis procedure.

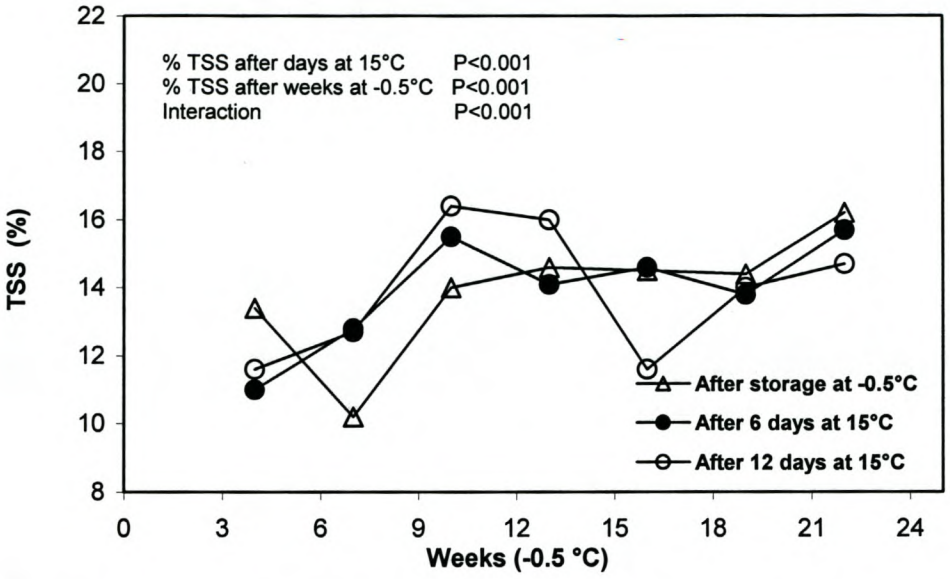

Fig. 20. TSS (%) of 'Forelle' pears harvested in the Theewaterskloof area and stored at -0.5°C for up to 21 weeks ($LSD_{5\%}$ = 0.94%)

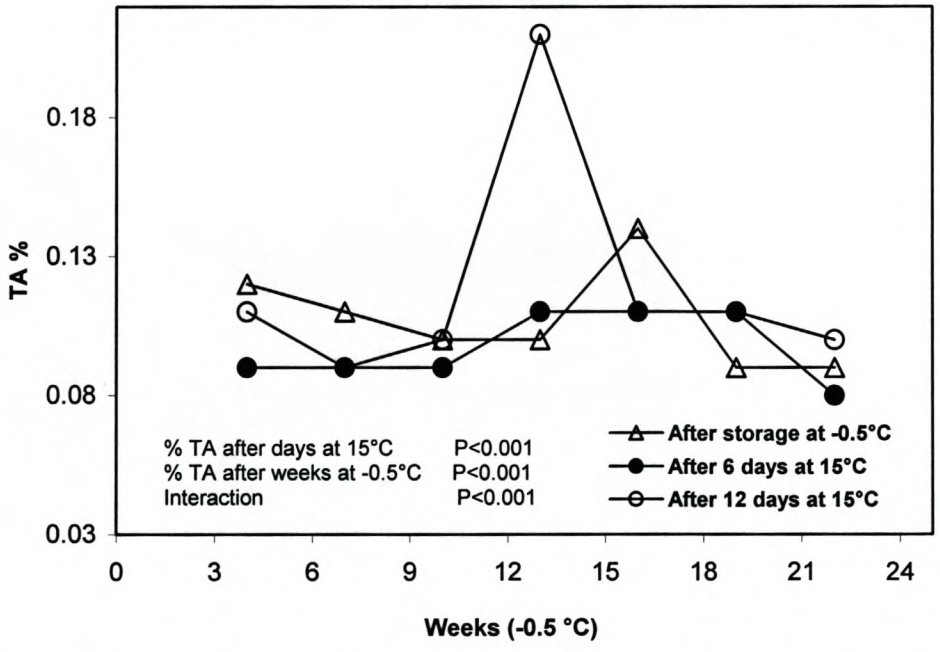

Fig. 21. TA (%) of 'Forelle' pears harvested in the Theewaterskloof area and stored at -0.5°C for up to 21 weeks ($LSD_{5\%}$ = 0.04%).

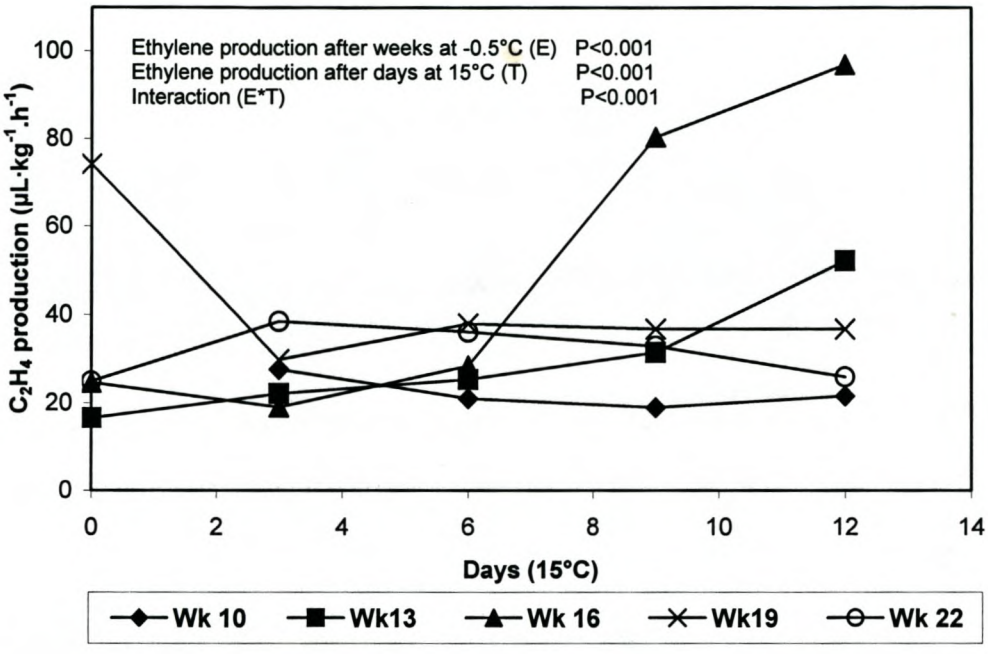

Fig. 22. Ethylene production rate ($\mu L \cdot kg^{-1} \cdot h^{-1}$) of 'Forelle' pears, harvested in the Theewaterskloof area, on days of storage at 15°C after weeks (Wk) of cold storage at -0.5°C ($LSD_{5\%} = 12.78$).

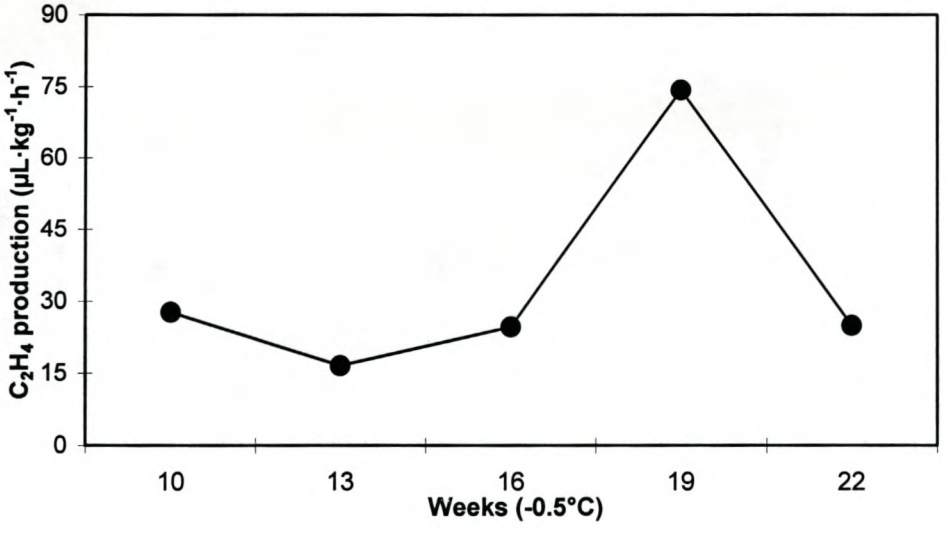

Fig. 23. Ethylene production rate (●) on day 0, 12h after transfer to 15°C ($LSD_{5\%} = 12.78$). 'Forelle' pears were sourced from the Theewaterskloof area in the 2000 season.

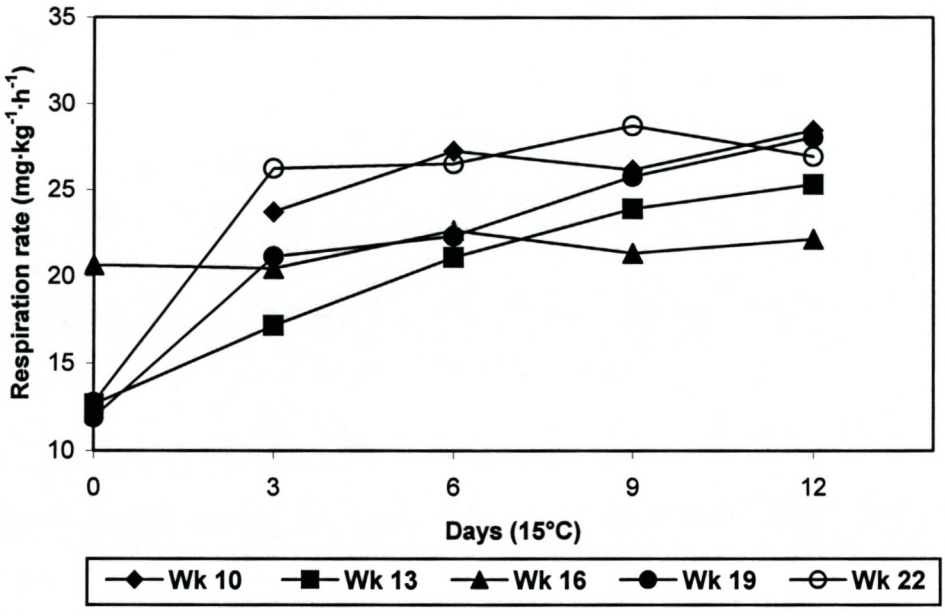

Fig. 24. Respiration rate ($mg \cdot kg^{-1} \cdot h^{-1}$ CO_2) of 'Forelle' pears from the Theewaterskloof area, on days at 15°C after weeks (Wk) of cold storage at -0.5°C ($LSD_{5\%} = 6.51$).

CHAPTER 3: PAPER 2

INFLUENCE OF HARVEST MATURITY ON RIPENING AND MEALINESS OF 'FORELLE' PEARS

ABSTRACT

'Forelle' pears produced in South Africa are prone to mealiness or low extractable juice content. Since harvest maturity affects the incidence of mealiness in other pear cultivars, its effect on 'Forelle' pears was investigated. Fruit were harvested at different maturities from the Ceres area, in weeks 8 (pre-optimum), 10 (optimum), 12 and 14 (both post optimum). Maturity indices, extractable juice content, mealiness, total ACC content and ethylene levels were monitored at harvest, after 6 weeks of storage at -0.5°C and again after 7 days at 15°C. Contrary to what was expected, fruit harvested 2 weeks before commercial harvest ripened and developed the highest mealiness (80%). However, fruit of all harvest maturities were mealy, except those harvested in week 10, which failed to ripen. This has been attributed to contamination by 1-methylcyclopropene in the coldroom. Although there was a decrease in the incidence of mealiness for fruit harvested in week 12 (50%), harvest maturity does not appear to have the same effect on 'Forelle' pears as it does on other winter pears. Factors other than harvest maturity must therefore play a more important role in the development of mealiness in 'Forelle' pears.

Keywords: (*Pyrus communis* L.) ethylene, 1-aminocyclopropane-1-carboxylic acid, flesh firmness.

1. INTRODUCTION

Pears are climacteric fruit and the stage of maturity at harvest is therefore very important. Fruit harvested immature do not have the ability to reach a climacteric and do not ripen, or ripen unevenly (Ben-Arie and Sonego, 1979). Pears harvested too late often develop with a mealy texture (Mellenthin and Wang, 1976). A climacteric reached after cold storage in pears is usually the time during ripening with the best flavour and eating quality (Ben-Arie and Sonego, 1979). Thus, harvest maturity does not only determine whether pears will ripen but also affects the quality during ripening (Elgar *et al.*, 1997).

Mealiness in 'd'Anjou' pears was found in fruit harvested overmature (Chen and Mellenthin, 1981) or stored for too long at too high a temperature (Hansen and Mellenthin, 1979). Consequently, mealiness was classified as a senescence related disorder (Murayama *et al.*, 1998). Hansen (1961) associated a 53-70% incidence of mealiness to growing seasons with high total heat units. This was confirmed by Mellenthin and Wang (1976), who reported that fruit grown in high daily-hour temperatures during the six weeks before harvest, failed to ripen uniformly and were susceptible to certain physiological disorders, including mealiness.

Ripening-associated increase in water soluble pectin (WSP) is well established in pears (Martin-Cabrejas *et al.*, 1994). Woolliness in peaches, which is texturally very similar to mealiness in pears, is the result of water-insoluble pectin and neutral sugars of pectic origin. These pectins are not broken down into the WSP form, which causes binding of free water and results in the dry mouth feel (Ben-Arie and Sonego, 1980). Chen and Borgic (1985) found that 'Bosc' pears that never developed a juicy and buttery texture also had low WSP. This was supported by Gerasopoulus and Richardson (1997). Murayama *et al.* (1998) found that 'Marguerite Marilatt' and 'La France' pears left on the tree for 28 days after optimum harvest had one third less WSP after ripening, than fruit that were optimally harvested and ripened off the tree. Harvest maturity plays an important role in the breakdown of pectin and hence the water binding capacity of pectin.

Since harvest maturity influenced ripening and mealiness of other pear cultivars, this study investigates the effect of harvest maturity on 'Forelle' pear ripening.

2. MATERIALS AND METHODS

2.1. *Fruit source*

'Forelle' pears *(Pyrus communis* L.*)* were harvested 2 weeks prior to commercial harvest (week 8) (H1), at commercial harvest (week 10) (H2), and 2 and 4 weeks after commercial harvest (weeks 12 (H3) and 14 (H4), respectively) in the Warm Bokkeveld area (33°15'S; 19°15'W), Western Cape, South Africa.

2.2 Experimental layout

Fruit samples were randomly harvested from the same 10 trees at each harvest date. Fruit were analysed at harvest, after 6 weeks of storage at -0.5°C and again after ripening at 15°C for 7 days. Six weeks of storage at -0.5°C, and ripening at 15°C proved to have maximised mealiness in fruit of 2 areas in the 2000 season. Six weeks at -0.5°C and 7 days at 15°C was thus used as a storage period, to determine the effect of harvest maturity on mealiness. At each examination date 40 fruit were used for maturity indexing (5 replicates of 8 fruit per replicate). A further 8 fruit were used to determine ethylene production and internal ethylene and total ACC concentration.

2.3. *Maturity indices*

Ground colour changes from green to yellow, were measured as hue angle (h°) using a colorimeter (Nippon Denshoku Model HR-3000, Tokyo, Japan) and a Unifruco colour chart for pears (where 0.5 = dark green, 5 = deep yellow). Flesh firmness (kg) was measured using a penetrometer (Southtrade fruit pressure tester, model FT 327, Alphonsine, Italy) fitted with an 8 mm probe. Total soluble solids (TSS; %) of a pooled juice sample of each replicate was measured by a hand held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Titratable acidity (TA) was calculated as a percentage malic acid, by titrating the pooled juice sample with 0.1 N NaOH to a pH end point of 8.2, using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland).

2.4. *Extractable juice*

Extractable juice content (EJ) ($mL \cdot cm^{-3}$) was measured with a Chylofel (COPA - Technologie S.A., St. Etienne du Gress, France) which best estimates release of juice on chewing. The instrument consists of a spring-mounted base, which is mobile along the vertical axis. The peeled fruit is placed on this base. When fruit is pressed into the instrument, a fixed, 25mm cylindro-conical nozzle with a stop ring mounted on the lower part of the shaft is inserted into the fruit and explores a constant volume of 3 cm^3. The droplets of juice released by the fruit are collected in a graduated beaker placed under the base plate. The Chylofel was used on opposite cheeks, and juice of the fruit in each of the 5 replicates was pooled.

2.5. *Mealiness*

Mealiness was determined on each fruit subjectively. Fruit were cut equatorially and squeezed. Fruit with a dry, floury texture were classified as mealy.

2.6. *Ethylene*

Ethylene production and internal ethylene concentration were measured at harvest (0 weeks), after 6 weeks at -0.5°C, and again after ripening at 15°C for 7 days. Four replicates of 2 fruit each were placed in 5 L containers at 20°C, to measure ethylene production in the static system. Samples were taken after 1 h. Internal ethylene was extracted from each pear under vacuum. Ethylene was measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian Instrument Group, Palo Alto, California, USA).

2.7. *ACC (1-aminocyclopropane-1-carboxylic acid)*

Peeled fruit disks from each replicate were frozen in liquid nitrogen and stored at -80°C for ACC quantification. The frozen disks were chopped with an onion chopper, and ground further with a pestle and a mortar while adding liquid nitrogen. Fifteen mL of an 80% ethanol solution was then added to 3 g of ground frozen pulp and homogenized by Ultra-Turrax (pp/10, Janke and Kunkel Ika, Staufn, Germany), for 30 seconds. Samples then stood for an hour, whereafter they were centrifuged at 500 g_n for 10 min. An aliquot (5mL) of the supernatant was concentrated in vacuo with a savant (Speedvac concentrator, SVC, 200H, Farmdale, N.Y.), and used for ACC analysis according to the method of Lizada and Yang (1979).

2.8. *Data analysis*

Data were analysed using the GLM (General Linear Means) procedure in the SAS (Statistical Analysis System) programme (SAS Institute Inc., 1990). ANOVA-generated *P*-values illustrate the significant differences. Significant differences between parameters within cultivars were determined using Fisher's protected least significant difference test with a 95% confidence interval.

3. RESULTS

3.1 *Maturity indices*

Ground colour values for the interaction between harvest maturity and storage time were non-significant. Ground colour values measured with a colour chart, were higher (more yellow) for fruit harvested 2 weeks prior to commercial harvest. For fruit of the other harvest dates ground colour did not change (data not shown).

Hue angle is an objective, and consequently more sensitive measure for ground colour. Unlike colour chart indices, small differences in colour during storage were observed, and the interaction between harvest maturity and storage time was significant (Fig. 1). Hue angle at harvest was the same for fruit harvested in weeks 8 (H1) and 10 (H2) (± 111°). Fruit harvested in weeks 12 (H3) and 14 (H4) were the same at harvest (± 107°), but the fruit harvested in weeks 8 (H1) and 14 (H4) ripened with slightly more yellow colour, than fruit harvested in weeks 10 and 12.

Flesh firmness (kg) was lowest for fruit harvested 2 weeks prior to commercial harvest (6.0 kg) (Fig. 2). Flesh firmness for fruit harvested on all the other harvest dates was the same (± 6.7 kg). Only the fruit harvested in weeks 8 (H1) and 14 (H4) ripened to an edible firmness after 6 weeks of storage at -0.5°C followed by 7 days at 15°C. Fruit harvested during commercial harvest (week 10) maintained their firmness (5.6 kg) while fruit harvested two weeks after commercial harvest had a firmness of 4.1 kg.

Total soluble solid (TSS) content for fruit harvested in weeks 8 (H1) and 10 (H2) did not differ at harvest (±14.0%), but was slightly higher in fruit harvested in weeks 12 (H3) and 14 (H4) (Fig. 3). After 6 weeks of storage at -0.5°C followed by ripening at 15°C for 7 days, TSS of all fruit had increased (>15%).

Titratable acidity was highest for fruit harvested in weeks 10 (H2) (commercial harvest) and 14 (H4) (post-optimum), but TA in all fruit decreased with increasing storage duration at -0.5°C (Fig. 4). The interaction between TA for harvest maturity and storage period was not significant.

3.2. *Extractable juice content and mealiness*

Extractable juice content ($mL \cdot cm^{-3}$) only decreased when fruit had softened, which was after 7 days at 15°C (Fig. 5). For fruit of all harvest maturities there was no EJ present after 7 days at 15°C, with the exception of the commercial harvest maturity (Fig. 5). Mealiness was unacceptably high (≥50%) for fruit harvested from weeks 8 (H1), 12 (H3) and 14 (H4) (Fig. 6). Mealiness could not be detected in fruit harvested at the commercial harvest date (week 10; H2) since fruit failed to soften due to contamination with 1-methylcyclopropene (1-MCP).

3.3. *Ethylene production, internal ethylene and total ACC concentration*

No ethylene production was detected directly after harvest for fruit from all harvest dates (Fig. 7). After 6 weeks at -0.5°C, a small concentration of ethylene was measured for fruit harvested 2 weeks prior to commercial harvest (week 8) (H1). Ethylene production was even less in weeks 12 and 14 (± 1.6 $\mu L \cdot kg^{-1} \cdot h^{-1}$) than in week 8 (H1) (11.1 $\mu L \cdot kg^{-1} \cdot h^{-1}$). Fruit harvested in week 8 (H1) and stored for 6 weeks at -0.5°C and 7 days at 15°C had the highest ethylene production (37.9 $\mu L \cdot kg^{-1} \cdot h^{-1}$), but fruit harvested in weeks 12 (H3) and 14 (H4) also produced ethylene (25.6 and 23.1 $\mu L \cdot kg^{-1} \cdot h^{-1}$, respectively). Only a trace amount of ethylene could be detected from the 1-MCP contaminated fruit at commercial harvest maturity, after ripening (1.74. $\mu L \cdot kg^{-1} \cdot h^{-1}$).

The same trend as in ethylene production could be noticed in internal ethylene with the exception that internal ethylene concentration from fruit harvested in week 14 (H4) was as high as in fruit from the earliest harvest maturity (± 61.0 $\mu L.L^{-1}$) (Fig. 8).

The total ACC concentrations at harvest, measured for all maturities were low (Fig. 9). When the mean total ACC for harvest maturity over all storage times are compared (Main effect harvest maturity), 'Forelle' harvested 2 weeks prior to commercial harvest (week 8) (H1) had significantly higher total ACC concentrations than fruit harvested later. Mean total ACC concentration of storage time over all harvest maturities (Main effect storage time), showed that fruit at harvest had significantly lower total ACC concentrations than fruit stored for 6 weeks at -0.5°C or for fruit that were stored for a further 7 days at 15°C.

4. DISCUSSION AND CONCLUSIONS

Harvest maturity of 'Forelle' pears, influenced hue angle, firmness, TSS and total ACC concentration significantly at harvest, and mealiness, and ethylene production after storage for 6 weeks at -0.5°C followed by 7 days at 15°C. The hue angle for the pre-optimum harvest (week 8) (H1) was higher than the last harvest (week 14) (H5), but ground colour value was the same after 6 weeks of storage at -0.5°C and 7 days at 15°C. The loss in flesh firmness followed the same softening pattern for fruit from these two harvest dates. Ethylene production and internal ethylene concentration were also at a maximum for these two harvest dates, after 6 weeks at -0.5°C, and 7 days at 15°C. On the basis of colour and firmness fruit harvested at both the start and end of the picking window were of a more advanced maturity after cold storage and ripening at 15°C, than the fruit picked on the other harvest dates. It is thought that the high incidence of mealiness measured for fruit after ripening is related to the mealiness assessment being more effective on softer fruit. Fruit seldom soften on the tree, although exposure to cool temperatures can cause premature ripening due to an accumulation of ACC causing ethylene production (Agar *et al.,* 1999). Climatic conditions before each harvest date play an important role in determining fruit quality. Mellenthin (1980) reported that pre-mature ripening of 'Bartlett' pears occur 30 days prior to harvest when there were 2 consecutive cold nights at a temperature of ± 7.2°C with daytime temperatures at ± 21.1°C. This might be the case for the first harvest date, which was 0.7 kg softer at harvest, had a higher total ACC concentration and higher ethylene production rate than later harvested fruit. This could indicate that the fruit harvested 2 weeks before commercial harvest, had already reached the stage of maturity where it had the potential for ripening. The conversion of ACC to ethylene for fruit of the later harvest maturities might have been inhibited by day temperatures higher than ± 38°C (Yu *et al.*, 1980; Lurie *et al.*, 1996), which is regularly reached in the orchard at that time of the season.

The change in maturity could not have been affected by protracted blossoming or position in the tree. Fruit were selected and marked prior to the first harvest to exclude the effect of these two factors.

Fruit harvested 2 weeks after commercial harvest (week 12) (H3) were 50% mealy after 6 weeks of storage at -0.5°C and 7 days at 15°C, which was lower than fruit harvested in weeks 8 (H1) and 14 (H4) (100% and 73%, respectively). However, firmness values were higher and ground colour did not reach the same deep yellow compared to the first and last harvest date. Since these fruit were firmer, and firmness affects mealiness, this may explain the lower incidence of mealiness. Fruit from the commercial harvest date (week 10; H1) did not develop a yellow colour and did not soften after 6 weeks at -0.5°C and 7 days at 15°C. Ethylene production for fruit from this harvest maturity was very low. The resistance to ripening of these fruit could possibly be related back to contamination with 1-MCP at the beginning of the storage period at 15°C.

1-MCP is an effective inhibitor of ethylene responses, due to its binding capacity to the ethylene receptor (Sisler and Serek, 1997). It prevents ethylene binding for many days after application, and is effective at very low concentrations (0.0005- 0.04 $\mu L \cdot L^{-1}$; 24h treatment). 1-MCP treated fruit that were not thoroughly ventilated and stored with other untreated unripe 'Forelle', could thus have contaminated the trial at that point. Ripening and senescence factors like rise in respiration, softening, development of flavour components and a loss in chlorophyll are retarded as a result of treatment with 1-MCP. In 'Forelle' it is thus possible that 1-MCP could have resulted in firm, less mature fruit which were not suitable for mealiness assessment.

In conclusion, fruit quality after ripening was influenced by harvest maturity, and fruit of all harvest maturities, except where contamination with 1-MCP occurred, were mealy. Fruit harvested 2 weeks prior to commercial (H1) harvest already had the potential to ripen. Although there was a decrease in mealiness for fruit harvested 2 weeks after commercial harvest (H3), it appears to be a function of the maturity (firmness) of the fruit at mealiness assessment. The influence of harvest maturity on 'Forelle' pear mealiness, cannot be explained. It would appear that factor(s), other than harvest maturity, play a more important role in the initiation of mealiness in 'Forelle' pears.

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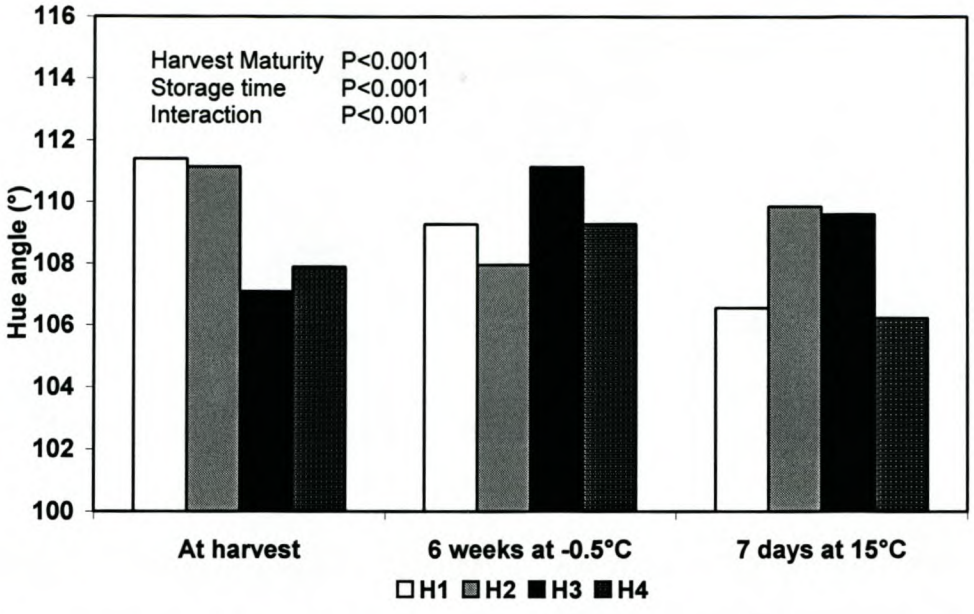

Fig. 1. Hue angle (°) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** ($LSD_{5\%}$ = 1.32).

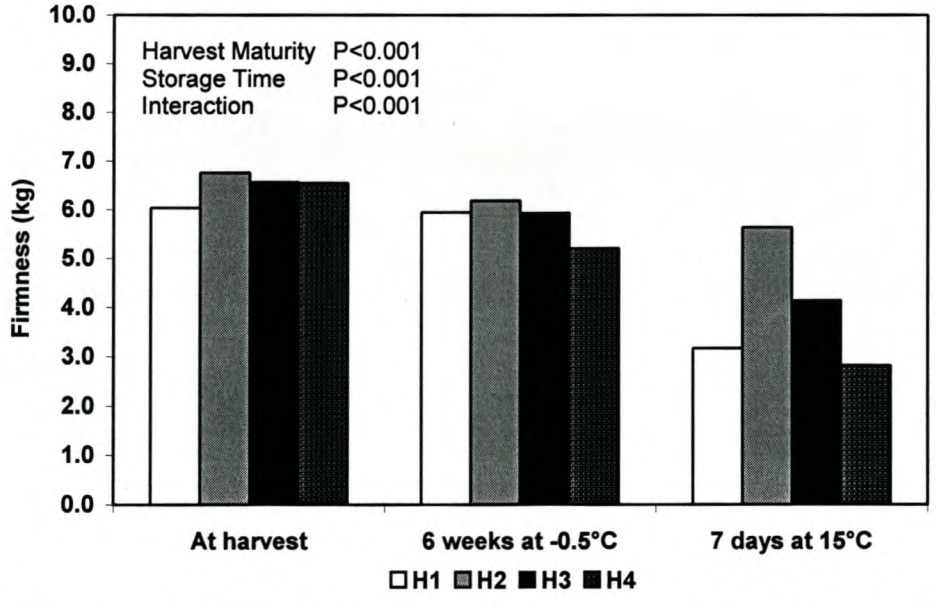

Fig. 2. Firmness (kg) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4).** ($LSD_{5\%}$ = 0.38).

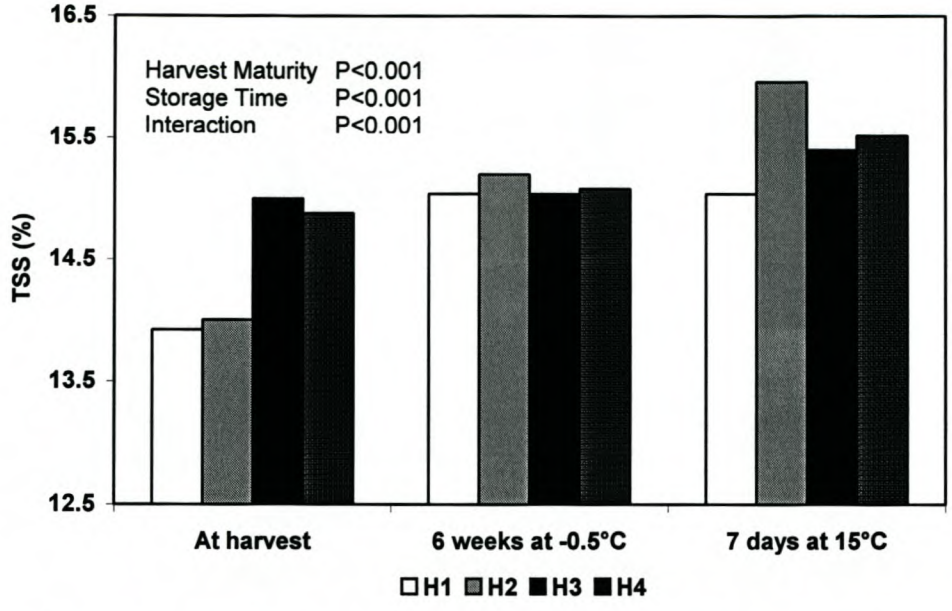

Fig. 3. Total soluble solids (%) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** ($LSD_{5\%} = 0.29$).

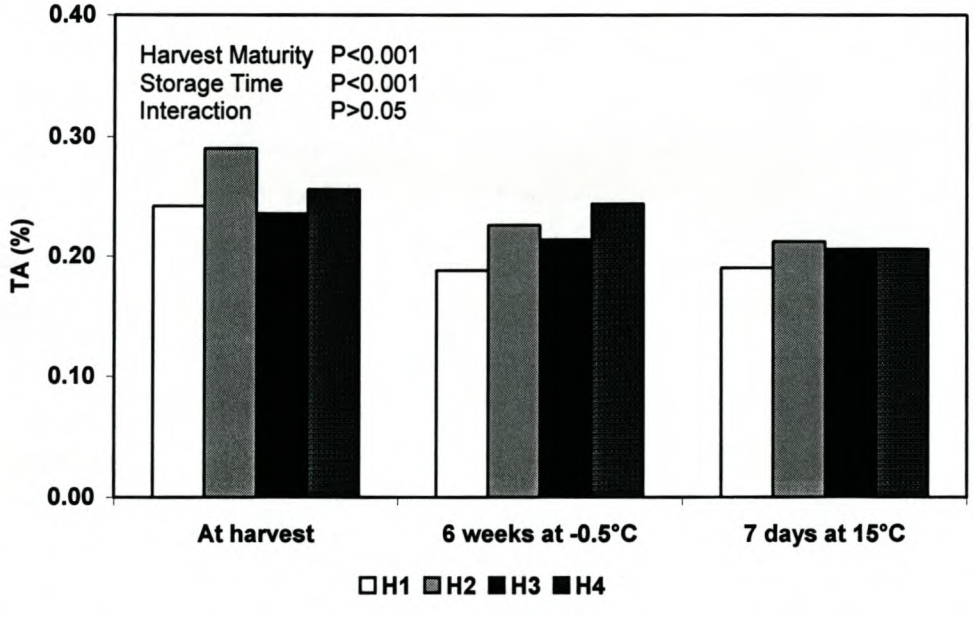

Fig. 4. Titratable acidity (%) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)**.

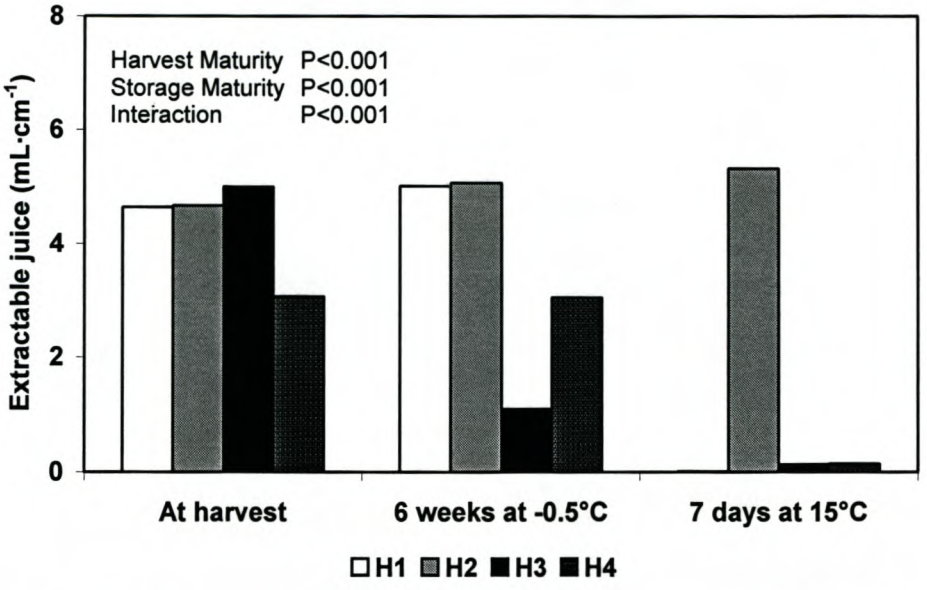

Fig. 5. Extractable juice ($mL \cdot cm^{-3}$) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** ($LSD_{5\%} = 0.48$).

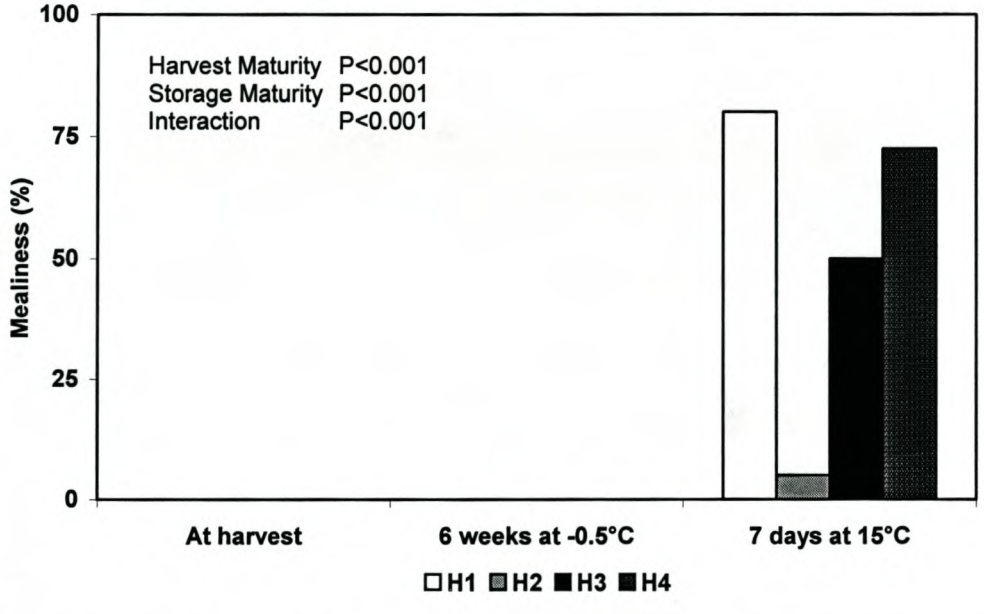

Fig. 6. Mealiness (%) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** (Logit transformed data)..

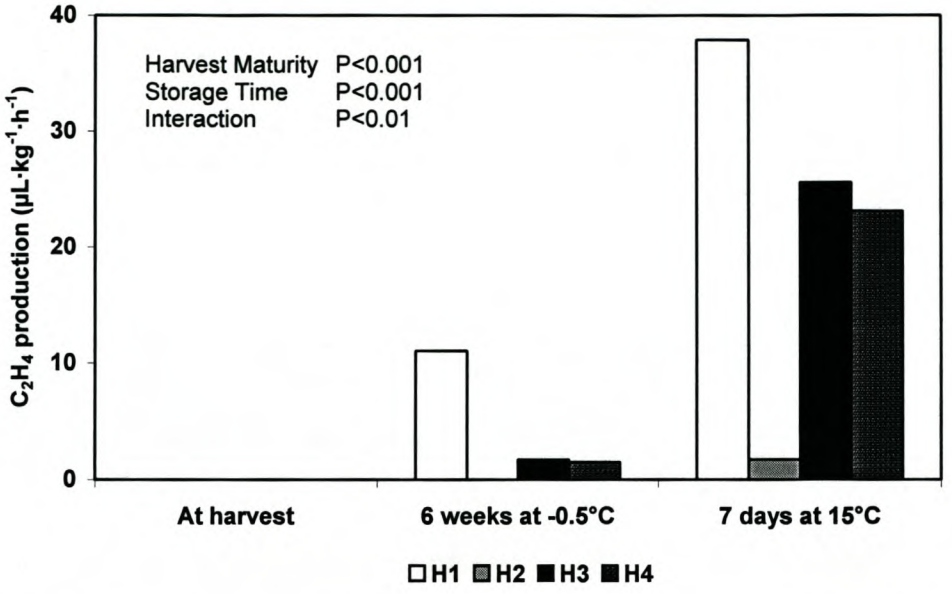

Fig. 7. Ethylene production ($\mu L \cdot kg^{-1} \cdot h^{-1}$) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** ($LSD_{5\%} = 10.72$).

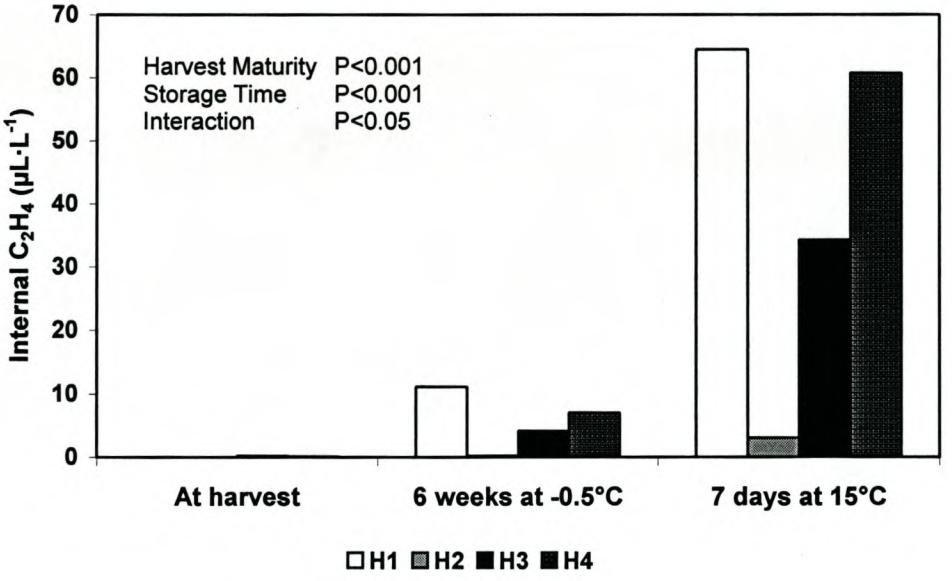

Fig. 8. Internal ethylene concentration ($\mu L \cdot L^{-1}$) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** ($LSD_{5\%} = 25.96$).

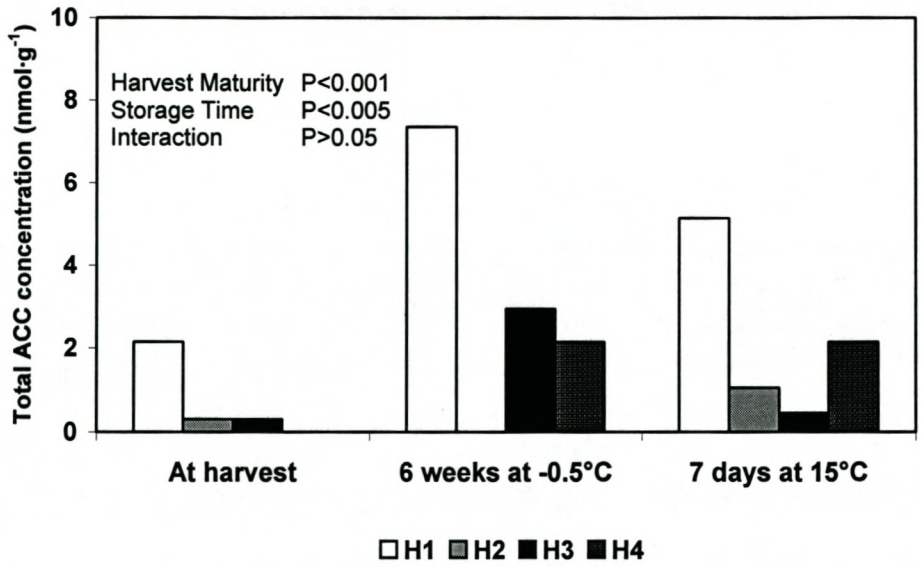

Fig. 9. Total ACC concentration (nmol·g^{-1}) of 'Forelle' pears harvested 2 weeks prior to commercial harvest **(H1)**, at commercial harvest **(H2)**, 2 weeks after commercial harvest **(H3)** and 4 weeks after commercial harvest **(H4)** (Main effect Harvest Maturity $LSD_{5\%}$ = 1.47; Main effect Storage Time LSD = 1.28).

CHAPTER 4: PAPER 3
EXOGENOUS ETHYLENE REDUCES THE COLD REQUIREMENT OF 'FORELLE' PEARS AT THE EXPENSE OF INTERNAL QUALITY

ABSTRACT

Although ethylene has been reported to shorten the cold requirement of 'Forelle', the effect on mealiness, an internal disorder, was not quantified. Consequently the aim of this study was to evaluate the effect of ethylene on both ripening and mealiness of 'Forelle' pears. Fruit harvested from the Elgin area at commercial maturity were stored for 3 weeks at -0.5°C, treated with ethylene (100 μ $L.L^{-1}$, 24h, 20°C) and held at 20°C for a further 2 days (without ethylene). Control fruit were held at 20°C for 3 days. Fruit were returned to -0.5°C for a further 3 weeks. After 3 days at 20°C, flesh firmness was 2.9 kg in treated fruit compared to 6.1 kg for control fruit. At that point all fruit treated with ethylene were mealy. Control fruit all exhibited mealiness after a further 3 weeks at -0.5°C followed by 7 days at 15°C. Ethylene treatment enhanced fruit maturity (firmness and ground colour), enabling the detection of mealiness earlier than in untreated fruit. Mealiness was neither prevented nor alleviated by exogenous ethylene treatment.

Keywords: (*Pyrus communis* L.), 1-aminocyclopropane-1-carboxylic acid, flesh firmness, mealiness.

1. INTRODUCTION

Most winter pears, including 'Forelle', do not ripen normally until they have been exposed to a critical period of cold storage (Hansen, 1961; Sfakiotakis and Dilley, 1974; Mellenthin and Wang, 1976; Puig *et al.*, 1996; De Vries, 2001). Cold storage prior to ripening of pears causes an accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor to ethylene, and is controlled by the ACC synthase (ACS) enzyme (Wang *et al.*, 1985). Ripening resistance is reduced and expression of autocatalytic ethylene results in normal ripening (Lelièvre *et al.*, 1997a). The induction of rapid ethylene production thus involves a temperature-dependent transition to an ethylene sensitive state (Jobling *et al.*, 1991).

In apples the low temperature storage for ripening can be substituted with exogenous ethylene or the analogue propylene (Jobling *et al.*, 1991). Ethylene (100 $\mu L \cdot L^{-1}$) applied prior to storage has been used to shorten the cold requirement of 'Bartlett' pears from 4 weeks to 7 days (Agar *et al.*, 1999), and of 'd'Anjou' pears from 6 weeks to 2-4 weeks (Chen *et al.*, 1997). Recent studies on `Forelle' pears demonstrated that exogenous ethylene treatments prior to storage can reduce the cold storage requirement of 12 weeks to 6 weeks at -0.5°C (Du Toit *et al.*, 2001). Since 'Forelle' is the last of the three blushed pear cultivars exported from South Africa, reducing this delay in marketing prevents discontinuity of the supply of blushed pears and results in premium prices being realised. However, industry trials demonstrated that both treated and untreated fruit had commercially unacceptable levels of mealiness (>50%) (Moelich and Crouch, 2001).

Lelièvre *et al.* (1997a) reported that ACC oxidase (*ACO)* gene expression and ACO enzyme activity can be induced by either chilling or short-term exogenous ethylene treatment in `Passe-Crassane' pears. They suggest that ACS and endogenous ethylene may not be regulated by applied ethylene alone, but that cold storage prior to ethylene treatment is required. To reach an ethylene climacteric both ACO and ACS are required (Lelièvre *et al.*, 1997b).

Consequently the hypothesis of this study is that cold storage of 'Forelle' pear, prior to ethylene treatment (100 $\mu L \cdot L^{-1}$, 24 h, 20°C), will result in a shorter cold requirement and fruit with an acceptable internal quality.

2. MATERIALS AND METHODS

2.1 *Fruit source*

'Forelle' pears *(Pyrus communis* L.*)* sourced in the Theewaterskloof area (33°15'S; 19°15'W), Western Cape, South Africa were harvested at a commercial maturity with an average flesh firmness of 6.0 kg and total soluble solids of 13.7 %. Ground colour, measured with a Unifruco colour chart for pears, was 2.6 (where 0.5 = dark green, 5 = deep yellow).

2.2 *Experimental layout*

Forty fruit were sampled for maturity assessment at harvest. Fruit were stored for 3 weeks at -0.5°C prior to ethylene treatment. Fruit samples were drawn after 1½ weeks and 3 weeks of storage at -0.5°C (on day 0) and at 15°C (on day 7). This was to determine mealiness development after these storage times without the confounding effect of ethylene. After 3 weeks of storage half the fruit were treated with 100 $\mu L \cdot L^{-1}$ ethylene (premixed in air by Afrox [Ltd]), at 20°C for 24 hours. A two stage ethylene regulator kept a constant flow of ethylene through tightly sealed plastic buckets that vented to the outdoors to prevent ethylene buildup in the room. After ethylene treatment, fruit was held at 20°C in air, with the same flow rate, for a further 48 hours. Control fruit were treated in the same manner with the exception that ethylene was substituted with air. Samples were taken after the 72 hour treatment for both ethylene treated and control fruit. Thereafter, all fruit were stored at -0.5°C for a further 3 weeks before sampling immediately and again after 7 days at 15°C. Each sampling date consisted of 5 replicates of 8 fruit per replicate. A further 8 fruit were used to determine ethylene production, internal ethylene and total ACC concentration.

2.3 *Maturity indices*

Ground colour changes from green to yellow were measured as hue angle (°) using a colorimeter (Nippon Denshoku Model HR-3000, Tokyo, Japan) and a Unifruco colour chart for pears (where 0.5 = dark green, 5 = deep yellow). Flesh firmness (kg) was measured using a penetrometer (Southtrade fruit pressure tester, model FT 327, Alphonsine, Italy) fitted with an 8 mm probe. Total soluble solids (TSS; %) of a pooled juice sample of each replicate was measured by a hand held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Titratable acidity was calculated as a % malic acid, by titrating the pooled juice sample with 0.1 N NaOH to a pH of 8.2, using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland).

2.4 *Mealiness*

Mealiness was determined on each fruit subjectively. Fruit were cut equatorially and squeezed. Fruit with expressed juice on the compacted equatorial surface were classified as non-mealy, whereas those with a dry, floury texture were considered mealy.

2.5 *Extractable juice*

Extractable Juice (EJ) ($mL \cdot cm^{-3}$) was measured with a Chylofel (COPA -Technologie S.A., St. Etienne du Gress, France) which best estimates release of juice on chewing. The instrument consists of a spring mounted base, which is mobile along the vertical axis. The peeled fruit, is placed on this base. When fruit is pressed onto the instrument, a fixed, 25mm-long cylindro-conical nozzle with a stop ring mounted on the lower part of the shaft is inserted into the fruit and explores a constant volume of 3 cm^3. The droplets of juice released by the fruit are collected in a graduated beaker placed under the base plate. The Chylofel was used on opposite cheeks, and juice of the fruit in each of the 5 replicates was pooled.

2.6 *Ethylene*

Ethylene production and internal ethylene content were measured before storage (0 weeks) and after 1½ and 3 weeks at -0.5°C, before ethylene treatment. Ethylene production and internal ethylene content were measured after 72 hours at 20°C for control and treated fruit, on removal from 3 weeks at -0.5°C and again after 7 days at 15°C. Four replicates of 2 fruit each were placed in 5 L containers at 20°C to measure ethylene production in the static system. Samples were taken after 1 h. Internal ethylene was extracted from each pear under vacuum. Ethylene was measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian Instrument Group, Palo Alto, California, USA).

2.7 *ACC (1-aminocyclopropane-1-carboxylic acid)*

Peeled fruit disks from each replicate were frozen in liquid nitrogen and stored at -80°C for ACC quantification. The frozen disks were chopped with an onion chopper, and then ground further with a pestle and a mortar, while adding liquid nitrogen. Fifteen mL of an 80% ethanol solution was then added to 3 g of ground frozen pulp and homogenized by Ultraturrax (pp/10, Janke and Kunkel Ika, Staufn, Germany) for 30 seconds. Samples then stood for an hour, whereafter they were centrifuged at 500 g_n for 10 minutes. An aliquot (5 mL) of the supernatant was concentrated in vacuo with a savant (Speedvac

concentrator, SVC, 200H, Farmdale, N.Y.) and used for ACC analysis according to the method of Lizada and Yang (1979).

2.8 *Data analysis*

Data were analyzed using the GLM (General Linear Means), procedure in the SAS (Statistical Analysis System) programme (SAS Institute Inc., 1990). *P*-values in graphs illustrate the significant differences. Significant differences between parameters within cultivars were determined using Fisher's protected least significant difference test with a 95% confidence interval.

3. RESULTS

3.1 *Ground colour and hue angle*

Ground colour for fruit stored for 3 weeks at -0.5°C prior to treatment did not show any change from colour at harvest (Fig. 1). Three days after ethylene treatment, ground colour indicated a slight progression from green to yellow. Untreated fruit only changed from green to yellow after a further 3 weeks at -0.5°C. Ethylene treated fruit were deep yellow (5.0) after the 7 day shelf life simulation at 15°C, whereas control fruit were light yellow (4.0).

Hue angle, an objective measure for ground colour change, was a more sensitive measure (Fig. 2). Differences between treatments were smaller during the storage period, but still significant. The final hue angle after 7 days at 15°C for ethylene treated fruit was 98.1° compared to 102.8° for control fruit.

3.2 *Firmness*

The most pronounced loss in flesh firmness (kg) was noted after the 24 hour ethylene treatment and two further days at 20°C, from the initial 6.0 kg to 2.9 kg (Fig. 3). Control fruit showed a gradual change in firmness after the 72h at 20°C and 3 weeks at -0.5°C.

3.3 *Mealiness and extractable juice content*

Fruit treated with ethylene were 100% mealy when evaluated 48 h after treatment and remained 100% mealy for the remainder of the storage period (Fig. 4). Untreated fruit

also became 100% mealy, but only after another 3 weeks at -0.5°C and 1 week at 15°C. Extractable juice was initially lower in ethylene treated fruit than in control fruit, but both had no juice at the end of the storage period at -0.5°C followed by 7 days at15°C (Fig. 5).

3.4 *TSS and TA*

Total soluble solids content for ethylene treated fruit increased immediately after the treatment and remained at that level for the rest of the storage period at -0.5°C (Fig. 6). After 3 days at 20°C levels of TSS decreased in control fruit but increased after a further 3 weeks storage at -0.5°C. Ethylene treatment had no significant effect on TA (Fig. 7). The interaction between storage period and ethylene treatment was also non significant. Only storage time affected TA significantly, but this effect was small.

3.5 *Ethylene production, internal ethylene and total ACC concentration*

Little to no internal ethylene or ethylene production could be measured during the 3 weeks of storage at -0.5°C prior to the exogenous ethylene treatment (Fig. 8). After exogenous ethylene treatment and a further 3 weeks of storage at -0.5°C, ethylene production rate and internal ethylene concentration reached a maximum (251.76 $\mu L \cdot kg^{-1} \cdot h^{-1}$ and 319.13 $\mu L \cdot L^{-1}$, respectively). Control fruit showed very low levels of ethylene production and internal ethylene even after intermittent warming, and a further 3 weeks at -0.5°C and 7 days at 15°C.

Total ACC concentration was very low (0.75 $nmol \cdot g^{-1}$) directly after the ethylene treatment and 3 days at 20°C (Fig. 8). The same was noticed for control fruit (0.7 $nmol \cdot g^{-1}$). Only after a further 3 weeks at -0.5°C, did ACC concentration increase. The increase was much higher in ethylene treated fruit (175.7 $nmol \cdot g^{-1}$) than in control fruit (26.75 $nmol \cdot g^{-1}$). After the 7 days of ripening at 15°C both treated and control fruit had a lower concentration than on removal from -0.5°C ($\pm$ 3.5 $nmol \cdot g^{-1}$).

4. DISCUSSION AND CONCLUSIONS

Ethylene treatment for 1 day and intermittent warming for 2 days at 20°C influenced ripening of 'Forelle' pears, resulting in very rapid softening. For a preconditioning

treatment to be commercially feasible, fruit must remain green and firm with no risk of bruising during shipping (Chen *et al.*, 1997). Ground colour change from green to yellow could be acceptable for ethylene treated fruit, depending on the shipping and distribution time to the market. However, this ethylene treatment is not feasible for a pre-shipment treatment, as firmness was as low as 2.9 kg after treatment.

Pears placed at room temperature (20°C) immediately after harvest tend to ripen slowly, fail to change colour evenly, and develop a dry texture, with little to no flavour (Mitchell, 1990). The resistance to ripening in pears at ambient temperatures after harvest is related to the synthesis of ACC and ethylene. Storage at low temperatures prior to ripening stimulates ACS and ACO activity necessary for an ethylene climacteric and normal ripening to occur (Lelièvre *et al.*, 1997a). 'Forelle' pears stored for 21 weeks have a better internal qualitythan those stored for 12 weeks (Paper 1). This suggests that the cold requirement of 'Forelle' is longer than 12 weeks, and that the climacteric is not reached within this time frame.

Exogenous ethylene treatment can compensate for an incomplete cold requirement to initiate ripening (Chen and Mellenthin, 1981). This has also been found to improve textural and flavour qualities in pears where the cold requirement has not been satisfied (Mitchell, 1990). In 'Forelle', storage at higher temperatures (4.0 and 7.5°C) led to higher total ACC accumulation and ethylene production, with no mealiness (paper 4). In tomatoes PG has been reported to be ethylene regulated (Sitrit and Bennet, 1998). In nectarines, 1-methylcyclopropene treatment inhibited ACO and polygalacturonase (PG) expression and activity, resulting in woolly fruit after ripening. In contrast to this, ethylene treated fruit were juicy (Dong *et al.*, 2001). Woolliness was also previously related to fruit with low activity of enzymes involved in pectin degradation, e.g. PG (Ben-Arie and Lavee, 1971), which recently is known to be ethylene related (Zhou *et al.* 2000; Dong *et al.*, 2001).

In this study, ethylene treated 'Forelle' also had higher ethylene production rates and total ACC concentration after a total of 6 weeks of storage at -0.5°C. But even so, all the fruit,

both treated and untreated, were mealy. In the ethylene treated fruit, mealiness was observed 3 weeks earlier and was more pronounced than in the untreated control fruit. These fruit however, were of an advanced maturity, which made them more suitable for mealiness assessment than control fruit. The prevention and alleviation of mealiness in temperature treatments (paper 4), did not appear to be the result of higher ACC concentrations at the advanced stage of maturity. However, it is known that the ripening process of pome fruit involves many individual reactions which are not necessarily related to each other but occur more or less in parallel and in time relative to the climacteric (Frenkel *et al.*, 1968). This makes it difficult to draw any conclusion from the ethylene treated fruit since mealiness is only noticed after fruit ripened.

It was thought that ethylene treatment after 3 weeks of cold storage at -0.5°C would be sufficient for an increase in internal ethylene and total ACC concentration, specifically with regard to ACS activity; which Lelièvre *et al.* (1997b) found true for 'Passe-Crassane' pears. In contrast to the rapid change in firmness and mealiness, internal ethylene and ACC concentration were only detectable 3 weeks after storage at -0.5°C. It is possible that the 3 days at 20°C were insufficient time for noticeable increases in ethylene production. However, the ethylene treatment must have stimulated ripening. The decline in flesh firmness and ground colour progression from green to yellow appears to have commenced earlier than increases in internal ethylene. It could also mean that cold storage of 'Forelle' prior to ethylene treatment does not necessarily affect ethylene production in the same way as in 'Passe-Crassane' pears, indicating specificity of each cultivar to the need of a cold storage and ethylene treatment at a certain time. However, this remains to be confirmed in a separate experiment where fruit are treated before and after a cold storage period and then ripened.

In conclusion, 'Forelle' pears stored for 3 weeks at -0.5°C and treated with 100 $\mu L \cdot L^{-1}$ of ethylene (24h, 20°C) and held for a further 48 h at 20°C, served the purpose of reducing the cold storage requirement of 12 weeks to 3 weeks, from a ripening point of view. However, ethylene treated fruit were too soft. Mealiness was high in ethylene treated and untreated fruit. This indicates that internal quality judged by mealiness is influenced by a

factor other than exogenous ethylene treatment, total ACC concentration and internal ethylene production. Although there is a considerable financial motivation for ethylene treatment to attain earlier availability of 'Forelle' pears in the market, this cannot be accomplished at the expense of internal quality.

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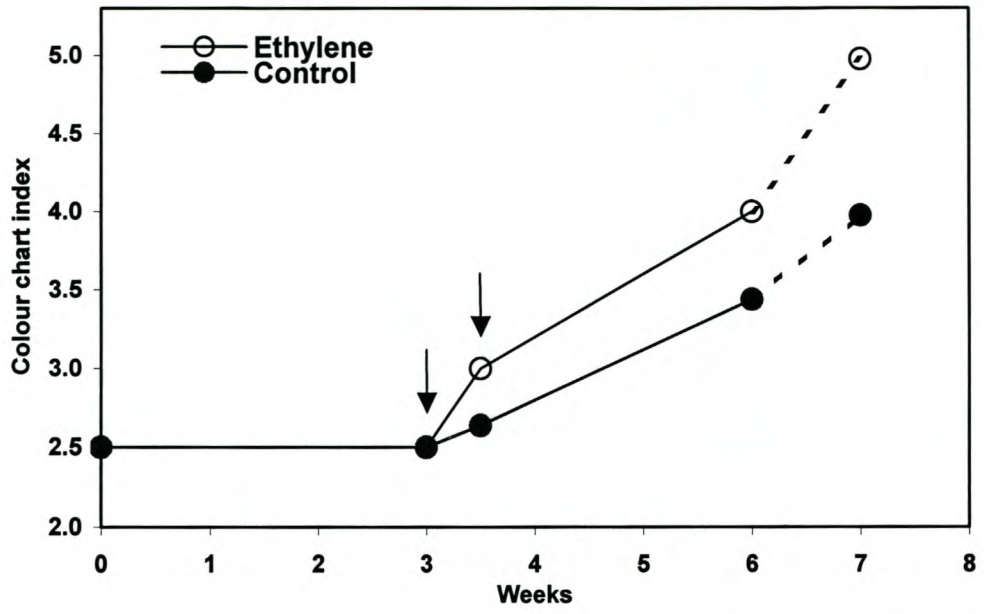

Fig. 1. Colour index of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↓) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↓). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 0.19$) (Dashed lines indicate ripening at 15°C.)

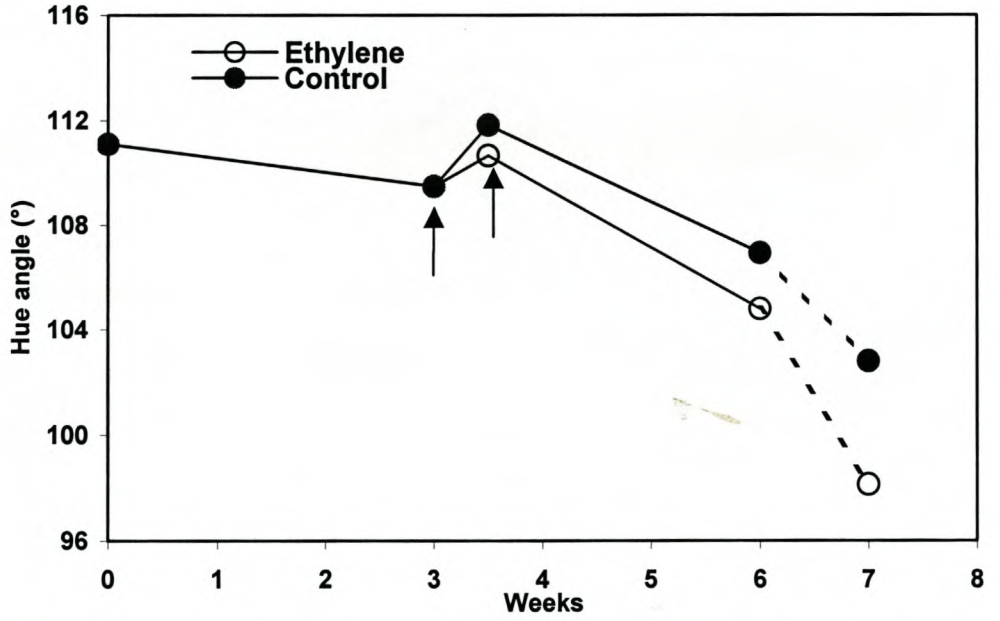

Fig. 2. Hue angle (°) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↑) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↑). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 1.13$) (Dashed lines indicate ripening at 15°C).

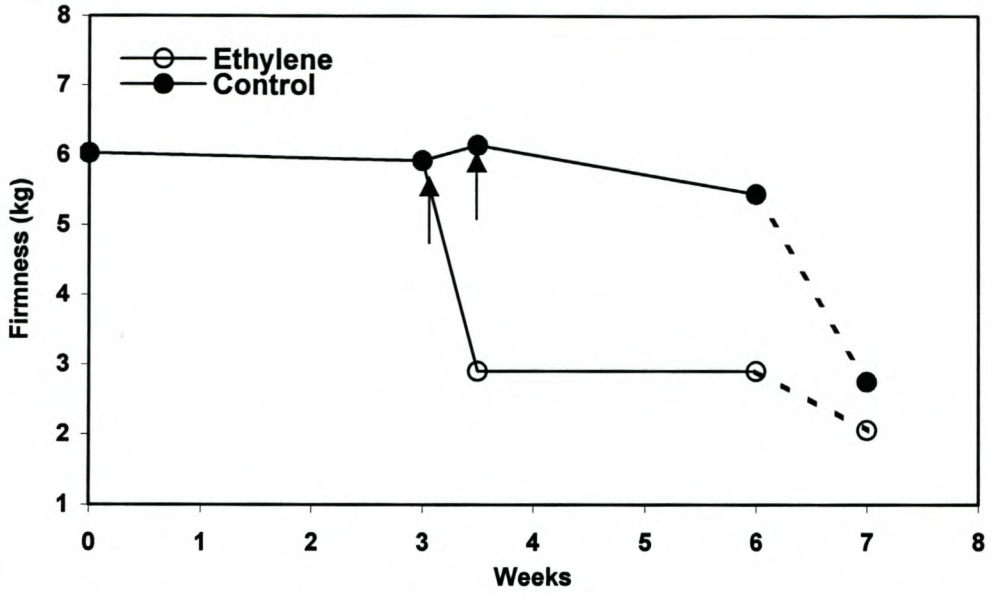

Fig. 3. Firmness (kg) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↑) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↑). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 0.21$) (Dashed lines indicate ripening at 15°C).

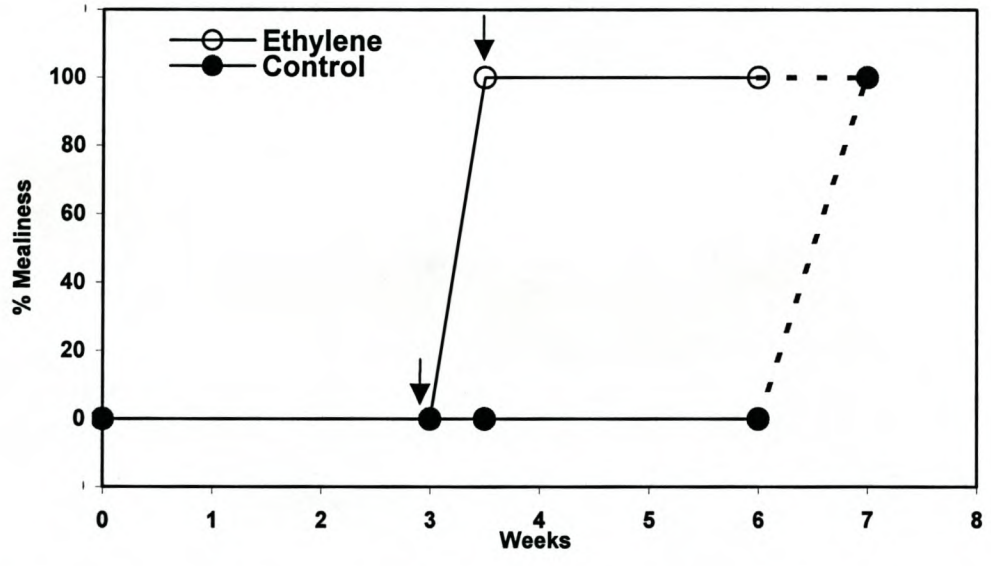

Fig. 4. Mealiness (%) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↓) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↓). Control fruit were stored at same temperatures without the ethylene treatment (logit transformed data) (Dashed lines indicate ripening at 15°C).

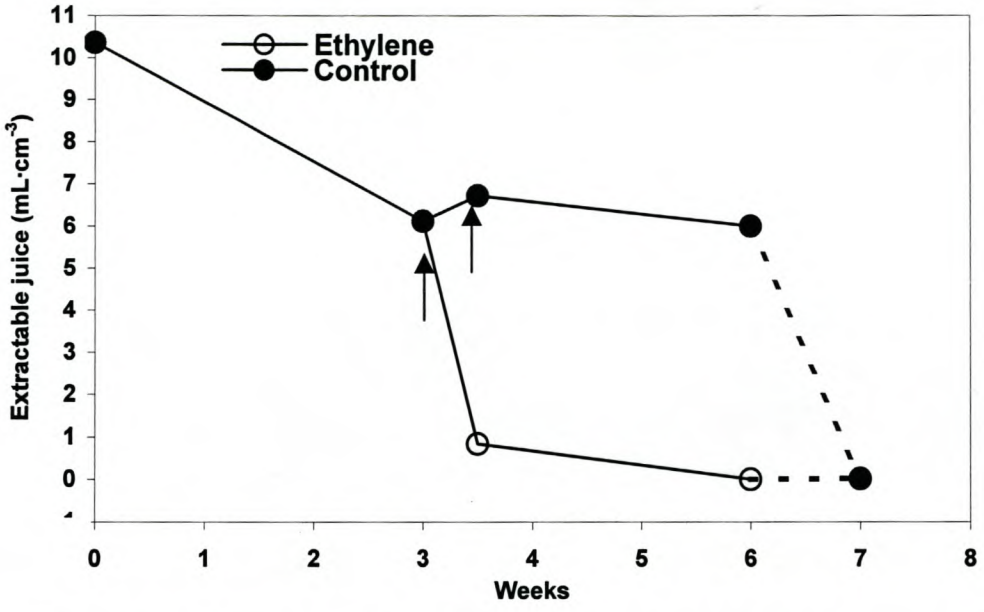

Fig. 5. Extractable juice ($mL \cdot cm^{-3}$) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↑) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↑). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 0.12$) (Dashed lines indicate ripening at 15°C).

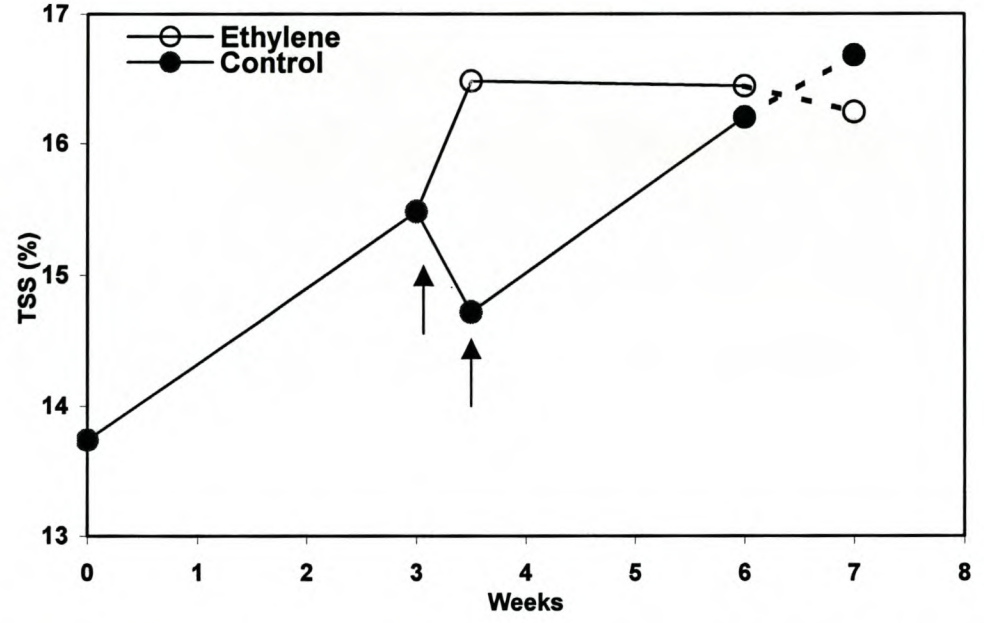

Fig. 6. Total soluble solids (%) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↑) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↑). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 0.95$) (Dashed lines indicate ripening at 15°C).

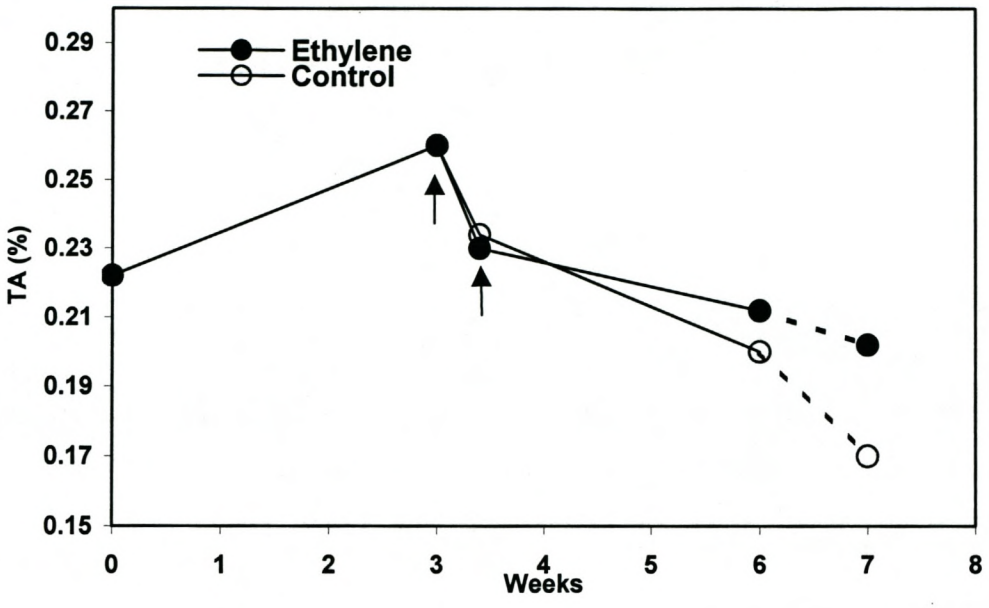

Fig. 7. Total acidity (%) of 'Forelle' pears, treated with ethylene ($100\mu L \cdot L^{-1}$) (↑) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↑). Control fruit were stored at same temperatures without the ethylene treatment ($LSD_{5\%} = 0.03$) (Dashed lines indicate ripening at 15°C).

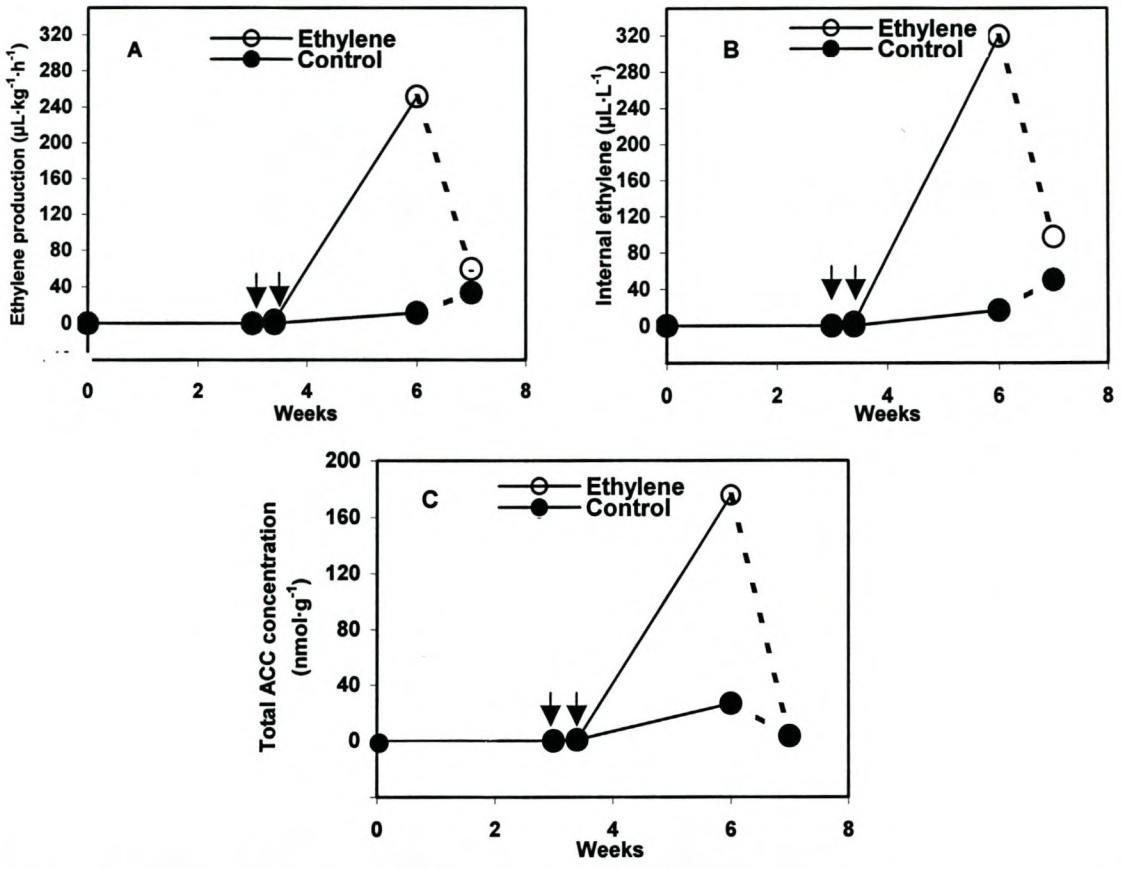

Fig. 8. Ethylene production rate (A), internal ethylene concentration (B) and total ACC concentration (C) of 'Forelle' pears, treated with ethylene (100µL·L^{-1}) (↓) for 24h, followed by 48h at 20°C in air before being returned to -0.5°C for a further 3 weeks (↓). Control fruit were stored at same temperatures without the ethylene treatment (Dashed lines indicate ripening at 15°C) (Ethylene production: $LSD_{5\%}$ = 28.68; Internal ethylene: $LSD_{5\%}$ = 63.04; Total ACC concentration: $LSD_{5\%}$ = 7.27).

CHAPTER 5: PAPER 4

RIPENING OF 'FORELLE' PEARS AS INFLUENCED BY STORAGE TEMPERATURES

ABSTRACT

'Forelle' pears produced in South Africa are prone to mealiness, or low extractable juice content. Mealiness can result from short duration postharvest cold storage, insufficient to induce ethylene biosynthesis and ripening, but may also be the result of a chilling injury. Consequently, the purpose of this study was to determine the role of storage temperature on ripening, and specifically mealiness of 'Forelle'. Fruit harvested from the Elgin area at commercial maturity were stored at -0.5°C, 4.0°C and 7.5°C for 0, 3 and 6 weeks. Samples were removed every third week, placed at 15°C, and maturity indices, juice content, mealiness, total ACC content and ethylene levels were monitored on removal and after 7 days. Flesh firmness of the 4°C-stored fruit was only 0.5 kg lower than fruit stored at -0.5°C on removal from storage. Fruit stored at 4°C and 7.5°C ripened with little to no mealiness (0 and 8% respectively) in contrast to fruit stored at -0.5°C (70% mealy). ACC accumulation and ethylene production were higher after 6 weeks and 7 days at 15°C, for fruit stored at 4°C and 7.5°C than fruit stored at -0.5°C. The higher storage temperatures (4°C and 7.5°C) allow sufficient accumulation of ACC and ethylene production for normal ripening resulting in a juicy, aromatic fruit.

Keywords: (*Pyrus communis* L.), ethylene, 1-aminocyclopropane-1-carboxylic acid, flesh firmness, mealiness.

1. INTRODUCTION

'Forelle' is one of three blushed pear cultivars produced in South Africa. The blushed pear market begins with 'Rosemarie', followed by 'Flamingo' and then 'Forelle'. Discontinuity in the availability of the blushed pears occurs between 'Flamingo' and 'Forelle'. A mandatory minimum cold storage requirement of 12 weeks at -0.5°C prevents 'Forelle' from being marketed earlier (De Vries and Hurndall, 1993). There is a strong financial incentive to reduce the 12 week cold storage requirement of 'Forelle' since earlier marketing can result in prices in excess of 50% more per carton,

and this incentive has directed research in recent years. Intermittent warming treatments (De Vries and Hurndall, 1993), controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals (De Vries and Hurndall, 1994; De Vries and Moelich, 1995), and ethylene treatments (Du Toit *et al.*, 2001), have all been tested as a means of reducing storage period. Ethylene treatment successfully reduced storage period, if judged by colour and firmness, but was not able to improve the internal quality.

A good quality pear should ripen with a juicy texture. Most winter pears, and in particular 'Forelle', do not ripen normally until they have been exposed to a critical period of cold storage (Hansen, 1961; Sfakiotakis and Dilley, 1974; Mellenthin and Wang, 1976; Puig *et al.*, 1996, De Vries, 2001). Cold storage prior to ripening of pears causes an accumulation of ACC, the precursor to ethylene (Wang *et al.*, 1985), to the point where the ripening resistance is reduced and expression of autocatalytic ethylene results in normal ripening (Lelièvre *et al.*, 1997a).

Mealiness, a disorder characterised by low extractable juice with low firmness, develops in 'Forelle' pears as they ripen and can reach unacceptably high levels (> 50%). This internal disorder reaches a maximum after about 6 weeks of storage at -0.5°C, during ripening, and gradually decreases as the storage period increases. 'Forelle' reaches an ethylene climacteric of 59.44 $\mu L.kg^{-1}.h^{-1}$ after 12 weeks of storage at -0.5°C (Paper 1). Although this stage is known to have the potential for maximum taste and quality (Beaudry and Ferenczi, 2001; Lelièvre *et al.*, 1997b; Wang *et al.*,1985), 'Forelle' still ripened unevenly, with little extractable juice (mealy) and no characteristic aroma or flavour.

Mealiness in 'Forelle' appears to be similar to woolliness in peaches and nectarines. In contrast to the cold requirement for ripening, woolliness in nectarines occurs as a consequence of the inactivation of enzymes like polygalacturonase (PG), involved in solubilization of polyuronides, and is attributed to a chilling injury (Ben-Arie and Lavee, 1971). Inactivation of ACC-oxidase and suppression of ethylene production in nectarines by chilling temperatures was recently shown by Zhou *et al.* (2000a) in nectarines.

The aim of the study was to determine the role of cold storage on 'Forelle' pear ripening with the hypothesis that higher storage temperatures will result in a reduction of storage time and an improvement in internal quality, especially with regard to mealiness.

2. MATERIALS AND METHODS

2.1 *Fruit source*

'Forelle' pears *(Pyrus communis* L.*)* sourced in the Theewaterskloof area (33°15'S; 19°15'W), Western Cape, S.A., were harvested at a commercial maturity with an average flesh firmness of 6.0 kg and total soluble solids of 13.7 %. Ground colour, measured with a Unifruco colour chart for pears, was 2.5 (where 0.5 = dark green, 5 = deep yellow).

2.2 *Experimental layout*

Forty-eight fruit were sampled for initial maturity assessment at harvest. The remainder of the fruit were stored for 3 and 6 weeks at -0.5°C, 4°C and 7.5°C (192 fruit per storage temperature). Forty-eight fruit were used for maturity indexing after cold storage and again after ripening at 15°C for 7 days. Each sampling date consisted of 5 replicates of 8 fruit per replicate. A further 8 fruit were used to determine ethylene production rates and internal ethylene and total ACC content.

2.3 *Maturity indices*

Ground colour changes from green to yellow, were measured as hue angle (h°) using a colorimeter (Nippon Denshoku Model HR-3000, Tokyo, Japan) and a Unifruco colour chart for pears (where 0.5 = dark green, 5 = deep yellow). Flesh firmness (kg) was measured using a penetrometer (Southtrade fruit pressure tester, model FT 327, Alphonsine, Italy) fitted with an 8 mm probe. Total soluble solids (TSS) of a pooled juice sample of each replicate was measured by a hand held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Titratable acidity (TA) was calculated as % malic acid, by titrating the pooled juice sample with 0.1 N NaOH to a pH of 8.2, using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland).

2.4 *Mealiness*

Mealiness was determined on each fruit subjectively. Fruit were cut equatorially and squeezed. Fruit with a dry, floury texture were classified as mealy.

2.5 *Extractable juice*

Extractable juice ($mL \cdot cm^{-3}$) was measured with a Chylofel (COPA -Technologie S.A., St. Etienne du Gress, France) which best estimates release of juice on chewing. The instrument consists of a spring-mounted base, which is mobile along the vertical axis. The peeled fruit is placed on this base. When fruit is pressed into the instrument, a fixed, 25 mm cylindro-conical nozzle with a stop ring mounted on the lower part of the shaft is inserted into the fruit and explores a constant volume of 3 cm^3. The droplets of juice released by the fruit are collected in a graduated beaker placed under the base plate. The Chylofel was used on opposite cheeks, and juice of the fruit in each of the 5 replicates was pooled.

2.6 *Ethylene*

Ethylene production and internal ethylene content were measured before storage (0 weeks) and after 3 and 6 weeks at -0.5°, 4°C and 7.5°C, and again after ripening at 15°C for 7 days. Four replicates of 2 fruit each were placed in 5 L containers at 20°C to measure ethylene production in the static system. Samples were taken after 1 h. Internal ethylene was extracted from each pear under vacuum. Ethylene was measured with a flame ionization gas chromatograph (Varian, Model 3300, Varian Instrument Group, Palo Alto, California, USA).

2.7 *ACC (1-aminocyclopropane-1-carboxylic acid)*

Peeled fruit disks from each replicate were frozen in liquid nitrogen and stored at -80°C for ACC quantification. The frozen disks were chopped with an onion chopper, and then ground further with a pestle and a mortar while adding liquid nitrogen. Fifteen mL of an 80% ethanol solution was then added to 3 g of ground frozen pulp and homogenized by ultra Turrex (model pp/10, Janke and Kunkel Ika, Staufn, Germany), for 30 seconds. Samples then stood for an hour, whereafter they were centrifuged at 500 g_n for 10 minutes. An aliquot (5 mL) of the supernatant was concentrated in vacuo with a savant (Speedvac concentrator, SVC, 200H, Farmdale, N.Y.) and used for ACC analysis according to the method of Lizada and Yang (1979).

2.8 *Data analysis*

Data were analyzed using the GLM (General Linear Means) procedure in the SAS (Statistical Analysis System) programme (SAS Institute Inc., 1990). ANOVA-generated *P*-values illustrate the significant differences. Significant differences between parameters within cultivars were determined using Fisher's protected least significant difference test with a 95% confidence interval.

3. RESULTS

3.1 *Ground colour and hue angle*

Ground colour for fruit stored at -0.5°C changed from 2.6 colour index to 2.8 colour index during the 6 week storage period (Fig. 1). After 7 days at 15°C the ground colour for fruit stored for 3 and 6 weeks at -0.5°C changed from 2.6 and 2.8 colour index, respectively to a 3.1 and 3.8 colour index, respectively. Colour of fruit stored at 4°C changed from the initial colour index value of 2.6 to 3.1 after 3 weeks of storage and remained the same after a further 3 weeks at 4°C. Ground colour for fruit stored at 7.5°C changed from green (2.6) to light green (3.0) after 3 weeks, and to a maximum of 5.0 after 6 weeks of storage.

Hue angle is an objective measure for ground colour. However, unlike colour chart indices, small differences in colour during storage were observed. While the hue angle for fruit stored at -0.5°C did not change after 3 weeks of storage (110.6°), a significant difference was measured after 6 weeks of storage (108.0°) (Fig.2). Hue angle of fruit stored at 4°C and at 7.5°C was significantly lower after 3 weeks of storage. After 6 weeks at 7.5°C and a further 7 days at 15°C, the dark yellow fruit had hue angles of 97.9° and 88.0°, respectively.

3.2 *Flesh firmness*

The loss in flesh firmness (kg) was most rapid for fruit stored at 7.5°C (Fig. 3). After 3 weeks at 7.5°C flesh firmness was 5.9 kg, and a further decline to 2.6 kg was measured after 7 days at 15°C. Flesh firmness after 6 weeks at 7.5°C, was 2.2 kg. Fruit stored at 4°C and -0.5°C only showed a significant drop in flesh firmness after 6 weeks at -0.5°C followed by 7 days at 15°C.

3.3 *Mealiness and extractable juice*

Fruit stored at 4°C and 7.5°C for 6 weeks ripened at 15°C for 7 days with little to no mealiness in contrast to fruit stored at -0.5°C, which was 70% mealy after storage and ripening (Fig. 4). Extractable juice ($mL \cdot cm^{-3}$) was lowest after 6 weeks at -0.5°C followed by 7 days at 15°C, and decreased from 3.5 $mL \cdot cm^{-3}$ at the beginning of storage to 0.6 $mL.cm^{-3}$ after 7 days at 15°C (Fig. 5). A similar decrease was noted for fruit stored for 6 weeks at 7.5°C which reached a minimum of 0.5 $mL \cdot cm^{-3}$ at the end of storage followed by 7 days at 15°C. After 6 weeks of storage at 4°C, and ripening for 7 days at 15°C fruit exhibited about 133% more extractable juice than fruit stored at -0.5°C and 7.5°C, and examined at the same time.

3.4 *TSS and TA*

The total soluble solids (%) of fruit stored for 3 weeks at 7.5°C increased from 13.7% to 16.1% (Fig. 6). Fruit stored at -0.5°C did not show any changes in TSS during this period but increased after 6 weeks in storage to 15.2 %, and after 7 days at 15°C to 16.0%. The change in TSS of fruit stored at 4°C was more rapid during the first 3 weeks of storage (13.7 % to 15.7%) than fruit stored at -0.5°C. After 6 weeks of storage at 4°C, TSS reached a maximum of 16.6%. Titratable acidity (%) for fruit stored at -0.5°C decreased from an initial value of 0.22% to 0.19% after 6 weeks of storage followed by 7 days at 15°C (Fig. 7). No significant change in TA was noted during the storage period at 4°C. For fruit stored at 7.5°C, TA decreased to 0.16% after 6 weeks of storage followed by 7 days at 15°C.

3.5 *Ethylene production, internal ethylene and total ACC concentration*

Ethylene production rate was highest for fruit stored at 7.5°C for 6 weeks (216.1 $\mu L \cdot kg^{-1} \cdot h^{-1}$). Ethylene production in fruit stored at -0.5°C and 4°C was delayed in comparison to fruit stored at 7.5°C (Fig. 8). After 6 weeks of storage and 7 days at 15°C, ethylene production rate of the 4°C stored fruit increased to 30.9 $\mu L \cdot kg^{-1} \cdot h^{-1}$, which was significantly higher than for fruit stored at -0.5°C (13.4 $\mu L \cdot kg^{-1} \cdot h^{-1}$), and significantly lower than for fruit stored at 7.5°C (61.10 $\mu L \cdot kg^{-1} \cdot h^{-1}$). Even after ripening, fruit stored at 4°C and -0.5°C, did not reach the high levels in ethylene production rate than measured during storage at 7.5°C.

Internal ethylene concentration was very low for fruit stored at -0.5°C and 4°C. Fruit stored at 7.5°C however, reached a maximum after 6 weeks (466.7 $\mu L.L^{-1}$), but decreased after storage at 15°C (Fig. 9). Internal ethylene for fruit stored at 4°C increased from 0 $\mu L \cdot L^{-1}$ before storage to 136.9 $\mu L \cdot L^{-1}$ after 6 weeks at 4°C followed by 7 days at 15°C, which was significantly higher (5.49 $\mu L \cdot L^{-1}$) than that of fruit stored at -0.5°C for the same time.

The change in total ACC concentration ($nmol \cdot g^{-1}$) was significant for the storage temperature as main effect (Fig. 10). Fruit stored at 7.5°C and 4°C had a higher total ACC content than fruit stored at -0.5°C. The total ACC concentration for fruit stored for 6 weeks was significantly higher than for fruit stored for 3 weeks (storage time main effect). Fruit stored for 6 weeks (storage time main effect) had a significantly higher total ACC concentration than fruit stored for 6 weeks followed by 7 days at 15°C.

4. DISCUSSION AND CONCLUSIONS

Storage temperature influenced skin colour, firmness, mealiness, TSS, ethylene production rates and total ACC concentration of 'Forelle' pears. Fruit stored for 6 weeks at -0.5°C were only 0.5 kg firmer than fruit stored at 4°C. Marked losses in firmness for fruit stored at these temperatures occurred only after transfer to 15°C, whereas fruit stored at 7.5°C softened considerably after only 3 weeks of storage followed by 7 days at 15°C. Fruit stored at the higher temperatures had little to no mealiness, whereas fruit stored at -0.5°C were 70% mealy. The 4°C storage temperature has the potential to reduce the duration of the cold requirement, with the added benefit of good internal quality and no marked loss in firmness during storage.

Flesh firmness decreased at the same time that ethylene production increased for fruit stored at all temperatures and also after 3 weeks of storage at -0.5°C (paper 1). This was also observed when 'Forelle' pears were treated with exogenous ethylene before storage (Du Toit *et al.,* 2001). Therefore, loss in flesh firmness in 'Forelle' appears to be an ethylene dependent process.

The onset of ethylene production was earlier for fruit stored at the higher temperature of 7.5°C. Fruit stored at -0.5°C did not have the ability to produce high levels of

ethylene, and internal content was low. A similar trend was observed by Sfakiotakis and Dilley (1974), who reported that 5°C and 7.5°C for seven days in preclimacteric 'Bosc' pears, were more effective than 0°C in developing competency to produce ethylene. This higher ethylene production rate was reflected in the total ACC concentration. The higher storage temperatures (4°C and 7.5°C) promoted ACC accumulation and conversion of ACC to ethylene.

Ethylene production for fruit stored at 7.5°C, declined after 7 days at 15°C, whereas ethylene production for 4°C and -0.5°C stored fruit increased at this point. The climacteric of the 7.5°C stored fruit was thus reached earlier than in the fruit stored at 4°C and -0.5°C. The increase in the ethylene production rate towards a climacteric, was still taking place after 7 days at 15°C. This could explain why the ethylene production rate of fruit stored at these lower temperatures (4°C and -0.5°C), had not reached the same levels of ethylene than fruit stored at 7.5°C, during this storage period.

The influence of higher total ACC concentration and ethylene production on mealiness of 'Forelle' is not known. However, in nectarines, higher levels of ACC oxidase and ethylene activate the enzymatic protein regulating translation of polygalacturonase (PG). Polygalacturonase inactivation at low temperatures leads to impaired pectin degradation, which in turn leads to woolly fruit e.g. low extractable juice in nectarines. This is a clear indication of chilling injury (Ben-Arie and Lavee, 1971; Zhou *et al.*, 2000b). In pears, PG also plays a key role in the degradation of pectin during ripening (Ben-Arie and Kislev, 1979; Bartley *et al.*, 1982). However, unlike in nectarines where woolliness was alleviated by the application of exogenous ethylene (Dong *et al.*, 2001), exogenous ethylene promoted ripening in 'Forelle' and as a consequence, mealiness could be observed earlier than in untreated fruit (Paper 3). Mealiness was thus induced during storage, before ethylene treatment, and the disorder is not reversed by exogenous ethylene. It appears that ACC accumulation at higher temperatures is thus not related to mealiness. Mealiness may rather be the result of a physical effect of chilling injury or temperature on pectin degradation in the ripening process of 'Forelle' pears.

Another interesting result of the study is that 'Forelle' stored at 4°C and 7.5°C for 6 weeks and ripened at 15°C for 7 days, had a better aroma and flavour than fruit stored at -0.5°C (personal observation). It is possible that the threshold ethylene concentration necessary for optimal development of aroma and flavour was reached at these higher temperatures.

In conclusion, fruit stored at 7.5°C ripened after 3 weeks of storage followed by 7 days at 15°C; and fruit stored at 4°C and -0.5°C ripened after 6 weeks of storage followed by 7 days at 15°C. Fruit stored at the higher temperatures had little to no mealiness compared to fruit stored at -0.5°C, indicating that 'Forelle' pears may exhibit mealiness as a result of a chilling injury.

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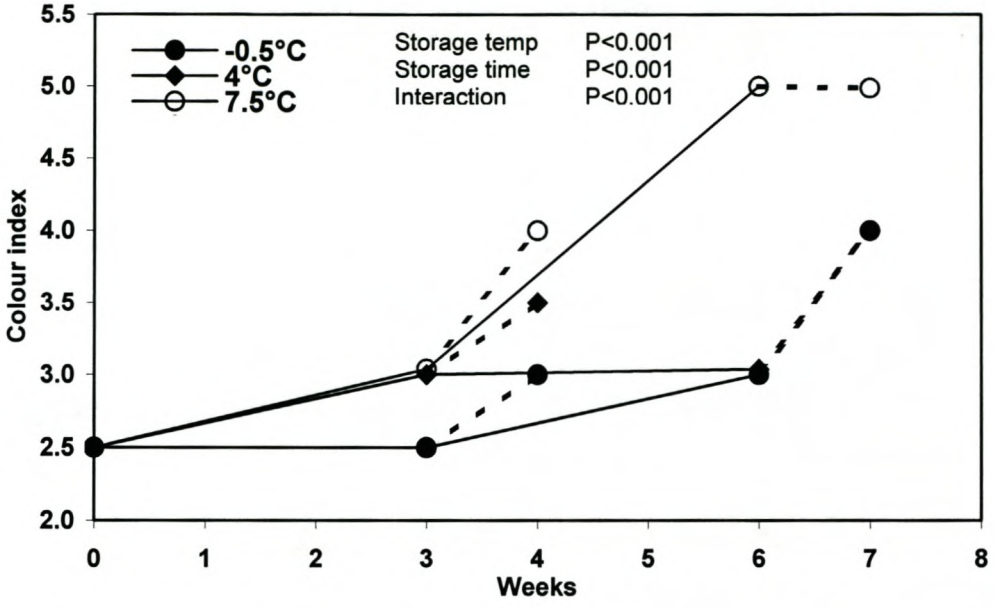

Fig. 1. Colour index (Unifruco colour chart) of 'Forelle' pears stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 0.03) (dashed lines indicating days at 15°C).

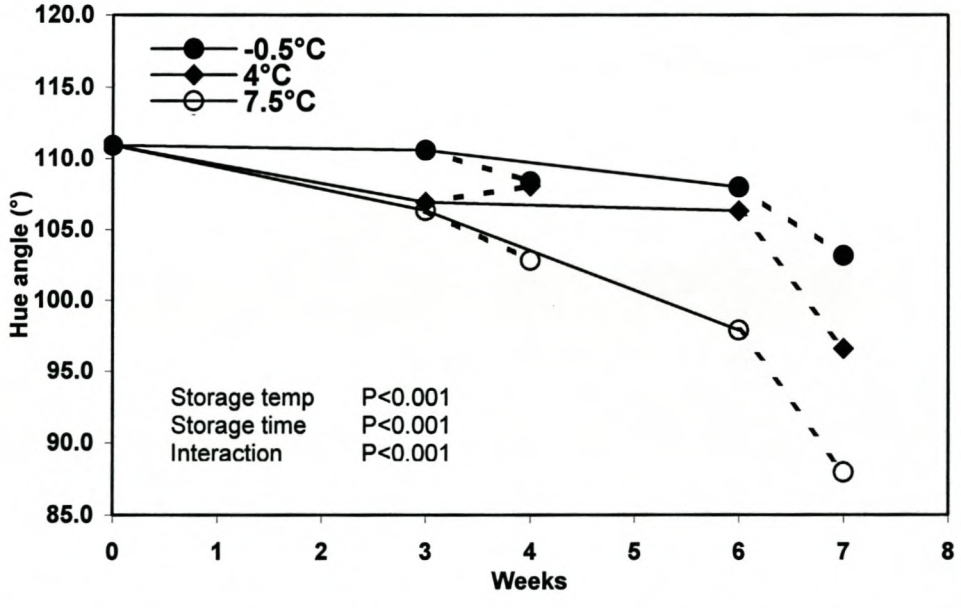

Fig. 2. Hue angle (°) of 'Forelle' pears stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 1.38) (dashed lines indicating days at 15°C).

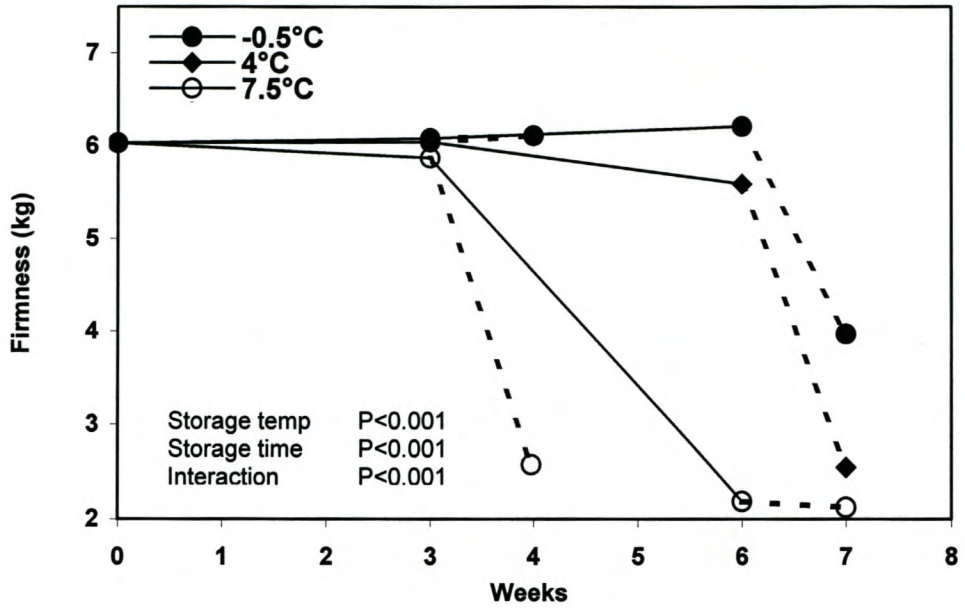

Fig. 3. Firmness (kg) of 'Forelle' pears stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 0.29) (dashed lines indicating days at 15°C).

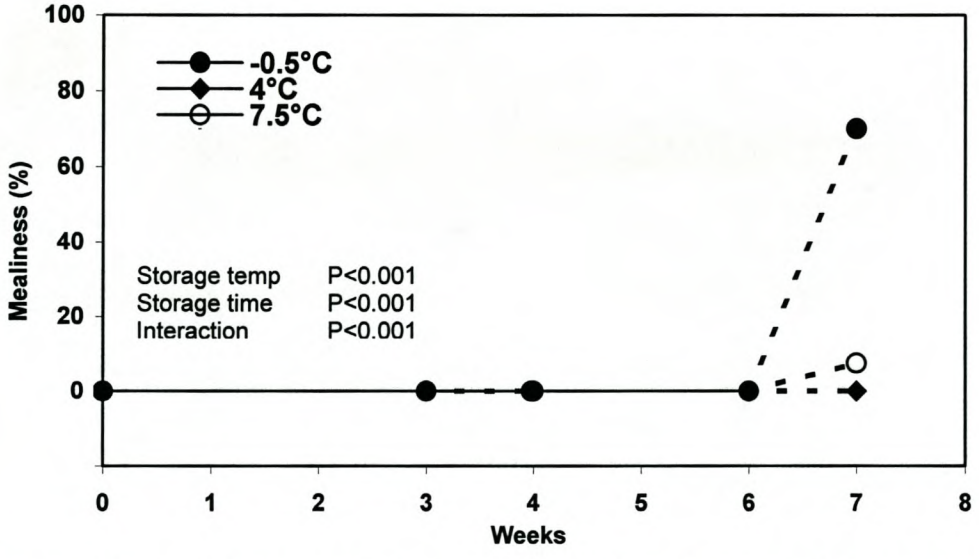

Fig 4. Mealiness (%) of 'Forelle' pears sourced from Theewaterskloof area in the 2001 season and stored at -0.5°C, 4°C and 7.5°C (dashed lines indicating days at 15°C) (Logit transformed data).

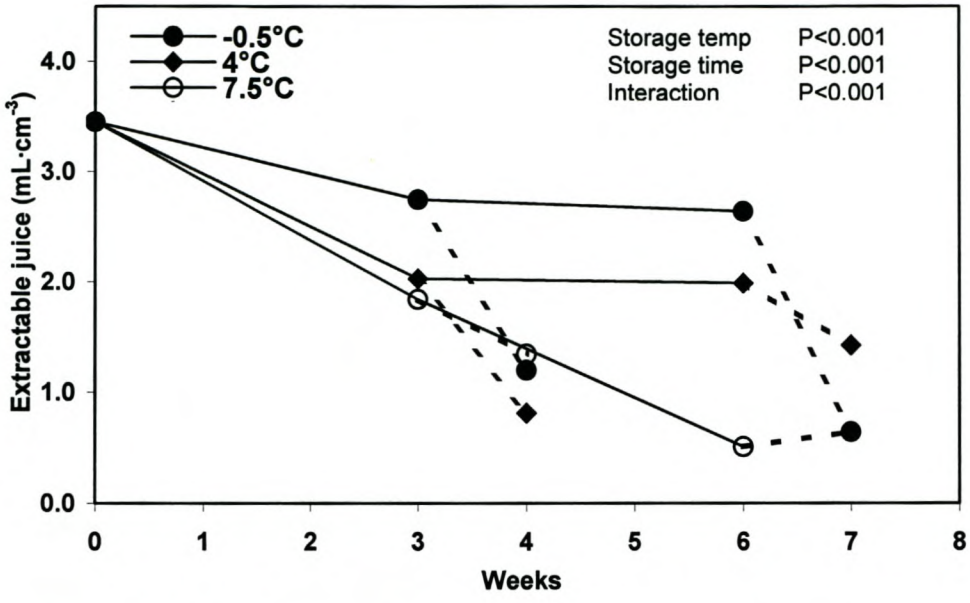

Fig. 5. Extractable juice ($mL \cdot cm^{-3}$) of 'Forelle' pears sourced from Theewaterskloof area in the 2001 season and stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%} = 0.42$) (dashed lines indicating days at 15°C).

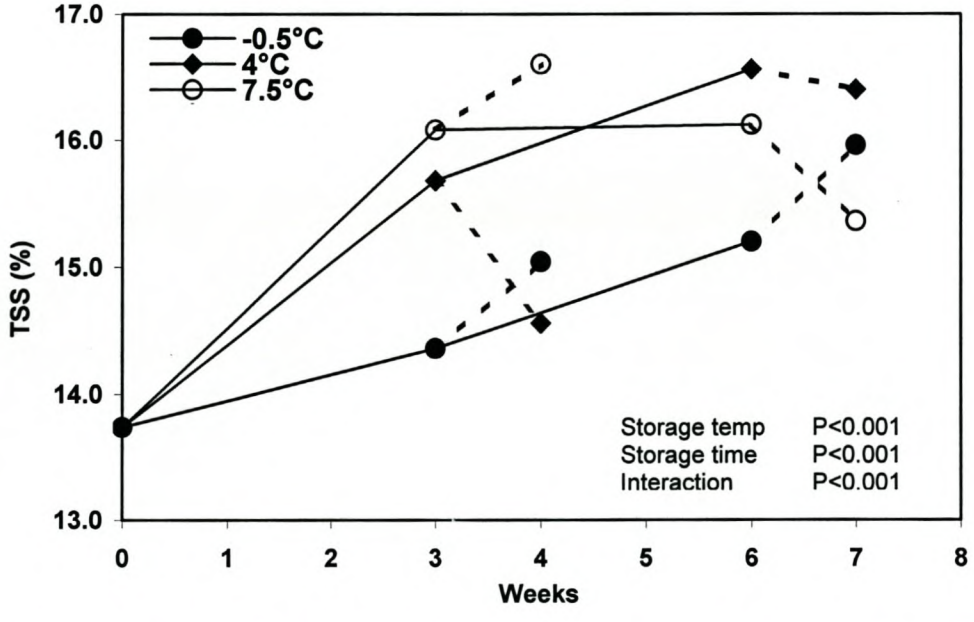

Fig. 6. Total soluble solids (%) of 'Forelle' pears stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%} = 0.65$) (dashed lines indicating days at 15°C).

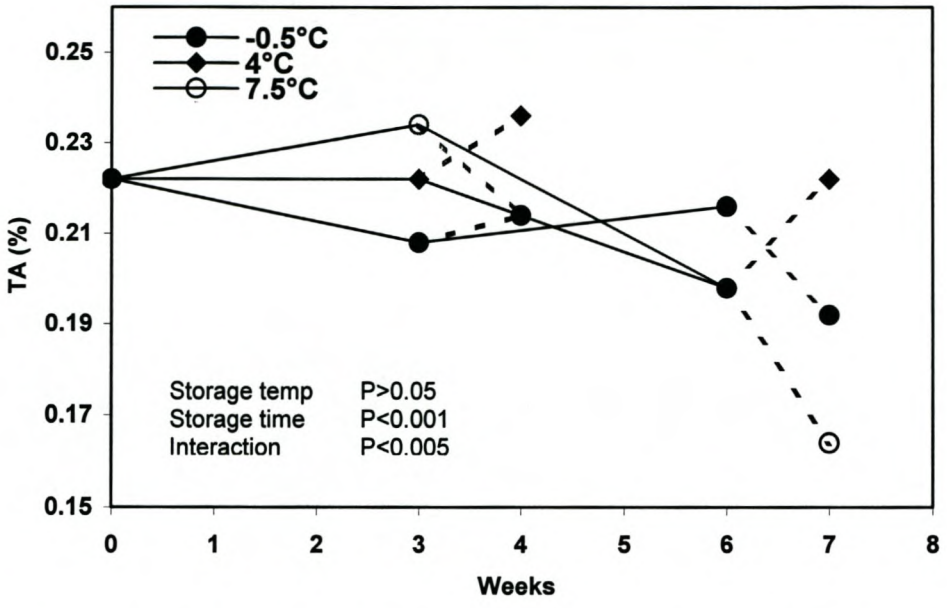

Fig. 7. Titratable acidity (%) of 'Forelle' pears stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 0.025) (dashed lines indicating days at 15°C).

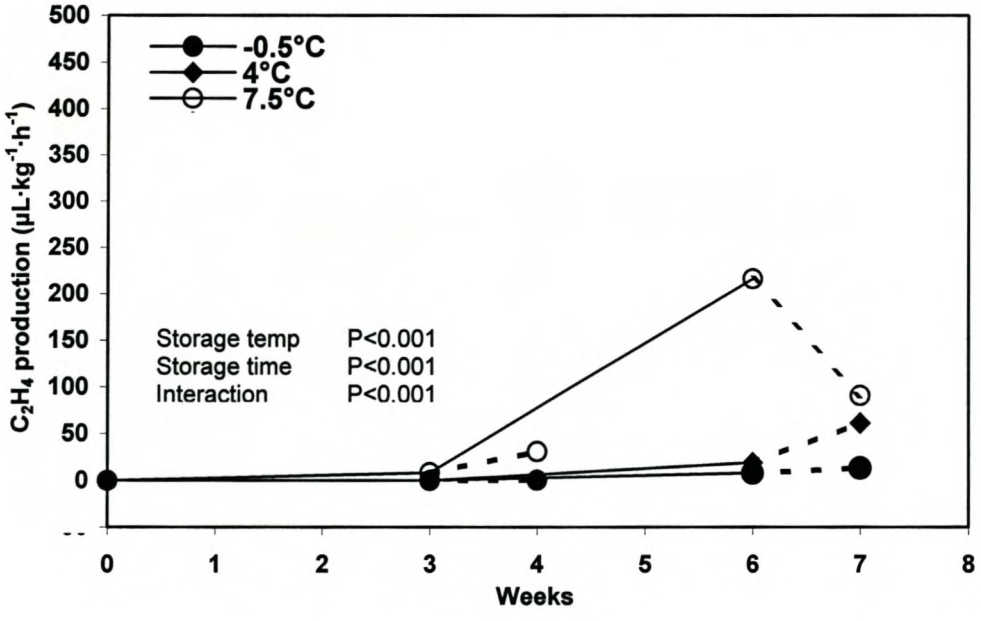

Fig. 8. Ethylene production rate ($\mu L \cdot kg^{-1} \cdot h^{-1}$) of 'Forelle' pears sourced from Theewaterskloof area in the 2001 season and stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 13.64) (dashed lines indicating days at 15°C).

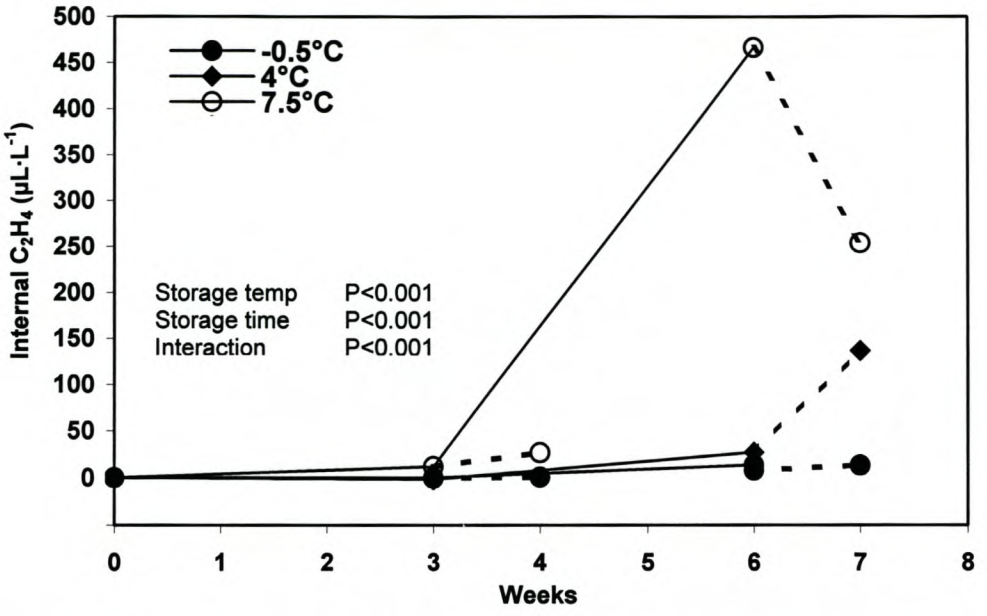

Fig. 9. Internal ethylene (µ L·L^{-1}) of 'Forelle' pears sourced from Theewaterskloof area in the 2001 season and stored at -0.5°C, 4°C and 7.5°C ($LSD_{5\%}$ = 68.25) (dashed lines indicating days at 15°C).

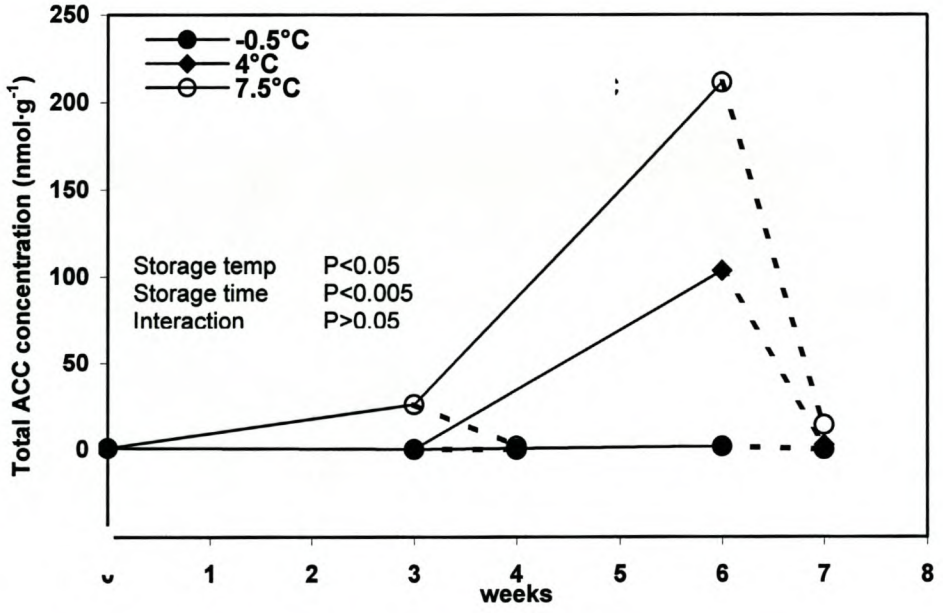

Fig. 10. Total ACC concentration (nmol·g^{-1}) of 'Forelle' pears sourced from Theewaterskloof area in the 2001 season and stored at -0.5°C, 4°C and 7.5°C (Main effect temperature LSD = 39.43) (Main effect storage time $LSD_{5\%}$ = 50.9) (dashed lines indicating days at 15°C).

GENERAL DISCUSSION AND CONCLUSION

This study investigated the effects of storage time (at -0.5°C), harvest maturity, exogenous ethylene application and storage temperatures on ripening and mealiness development in 'Forelle' pears, with the aim of understanding the ripening physiology of 'Forelle', and to reduce the mandatory 12 week cold storage period at -0.5°C to 6 weeks without compromising fruit quality.

'Forelle' pears harvested in the Warm Bokkeveld and the Theewaterskloof areas in the 2000 season, showed the same trend in the development of mealiness during prolonged storage at -0.5°C. Fruit from the Theewaterskloof area had a lower incidence of mealiness. The maximum incidence of mealiness for fruit from the Warm Bokkeveld and Theewaterskloof area was measured after 6 and 7 weeks at -0.5°C, respectively, and started declining after 12 and 15 weeks at -0.5°C, respectively, but never disappearing during the duration of this trial.

Mealiness levels in fruit from the Warm Bokkeveld, in the 1999 season, were much lower (maximum 76%), with a decline apparent after 3 weeks of cold storage (Crouch, 2000). This was not the case for either of the two areas studied in the 2000 season. Mealiness percentage should therefore not be thought of as a definitive value, since seasonal fluctuations in climate influence the incidence in mealiness in each area. Results from this study suggest that the longer the storage period at -0.5°C, the less the incidence of mealiness in 'Forelle' pears. The same conclusion was made by De Vries and Hurndall (1993). Further work is needed to understand seasonal influences on mealiness, before the mandatory 12 week storage period at -0.5°C is revised.

Development of mealiness during ripening at 15°C followed the same pattern as in fruit that were stored at -0.5°C. Crisp fruit did not show mealiness. However, once the fruit were softer, mealiness developed to a maximum. In the 2000 season this occurred between 6 to 9 days at 15°C. Mealiness declined after excessive ripening (11 days at -0.5°C). This pattern was also noticed in woolly nectarines, and was related to pectin gels that decreased in viscosity and released juice again (Von Mollendorf and De Villiers, 1988).

Minimum ethylene production measured for 'Forelle' after storage at -0.5°C was 25-32 $\mu L \cdot kg^{-1} \cdot h^{-1}$. This production rate was still relatively high compared to other winter pears; e.g. 'd'Anjou' peak in ethylene production after storage at -0.5°C at these ethylene levels (Chen *et al.,* 1983; Chen *et al.,* 1997; Gerasopoulos and Richardson, 1997). Although the threshold ethylene concentration for certain ripening processes in 'Forelle' is not yet known, the ethylene production rates recorded in this study seemed sufficient for ripening to occur after 6 weeks at -0.5°C.

The effect of harvest maturity on the development of mealiness was investigated. Fruit sampled at a range of harvest maturities were all found to exhibit mealiness regardless of maturity at harvest, except for the commercial harvest where contamination with 1-MCP resulted in non ripened fruit. Fruit harvested 2 weeks prior to commercial harvest (week 8) were 0.7 kg softer at harvest, had a higher total ACC concentration and higher ethylene production rate than later harvested fruit and had the potential to ripen. Agar *et al.* (1999) described the pre-mature ripening of pears on the tree as the result of cool temperatures causing accumulation of ACC which stimulates ethylene production. This might be the case for the first harvest date. The conversion of ACC to ethylene for fruit of the later harvest maturities might have been inhibited by day temperatures higher than ± 38°C (Yu *et al.*, 1980; Lurie *et al.*, 1996), which is regularly reached in the orchard at that time of the season.

Although a decrease in mealiness was noted in fruit harvested 2 weeks after commercial harvest (week 14), this appeared to be a function of the maturity (high flesh firmness) of the fruit at the time of mealiness assessment, which made mealiness impossible to measure. The results suggest that factor(s), other than harvest maturity, play a more important role in the initiation and development of mealiness in 'Forelle' pears.

Exogenous ethylene treatment on fruit is known to stimulate fruit ripening. 'Forelle' pears were treated with ethylene in an attempt to initiate early ripening and reduce the development of mealiness during ripening. Treatment of 'Forelle' pears, previously stored for 3 weeks at -0.5°C, with 100 $\mu L \cdot L^{-1}$ of ethylene (24h, 20°C) and held for a

further 48 h at 20°C, served the purpose of reducing the cold storage requirement of 12 weeks to 3 weeks from a fruit ripening point of view, but were mealy. In addition, ethylene treated fruit were too soft for marketing purposes. Ethylene treated fruit also had a higher total ACC concentration and an increased rate of ethylene production after a total of 6 weeks of storage at -0.5°C. However, all the fruit, both treated and untreated, were mealy. In the ethylene treated fruit, mealiness was observed 3 weeks earlier than in the control fruit. Ethylene treated fruit were of a more advanced maturity, which made it possible to see mealiness earlier than in untreated fruit. Although there is a considerable financial motivation for ethylene treatment to attain earlier availability of 'Forelle' pears in the market, this must not be accomplished at the expense of internal quality.

Polygalacturonase (PG) inactivation at low temperature leads to impaired pectin degradation, which in turn leads to woolly fruit e.g. low extractable juice in nectarines. This is a clear indication of chilling injury (Ben-Arie and Lavee, 1971; Zhou *et al.*, 2000). Mealiness is also a textural disorder that is characterized by low extractable juice. Therefore, the effect of temperatures (-0.5°C, 4°C and 7.5°C) on the levels of mealiness in 'Forelle' were examined. Fruit stored at 4°C and 7.5°C compared to -0.5°C, posed some interesting results with regard to ripening, total ACC accumulation, and development of mealiness. Flesh firmness of the 4°C stored fruit was only 0.5 kg lower than fruit stored at -0.5°C, on removal from storage. Fruit stored at 4°C and 7.5°C ripened with little to no mealiness (0 and 8% respectively) in contrast to fruit stored at -0.5°C (70% mealy). Total ACC accumulation and ethylene production were higher for fruit stored at 4°C and 7.5°C than fruit stored at -0.5°C. The higher storage temperatures (4°C and 7.5°C) resulted in fruit ripening with a juicy texture, and no astringency. The higher the temperature, the higher the total ACC concentration measured after storage. When fruit were transferred to 15°C, ACC concentrations declined again.

The influence of higher ACC concentration and ethylene production on the development of mealiness of 'Forelle' is not known. However, in nectarines higher ACC oxidase and ethylene levels activate the enzymatic protein regulating translation of PG. As previously mentioned, PG inactivation at low temperature leads to impaired pectin degradation, which in turn leads to woolly fruit e.g. low extractable juice in

nectarines which is a clear indication of chilling injury (Ben-Arie and Lavee, 1971; Zhou *et al.*, 2000).

In pears, PG is also known to play a key role in the degradation of pectin during ripening (Ben-Arie and Kislev, 1979; Bartley *et al.*, 1982). However, unlike in nectarines where woolliness was alleviated (Dong *et al.*, 2001), high total ACC levels and ethylene production after ethylene treatment promoted ripening of 'Forelle' pears, but did not prevent the development of mealiness (Paper 3). Mealiness might already have been induced preharvest or during the 3 weeks of cold storage prior to ethylene treatment, and the disorder could not be alleviated, by exogenous ethylene. Therefore, the prevention and alleviation of mealiness through temperature manipulation treatments (Paper 4), did not appear to be a result of the higher total ACC concentration.

Although higher postharvest storage temperature reduced the development of mealiness in 'Forelle', more research is needed to explain these findings on a basic level, to understand the underlying mechanism of action of mealiness development. Postharvest temperature treatment as a factor influencing mealiness must also be studied over a number of seasons. This is necessary to determine if postharvest temperature treatments are influenced by seasonal preharvest factors.

The influence of preharvest temperatures on the postharvest development of mealiness was not investigated in this study, and needs further research.

Future research should focus on mealiness development over time in storage and should concentrate on chemical analysis of isolated cell walls, to establish similarities of pectin degradation, viscosity and other factors between mealiness and other similar textural disorders.

Histochemical techniques using electron and light microscopy could prove a particularly useful tool to understand differences between non mealy and mealy tissues. It is known that fruit having textures with low juice content like woolly nectarines, mealy apples, and gel breakdown in plums, develop big intercellular spaces, and experience disintegration of the middle lamella. This is discussed in detail in the literature review. Ben-Arie and Kislev (1979) found that plasmodesmata of ripe

'Spadona' pears were resistant to exogenously-applied PG. This means that plamodesmata keep cells together during extensive ripening, whereas in other fruit, cells are known to separate. Martin-Cabrejas *et al.* (1994) confirmed the persistence of plasmodesmata in pit fields of over mature 'Blanquilla' Spanish pear, that even showed signs of plasmolysis. They also concluded that this and the higher degree of sclereids associated with parenchyma might be responsible for maintaining tissues of pears even when very ripe. It would therefore be useful to know whether these stabilising factors also remained in mealy tissues. This could in turn lead to a better description and understanding of the development in mealiness.

This study has attempted to determine the physiological factors affecting the ripening and development of mealiness in 'Forelle' pears. By examining fruit grown in different climatical areas, harvested at different maturities, treated with exogenous ethylene and stored at different temperatures, this research has helped to better understand the role of these factors on the quality of 'Forelle'. While this study has succeeded in providing answers to certain questions, it has also opened the way forward to further research in this field.

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