EFFECT OF FRUIT MATURATION AND RIPENING POTENTIAL FOR

OPTIMUM EATING QUALITY OF 'FORELLE' PEARS

By

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DECLARATION

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SUMMARY

Climatic differences between production areas or seasons directly affect the rate of fruit maturation and the eating quality following storage and ripening. South African 'Forelle' pears are harvested at an optimum firmness of 6.4 kg and have mandatory cold storage duration of 12 weeks at -0.5°C to ensure even ripening. The firmness variable alone, however, is not a good indicator of ripening potential. Hence, various maturity variables (ethylene production, ground colour, firmness, total soluble solids (TSS) titratable acidity (TA), and starch breakdown) and their rates of change were evaluated to identify consistent maturity indices that can be reliably used in a prediction model to determine optimum harvest maturity (Chapter 2). This was then related to the ripening potential (Chapter 3) and eating quality (Chapter 4), defined by optimum 'edible firmness' (3.5 kg), presence or absence of astringency or mealiness.

Fruit were harvested from three main producing areas: Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Harvesting was done biweekly on five harvest dates over three successive seasons (2007-2009). At harvest, 20 of 240 fruit per block were used to determine maturity using all the mentioned parameters in order to understand their changes and behaviour pre-harvest. The remaining 220 fruit were stored at -0.5°C for three storage durations followed by ripening at 15°C.

At harvest, the 2007 season's fruit were more advanced in ground colour and were significantly softer (6.7 kg) than the 2008 (7.0 kg) and 2009 (7.1 kg) seasons. Firmness, ground colour, TSS and TA, all displayed a linear relationship with days after full bloom. For the firmness and ground colour, more than 90% and 73%, respectively, was explained by

the variation in the linear model, while for the TSS and TA less than 70% could be accounted for by the model.

Fruit harvested before commercial harvest (pre-optimum) in 2007 and 2009 failed to ripen to an 'edible firmness' when stored for eight weeks at -0.5°C plus 11 days at 15°C. In 2008, eight weeks storage was sufficient to induce ripening changes in pre-optimum harvested fruit. The development of ripening potential in the 2008 earlier harvested fruit, corresponded with a higher rate of change ($3.15 \ \mu L^{+}kg^{-1}h^{-1}day^{-1}$) in ethylene production at 15°C compared to the 2007 ($1.98 \ \mu L^{+}kg^{-1}h^{-1}day^{-1}$) and 2009 ($1.87 \ \mu L^{+}kg^{-1}h^{-1}day^{-1}$) seasons. The 2007 season fruit experienced maximum incidence of astringency (36.7%) on the first harvested fruit.

In all three seasons, fruit harvested at commercial harvest time and later (optimum and post-optimum), required an eight week storage period to induce ripening. However, the eight weeks storage period developed highest mealiness. More than 40% of the last harvested fruit were mealy after eight weeks at -0.5°C plus seven days at 15°C. Mealiness significantly reduced with prolonged storage at -0.5°C. Fruit from the WBV and Elgin, warmer areas than the KBV, were more prone to mealiness.

In conclusion, firmness was the most consistent variable at harvest and could be used in conjunction with ground colour to determine 'Forelle' harvest maturity. Furthermore, the study does not support shortening the current mandatory 12 weeks period at -0.5°C due to the higher incidence of astringency and mealiness.

OPSOMMING

Klimaats verskille tussen produksie areas of seisoene affekteer die tempo van vrugrypwording en eetkwaliteit na opberging en rypwording direk. Suid-Afrikaanse 'Forelle' word ge-oes by 'n optimum fermheid van 6.4 kg en het 'n verpligte opbergingstydperk van 12 weke by -0.5°C om egalige rypwording te verseker. Die veranderlike 'fermheid' is egter nie 'n goeie aanduiding van die rypheidspotensiaal op sy eie nie. Dus is verskeie rypheidsparameters (etileen produksie, agtergrond kleur, fermheid, total oplosbare vaste stowwe (TOVS), titreerbare suur (TS) en stysel afbraak) en die tempo van verandering ge-evalueer om konstante rypheidsverwysings te identifiseer wat met vertroue in 'n voorspellingsmodel gebruik kan word om optimum oes rypheid te kan bepaal (Hoofstuk 2). Dit is dan in verband gebring met die rypwordingspotensiaal (Hoofstuk 3) en eetgehalte (Hoofstuk 4), wat gedefiniëer is deur "eetbare fermheid" (3.5 kg), frankheid en melerigheid.

Vrugte is ge-oes uit drie, hoof verbouingsareas: Warm Bokkeveld (WBV), Elgin en Koue Bokkeveld (KBV). By oes is 20 van die 240 vrugte per blok gebruik om die vrug rypheid te bepaal, deur al die bogenoemde parameters te gebruik, om die verandering en reaksie voor oes te begryp. Die oorblywende 220 vrugte is opgeberg by -0.5°C vir drie opbergingstye, gevolg deur rypmaking by 15°C.

By oes was die vrugte van die 2007 seisoen verder gevorderd in agtergrond kleur en betekenisvol sagter (6.7 kg) as die van 2008 (7 kg) en 2009 (7.1 kg). Fermheid, agtergrond kleur, TOVS en TS het almal 'n linêere verband getoon met dae na volblom. In geval van fermheid en agtergrond kleur, is meer as onderskeidelik 90% en 73% verklaar deur die

variasie in die linêere model, terwyl in geval van die TOVS en TS, minder as 70% deur die model verklaar kon word.

Vrugte wat voor die kommersiële oes (pre-optimum) ge-oes is in 2007 en 2009, het nie daarin geslaag om ryp te word tot by 'eetbare fermheid' na ag weke by -0.5°C en 11 dae by 15°C nie. Daarteenoor kon vrugte wat pre-optimum ge-oes is in 2008, wel geïnduseer word om ryp te word met ag weke opbeging. Die ontwikkeling van die rypwordingspotensiaal van vrugte wat vroeër ge-oes is, stem ooreen met die hoër tempo van verandering (3.15 μ L'kg⁻¹·h⁻¹·dag⁻¹) in etileen produksie by 15°C in vergelyking met seisoene 2007 (1.98 μ L'kg⁻¹·h⁻¹·dag⁻¹) en 2009(1.87 μ L'kg⁻¹·h⁻¹·dag⁻¹). Die 2007 seisoen vrugte het die maksimum voorkoms van frankheid (36.7%) getoon vir vrugte van die eerste oes datum.

In al drie seisoene waar vrugte wat by kommersiële oes of later (optimum en post optimum) ge-oes is, was 'n ag weke periode van opgeberging voldoende om rypwording te inisiëer, alhoewel die ag weke opberging ook gelei tot die hoogste voorkoms van melerigheid. Meer as 40% van die laat ge-oeste vrugte was melering na ag weke opberging by -0.5°C en sewe dae by 15°C. Melerigheid is betekenisvol verlaag met 'n verlengde opbergingsperiode by -0.5°C. Vrugte vanaf die WBV en Elgin, warmer areas as die KBV, was meer onderhewig aan melerigheid.

Opsommend was fermheid die reëlmatigste veranderlike by oes en kan tesame met agtergrondkleur, gebruik word om vrugrypheid van 'Forelle' te bepaal. Verder het die studie nie 'n verkorting van die huidige, verpligte 12 week opberingsperiode by -0.5°C gesteun nie, weens die hoë voorkoms van frankheid en melerigheid.

DEDICATED TO MY LATE GRANDMOTHER, ESTHER SIPHIWE MATSENJWA, WITHOUT HER UPBRINGING, LOVE AND SUPPORT THROUGH OUT MY CHILDHOOD, THIS WOULD NOT HAVE BEEN POSSIBLE.

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This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetitions between chapters, therefore, have been unavoidable. The different styles used in this thesis are in accordance with the agreements of different journals used for submission of manuscripts from the thesis. Chapters 1 and 2 were written for Scientia Horticulturae, while Chapter 3 and 4 were written for Postharvest Biology and Technology.

General Introduction

'Forelle' (*Pyrus communis* L.) is a late season blush pear cultivar grown in South Africa. It is the third most important pear cultivar planted and occupies 25% of the area under pear production (Deciduous Fruit Producer's Trust (DFPT), 2009). 'Forelle' has a mandatory 12 weeks of cold storage at -0.5°C to allow even ripening, since it has a high cold requirement.

The quality and ripening potential of 'Forelle', a climacteric fruit, is closely related to harvest maturity (Kader, 1999; Crouch et al., 2005; Tromp, 2005). The degree of maturity at harvest has a direct effect on the period for which fruit can be stored without losing quality (Kader, 1999). Several techniques ranging from destructive (traditional) (Crisosto, 1994; Watkins, 2003) to non-destructive measures (Kawano, 1994; Costa et al., 2000; Peirs et al., 2001; Nicolaï et al., 2007) were evaluated on different maturity indices (firmness, total soluble solids, titratable acidity, ground colour and starch breakdown). These maturity indices are greatly influenced by prevailing climatic conditions and vary from season to season (Frick, 1995; Van Rensburg, 1995; Lotze and Bergh, 2005). Hence, it is of absolute importance that optimum harvest maturity is well defined to reduce postharvest losses and attain 'acceptable' eating quality after storage (Hansen and Mellenthin, 1979). Proper prediction for harvest maturity will also allow producers to plan for harvesting and marketing well in advance and capitalize on labour productivity.

Pears will not ripen normally until they are exposed to a low temperature for a critical period. The cold treatment induces accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), which is a close precursor to ethylene, to a degree that ripening resistance declines (Wang et al., 1985; Martin, 2002). The ACC is then oxidised to ethylene by ACC oxidase,

which is active after fruit is transferred to room temperature. The autocatalytic ethylene is then expressed, thus resulting in normal and even ripening.

Mealiness and astringency are the key internal quality disorders associated with 'Forelle' eating quality in South Africa (Martin, 2002; DFPT Technical Services, 2008; Crouch and Bergman, 2010). Mealiness in 'Forelle' decreases with extended storage period at -0.5°C (Martin, 2002). Astringency in pears and apples appears to be more of a maturity problem rather than that of storage (Eccher Zerbini and Spada, 1993; Young et al., 1999; Mielke and Drake, 2005), possibly due to high levels of tannins in less mature fruit (Ramin and Tabatabaie, 2003). Seasonal and geographic differences also influence eating quality related disorders, particularly mealiness. An incidence of 53 to70% mealiness was associated with growing seasons experiencing high total heat units (Hansen, 1961). This was further confirmed in 'd'Anjou' pears (Mellenthin and Wang, 1976) where fruit exposed to high daily temperatures six weeks before harvest ripened unevenly and were prone to mealiness. Cultural factors such as clay or heavy soils were observed to favour astringency in pears (Downing, 2009 unpublished observation).

The study was carried out in three major 'Forelle' growing areas in the Western Cape, South Africa, from 2007 to 2009 seasons. The three growing areas; Warm Bokkeveld (WBV), Elgin, and Koue Bokkeveld (KBV), experience considerable climatic differences in terms of annual accumulated heat and chill units. The KBV is known as a cooler area compared to the WBV (Wand et al., 2008). Fruit were harvested biweekly for five harvest dates. Thereafter, fruit were stored at -0.5°C for three storage periods and then ripened at 15°C for seven and 11 days. The aim of the study was to use various maturity indices and their rate of change to identify maturity variables that behave uniformly over the growing season and can be reliably used in a prediction model to determine optimum harvest maturity of 'Forelle' pears. This was then related to the ripening potential and eating quality of 'Forelle', which was defined by an optimum edible firmness of 3.5 kg and presence or absence of astringency or mealiness. The information gathered in this study will then be used in future in a prediction model that will combine both climatic indices and the maturity indices to see whether there is a correlation per season which could predict not only harvest maturity but ripening potential for even ripening with 'acceptable' eating quality. A lower predicted cold requirement for a particular season should compare to the present quality of the fruit after 12 weeks at -0.5°C protocol, also in terms of astringency and mealiness.

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Chapter 1: Literature Review

Biochemical and physiological changes during fruit maturation and ripening

1. Introduction

Climatic differences between cropping seasons and production areas influence harvest maturity and ripening capacity of climacteric fruit (Wang et al., 1971; Matthee, 1988; Frick, 1995). This impacts greatly on the fruit eating quality after storage and ripening. High spring temperature causes a faster decrease in flesh firmness of pear fruit (Lotze and Bergh, 2005). High accumulated heat units before harvest enhance total soluble solid levels in 'Bon Chretien' pears (Frick, 1995) due to increased carbohydrate assimilation. The eating quality together with fruit appearance, are two of the most essential factors that influence consumer acceptance (Manning, 2009). Consumer satisfaction depends mainly on taste of the commodity (Kader, 1999), which motivates consumers to come back and purchase more of the product.

Since pears are harvested pre-climateric as their ripening is dependent on the autocatalytic burst in ethylene (El-Sharkawy et al., 2003) to allow even ripening, harvesting must be done at the proper maturity (Garriz et al., 2008). Hence, proper prediction of optimum harvest maturity is crucial for producers to avoid losses during storage and maintain better post-storage quality (Kvikliene et al., 2008).

Maturity variables, viz. firmness, ground colour, starch breakdown, acid, sugars, ethylene and carbon dioxide production are useful aids for defining fruit quality traits (Truter et al., 1985; Little and Holmes, 2000; Watkins, 2003), used to predict harvest maturity for optimum eating quality. These maturity indices are based on the quality attributes that assist in interpreting the gradual change in fruit ripening (Garriz et al., 2008). The rate of change of

these maturity variables is dependent on the physiological and biochemical changes that occur during maturation and ripening, in which the environment (climate (Wang et al., 1971), soil patterns (Lötze and Bergh, 2005), light (Bramlage, 1993; Kappel and Neilsen, 1994) etc) also plays a vital role.

Variation in fruit quality may occur from season to season. This is a major concern to fruit producers around the world due to demand for consistent supply of best export quality fruit that offers premium prices. In order to supply the best quality, producers need to be aware of the optimum time to harvest for good eating quality in a particular cropping season.

Clear knowledge of fruit maturation and ripening is, therefore, necessary in order to assist growers make informed decisions regarding fruit handling practices. Hence, the reviewed literature covers biochemical and physiological changes that occur in fruit during maturation and ripening with special emphasis on harvest maturity variables. Factors related to fruit quality are also considered.

1.1 Physiological and biochemical changes related to harvest maturity variables

1.1.1 Ethylene and fruit ripening

Ethylene is a naturally synthesized plant hormone that plays a key role in initiating fruit ripening (Watkins, 2003). Ripening is the composite of processes that occur from the latter stages of fruit growth and development through the early stages of senescence (Kader, 1999). This leads to development of flavour, texture, aroma, and loss of astringency, which all contribute to optimum eating quality (Weatherspoon et al., 2005).

According to Watkins (2003), ethylene is at times used as a main deciding factor in terms of harvesting decisions especially in apples. However, this may not be reliable at all

times because this parameter can be significantly influenced by factors such as the production region, orchards within that region, cultivar, and growing season (Vendrell and Larrigadiere, 1997; Watkins, 2003). Due to this limitation, such a maturity variable will need to be used in conjunction with other maturity indices when predicting harvest maturity for optimum eating quality.

Postharvest cold treatment is a prerequisite for some of the late pear cultivars (El-Sharkawy, 2003), to allow production of autocatalytic ethylene for even ripening, and these include the 'Forelle'pear (Martin, 2002). The cold treatment prior to ripening is to allow accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), a close precursor to ethylene, to a degree that ripening resistance declines (Wang et al., 1985; Martin, 2002). The ACC is then oxidised to ethylene by ACC oxidase, which is active after fruit is transferred to room temperature. The autocatalytic ethylene is then expressed thus resulting in normal and even ripening (Leliévre et al., 1997).

1.1.2 Ground colour

Change in fruit colour is the most obvious signal of maturity (Wills et al., 2007). It is often one of the standards that consumers use to determine whether a fruit is ripe or unripe. Pears lose their green colour as they mature and ripen, through a catabolic process. The chlorophyll structure is degraded by the enzyme chlorophyllase (Dangl et al., 2000), which reveals the carotenoids present in the skin, hence fruit appearing greenish yellow.

Fruit ground colour is influenced to some degree by the environment independent of maturity. In trees that have a lot of leaves per fruit with high nitrogen levels in the fruit, the ground colour may be greener at optimum harvest (Little and Holmes, 2000). Furthermore, increased levels of nitrogen accompanied by high night temperatures will improve the

retention of chlorophyll and delay development of the yellow ground colour (Olsen and Martin, 1980). Due to these environmental influences the standard ground colour may not always indicate optimum maturity (Little and Holmes, 2000).

1.1.3 Starch

The maturation process in apples begins in the core part of the fruit and gradually spreads outwards until little starch remains underneath the fruit peel (Truter et al., 1985). As the fruit ripens, carbohydrate polymers are broken down and starch is converted to sugars. This affects both the taste and the texture of the fruit, and the rise in sugars makes the fruit much sweeter (Wills et al., 2007). Starch in plant tissue is metabolized by two amylases, and these are: α -amylase which hydrolyses the α -1, 4 linkage of amylose to release a combination of glucose and maltose and β - amylase, which breaks down the last but one linkage from the non-reducing end to release only maltose (Prasanna et al., 2007). This enzymatic hydrolysis of starch will cause the loosening of the cell structure and development of sweetness (Prasanna et al., 2007).

During maturation and ripening, the protopectin is gradually broken down to lower molecular weight fractions, which are more soluble in water. The rate of pectin substance degradation is directly correlated with the softening rate of the fruit (Wills et al., 2007). The degradation of pectin substances is linked to rising soluble polyuronides and a decline in the insoluble polyuronides (Yoshioka et al., 1992).

The use of starch as a maturity index to predict maturity has shown remarkable precision when predicting the rate of starch breakdown in 'Granny Smith' apples (Van Rensburg, 1995), regardless of seasonal differences. Furthermore, this is regarded as an

important maturity variable in apples, as it positively correlates with internal ethylene concentration (Lau, 1988; Tomala, 1999), an important indicator of maturity.

Temperature affects the rate of change in starch hydrolysis of apples. Low temperatures prior to harvest of apples favour the hydrolysis of starch to sugars, while high temperatures are inhibitory to this conversion (Smith et al., 1979).

1.1.4 Titratable acidity-Malic acid

The biosynthesis of malate in fruit flesh cells occurs in the cytoplasm and mitochondrion, this is then stored in the vacuole (Wills et al., 2007). Malic acid is the principal acid in most pear cultivars at maturity (Eccher Zerbini, 2002; Watkins, 2003; Colaric et al., 2007). Malic acid decreases during maturation, storage and ripening in apples (Truter et al., 1985; Ackermann et al., 1992) and pears (Martin, 2002). Ackermann et al. (1992) considered this decline a result of a dilution effect due to the mass increase during the cell growth phase and a rise in respiration after storage. Together with the sugars and aromatic compounds, malic acid contributes remarkably to the organoleptic quality (Wang et al., 1993). In apples high levels of acids at harvest were associated with good eating quality after storage (Truter and Hurndall, 1988).

Although the amount of titratable acidity is cultivar dependent, the climate, cultural practices and growing location play a role (Ackermann et al., 1992; Kingston, 1994). Lower titratable acidity was associated with fruit exposed to light and increased applications of nitrogen fertilizer (Kingston, 1994). Titratable acid levels are considered less reliable in determining harvest maturity, since in some apple varieties, the acid level at optimum harvest will vary greatly between seasons and growing regions (Olsen and Martin 1980; Little and Holmes 2000). Kingston (1994) recommended that the rate of change in the titratable acidity

be used rather than the absolute values, but very little change is observed in this variable (Frick, 1995), hence limiting its value as a maturity indicator

1.1.5 Flesh firmness

A decline in flesh firmness is one of the most noticeable changes occuring during fruit ripening (Eccher Zerbini, 2002). Firmness is highly correlated to the overall quality and texture of the fruit (Wills et al., 1989). A good eating quality pear has a buttery and juicy texture, generally accompanied by high extractable juice (Manning, 2009). Hence, the measure of flesh firmness is a good indicator of fruit maturity (Hansen and Mellenthin, 1979; Chen and Mellenthin, 1981), as it is strongly associated with the composite quality and texture of the fruit (Kingston, 1991).

During fruit ripening the middle lamella, a cementing material between cells, dissolves thus changing the cell sap and causing fruit to soften (Kingston, 1994). Several physiological factors have been linked with fruit texture, but to a larger extent the structural integrity of the primary cell wall and the middle lamella, storage polysaccharides accumulation and turgidity of the cells play a key role (Jackman and Stanely, 1995). The change in cell turgor pressure and breakdown of starch and cell wall polysaccharides directly affects the degree of fruit softening at ripening (Brady, 1987). Also, larger sized fruit in pears are associated with a lower firmness (Lötze and Bergh, 2005; Bai et al., 2008). This is possibly due to a higher proportion of intercellular airspace in the larger fruit, and such fruit therefore, are generally softer (Volz et al., 2004).

1.1.6 Total soluble solids (TSS)

The extractable juice in pears contains soluble compounds that include reducing-sugars and other carbohydrates, organic acids and amino acids (Wills et al., 1989). As fruit matures, the sugars become the main component of the soluble solids (Wills et al., 1989). TSS has a marked influence on the sensory attributes (Ackermann et al., 1992; Hudina and Štampar, 2005), as it contributes significantly to the flavour of pears (Vangdal, 1985).

The main sugars in most rosaceae species are fructose, sucrose, glucose and sorbitol (Fourie et al., 1991; Brady, 1993). Fructose is the dominating sugar in pears at maturity (Fourie et al., 1991; Chuji et al., 2001; Hudina and Štampar, 2005; Colaric et al, 2007), although, other researchers report glucose and fructose to be occurring in comparable amounts (Chapman and Horvart, 1990).

1.2 Factors influencing fruit quality

The environment and tree management practices have a significant influence on the internal and external characteristics of fruit (Wang et al., 1971; Matthee, 1988; Bramlage, 1993; Frick, 1995). Factors affecting fruit quality could occur both before harvest and after harvest.

1.2.1 Climatic effects

Climatic variables, particularly temperature (Frick, 1995; Van Rensburg, 1995) and light (Bramlage, 1993) prevailing during fruit growth and development have a fundamental role on postharvest quality of pome fruit. Low temperatures occurring four to five weeks before harvest cause premature ripening in 'Bartlett' pears (Wang et al., 1971). The premature ripening was linked with rising levels of abscisic acid (Wang et al., 1972).

Pre-harvest temperatures also affect the rate of ethylene production during ripening. High production rates of ethylene in 'Bartlett' pears were common in fruit produced in regions with lower temperatures prior to harvest (Mellenthin and Wang, 1976; Agar et al., 1999). The ethylene-forming enzyme (EFE) activity develops earlier in apples exposed to low night temperatures as opposed to fruit that mature under warm night conditions (Blankenship, 1987).

A variation in the rates of change in maturity indices occurs from season to season and within production regions (Frick, 1995). This indicated that maturity parameters are not completely synchronized and will not express a similar pattern from season to season.

Furthermore, the daily-hourly average (DHA) temperatures occurring during the last six weeks prior to harvest were found to influence the acid and sugar content of 'd'Anjou' pears after long cold storage periods. Increased acid and sugar levels were reported in pears produced at 17.2°C and 13.9°C DHA temperatures, whereas in pears grown at 20.0°C and 11.7°C, the ripening capacity was low (Mellenthin and Wang, 1976).

Increased exposure to light increases fruit size (Tahir et al., 2007), total soluble solids and flesh firmness (Woolf and Ferguson, 2000). In South Africa, Lötze and Bergh (2005) found that soluble solid content in pears was improved under conditions with higher heat unit accumulation, as a result of high photosynthetic rates and carbohydrate reserves.

1.2.2 Soil nutritional effects

The effect of soil on fruit quality is largely dependent on plant nutrient availability (Sharples, 1979; Hudina & Štampar, 2005; Calouro et al., 2008). High levels of nitrogen

application are linked with increased green colouration on ground colour and low levels of TSS, however, this also intensifies the susceptibility of fruit to premature drop (Bramlage, 1993). The high levels of nitrogen have a drastic effect on fruit calcium availability due to shoot-fruit competition, as influenced by high tree vigour (Bramlage, 1993; Sugar et al., 1998).

According to Sharples (1979), Perring in 1968 realized an increase of titratable acidity in apples when high potassium levels were used and this enhanced the eating quality, but such an improvement was only observed when fruit calcium levels were above the threshold of susceptibility to bitter pit. Likewise, Calouro et al. (2008) found a strong relationship in fruit potassium and titratable acidity in 'Rocha' pears and improved texture and juiciness in 'Conference' pears (Sharples, 1979). Furthermore, foliar fertilization of phosphorus and potassium resulted in increased amounts of sugars (glucose, sorbitol, soluble solids) and organic acids (malic and citric acid) (Hudina and Štampar, 2005) in 'Williams' pear.

An effect of fruit nutrition on ripening behaviour was reported in pear cultivars such as 'Alexander Lucas' (Tomala and Trzak, 1994) 'Passe-Crassane' and 'd'Anjou'(Richardson and Al-Ani, 1982). In these pear cultivars the rate of ripening was slower in fruit with consistently high levels of calcium, which was indicated by lower respiration rates and ethylene production. In addition, higher fruit firmness at harvest is associated with calcium treatments in 'd'Anjou' cultivar (Gerasopoulos and Richardson, 1997). Under such conditions, Richardson and Gerasopoulos (1993) and Gerasopoulos and Richardson (1997) then proposed that high chilling conditions will be necessary to stimulate the ripening potential. On the other hand, early fruit ripening is common in pome fruit with excess amounts of boron, and such fruit are more prone to premature drop (Bramlage, 1993).

Differences in soil patterns also affect the internal quality of pears. Fruit from sandy soils have lower firmness and TSS levels (Lötze and Bergh, 2005). This could possibly be

attributable to poor nutrition associated with heavy leaching of sandy soils or the influence of irrigation on fruit growth.

1.2.3 Irrigation and planting density

Although most postharvest studies generally emphasized harvest maturity as a key factor on fruit quality (Lau 1998; Kader 1999), cultural factors such as planting density (Predieri and Gatti., 2008) and irrigation (Crisosto et al., 1994; Crisosto et al., 1995; Verreynne et al., 2001) proved to have an important role on TSS levels in most fruit.

Low tree density is linked with higher levels of TSS in 'Abate Fetel' pears (Predieri and Gatti, 2008) due to reduced competition for available resources during plant development (Faust, 1989). Also, fruit with increased TSS levels were observed in moisture stressed trees during the last phase of fruit growth - just prior to harvest (Crisosto et al., 1995; Mpelasoka et al., 2001; Hudina and Štampar, 2005). Such an effect was a result of accumulation of glucose, fructose, sucrose and sorbitol (Behboudian et al., 1994).

1.2.4 Fruit bearing position

Fruit are produced throughout the canopy and this may affect the amount of light, ambient temperatures and endogenous hormone supply received by the fruit (Kingston, 1994). Less ethylene production in apple was associated with fruit that is borne at the terminal end as opposed to fruit within the canopy at any sampling date after full boom (Kingston, 1994). The bearing position will also impact on the flow of nutrients and water into the developing fruit, and consequently on the quality of the fruit. Apple fruit borne on terminal shoots rather than on laterals have higher calcium levels (Tomala, 1999), this has a direct effect of flesh firmness (Gerasopoulos and Richardson, 1997) and therefore fruit ripening (Richardson and Al-Ani, 1982; Tomala and Trzak, 1994).

Studies in Argentina confirmed that fruit bearing position influences the quality of pears. Fruit sampled in the upper part of the canopy were larger in size than those on the lower parts of the canopy and flesh firmness was generally higher (Benitez and Duprat, 1998). This was a result of increased photosynthesis as manipulated by light intensity (He et al., 2008).

Furthermore, Crisosto et al. (1997) correlated mealiness and flesh browning in peaches with low crop load and fruit found inside the canopy. Fruit borne on thinner bearing shoots have lower malic acid content than fruit borne on thick shoots (Genard and Bruchou, 1992).

1.2.5 Harvest maturity and postharvest effects

The degree of maturity at harvest is a prime factor with respect to fruit quality after storage and ripening (Tomala, 1999; Kader, 2002; Martin, 2002). Therefore, it is important that pears are harvested at the proper maturity (Hansen and Mellenthin, 1979; Tomala, 1999) because immature fruit do not ripen properly and have poor eating quality (Hansen and Mellenthin, 1979; Tromp, 2005). On the other hand, over mature fruit are prone to mealiness (Peirs et al., 2001; Martin, 2002).

Mealiness is one textural disorder related to storage duration and temperature in 'Forelle' pears. Mealiness in 'Forelle' decreases with storage duration longer than the mandatory 12 weeks at -0.5°C (Martin, 2002). Furthermore, fruit that were stored at 4°C had better quality and little or no mealiness compared to fruit stored at -0.5°C, which experienced 70% mealiness due to chilling injury (Martin, 2002). According to Hiu (2006), chilling

injury will cause mealiness as a result of increased intercellular spaces and accumulation of pectin substances in the intercellular matrix, caused by the splitting of the mesocarp parenchyma cells.

Although other pear cultivars such as 'd'Anjou' (Chen and Mellenthin, 1981) have shown influences of harvest maturity on textural related disorders (mealiness) after ripening, with 'Forelle' pears the harvest maturity did not necessarily show a similar effect as other winter pears (Martin, 2002). This implied that factors other than harvest maturity could be involved.

1.3 Conclusion

Among other maturity indices, flesh firmness is the present maturity parameter used by the South African industry on 'Forelle' pears to determine optimum harvest maturity. The ideal harvest maturity ranges from 4.5 to 6.8 kg firmness. A mandatory minimum 12 weeks of cold storage at -0.5°C (Hurndall, 2010) is necessary for normal and even ripening of 'Forelle' (Martin, 2002). Firmness alone is not a good indicator of the ripening potential, possibly due to it being affected by several factors prior to harvest (Gerasopoulos and Richardson, 1997; Benitez and Duprat, 1998; Lötze and Bergh, 2005).

'Forelle' pear in South Africa is produced in three climatically diverse areas; Warm Bokkeveld (WBV), Elgin, and Koue Bokkeveld (KBV). The KBV is known as a cooler area compared to the WBV (Wand et al., 2008). Fruit from these areas may differ in their maturity and ripening behaviour possibly due to the climatic effect (Mellenthin and Wang, 1976; Lötze and Bergh, 2005). Hence, the aim of the study was to use various maturity indices and their rate of change to identify maturity variables that behave consistently and uniformly over the growing season and can be reliably used in a prediction model to determine optimum harvest maturity of 'Forelle' pears. This was then related to the ripening potential and eating quality of 'Forelle' from the three areas.

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Chapter 2: Paper 1

Evaluation of maturity indices and their rates of change to determine optimum harvest maturity of 'Forelle' pears

Abstract

'Forelle' (Pyrus communis L.) is a late season blush pear cultivar grown in South Africa and has a high market value. It requires a mandatory 12 weeks of cold storage at -0.5°C, since it has a high cold requirement for even ripening and good eating quality. This limits producers from accessing earlier markets. Among the various indices used to determine harvest maturity (the release date), flesh firmness is one variable used by the South African deciduous fruit industry. This parameter alone, however, does not give a good indication of ripening potential. Various maturity indices and their rates of change were used to predict optimum harvest maturity, and relate this to the ripening potential and eating quality of 'Forelle'. Fruit were sourced from three climatically different production areas: Warm Bokkeveld, Koue Bokkeveld and Elgin. Fruit were harvested biweekly for five harvest dates over a period of three consecutive seasons (2007-2009). Findings showed that flesh firmness was changing at a faster rate than all the other variables, but was comparable to the rate of change in ground colour. Furthermore, these two variables were more reliable and could be fitted in a linear model and used to predict harvest maturity of 'Forelle' pears. Data for total soluble solids and titratable acidity were inconsistent; hence these parameters may need to be coupled with other maturity indices in order to increase precision when predicting optimum harvest maturity.
Keywords: Firmness; Ground colour; Heat units; Prediction model; Pre-harvest temperatures; *Pyrus communis* L.

1. Introduction

The prediction of harvest maturity of climacteric fruit has interested many researchers for many years. Several techniques ranging from destructive (traditional) to non-destructive measures have been evaluated on different maturity indices and their rates of change. These maturity indices are greatly influenced by prevailing climatic conditions and vary from season to season (Frick, 1995; Van Rensburg, 1995; Lötze and Bergh, 2005). Furthermore, the eating quality of pears is associated with the time of harvest, cold storage duration and post-storage ripening as well as climatic factors (Eccher Zerbini, 2002).

Quality and ripening potential of pears is closely related to harvest maturity of the fruit (Kader, 1999; Crouch et al., 2005; Tromp, 2005), such that the degree of maturity at harvest has a direct bearing on the period for which it can be stored without losing quality (Kader, 1999). Therefore, it is of absolute importance that optimum harvest maturity is well defined to reduce postharvest losses and attain acceptable eating quality after storage (Hansen and Mellenthin, 1979). This will also allow producers to plan well in advance and capitalize on labour productivity. In general, climacteric fruit that are harvested immature will not ripen properly upon removal from cold storage, and will possess poor organoleptic quality. Conversely, if harvested at an advanced maturity stage, they will soften rapidly during ripening and develop mealiness rapidly (Peirs et al., 2001).

The common and traditionally used maturity indices in determining harvest maturity are ground colour, starch breakdown, flesh firmness, total soluble solids (TSS), full bloom dates and days after full bloom (DAFB), fruit size and ethylene production (Truter et al., 1985; Crisosto, 1994; Watkins, 2003). An innovative method referred to as the 'NSure' has recently been introduced to determine the ripening stage for apples and pears (www.nsure.eu). This technique is based on measuring the activity profile of fruit genes to determine the ripening stage of the fruit. It is claimed that NSure testing offers reliable prediction of the maturation stage of the fruit, hence helping growers to plan harvest and sales in time.

The rate of change in firmness is regarded as the most reliable and seasonally consistent when used in conjunction with other indices such as fruit ground colour and chemical constituents to predict harvest maturity in most pear cultivars (Hansen and Mellenthin, 1979; Wang, 1982). Total soluble solids proved to be unreliable when used as sole indicator of maturity (Hansen and Mellenthin, 1979), but when it is combined with flesh firmness, reliable results are obtained (Little and Holmes, 2000).

Days after full bloom (DAFB) is a better maturity index in predicting harvest time (Truter and Hurndall, 1988), when compared with calendar date in apples. This applies when the number of days used is obtained from the region where it is being used as an index (Salunkhe and Desai, 1984). In regions that experience great temperature fluctuations like the Western Cape, DAFB are inaccurate as a maturity indicator (Truter and Hurndall, 1988).

There are also several non-destructive methods that were developed and could be used to evaluate fruit quality attributes (Kawano, 1994; Costa et al., 2000; Nicolaï et al., 2007; Rutkowski et al., 2008). For instance, the use of the near infrared spectroscopy (NIRs) in combination with reliable sampling procedures was evaluated on fruits and vegetables to determine parameters that predict maturity more accurately (Kawano, 1994). Bobelyn et al. (2010) reported poor performance of NIR calibration model with lower R² values for apple firmness compared to soluble solids content. Reasonable results were achieved for dry matter, texture attributes and sugars in fruits, but prediction of acidity was not easy as it is too small to significantly affect the NIR spectrum (Nicolaï et al., 2007).

'Forelle' (*Pyrus communis* L.) is a high value cultivar ranking third largest in exporting volume of pears in South Africa (DFPT, 2009). It is mainly produced in the Western Cape where the growing areas have varying climatic factors. These climatic differences may influence harvest maturity and ripening potential of the fruit with regard to rates of change of the different maturity indices. The Koue Bokkeveld, for instance, will experience lower daily and seasonal minimum temperatures compared to the Warm Bokkeveld, which is at a lower altitude (Wand et al., 2008).

Flesh firmness is one variable used by the South African deciduous fruit industry to determine harvest maturity of 'Forelle' pears and therefore release dates (DFPT technical services, 2010). This variable has, over the past seasons, been recommended as the most reliable and seasonally stable technique to determine time of harvest of most pear cultivars (Hansen and Mellenthin, 1979). It is based on the assumption that during the maturation phase, there is a time when the cells enlarge rapidly and cell wall thickness decreases, which is related to a decline in flesh firmness (Murneek, 1923). This parameter alone, however, does not to give a good indication of ripening potential in 'Forelle' pears.

Hence, the aim of this study was to use various maturity indices and their rate of change to identify maturity variables that behave uniformly over the growing season and can be reliably used in a prediction model to determine optimum harvest maturity of 'Forelle' pears. This will then be related to the ripening potential and eating quality of 'Forelle', that we defined by optimum edible firmness (3.5 kg), presence or absence of astringency and mealiness (Chapter 3 and 4).

2. Materials and methods

2.1 Fruit source and experimental lay-out

'Forelle' pears were obtained from three climatically different production areas: Warm Bokkeveld (WBV) (33°15'S; 19°15'E), Koue Bokkeveld (KBV) (33°8'S; 19°23'E) and Elgin (33° 54'S; 19°4'E), located in the Western Cape, South Africa. On average, KBV accumulates 1477 daily positive chill units (DPCU) annually (DFPT- climate data base, 2006 - 2008), and is cooler than Elgin (768 DPCU) and WBV (1007 DPCU). Five harvest dates were used. Fruit were harvested biweekly from week five (H1), week seven (H2), week nine (H3), week 11 (H4), and week 13 (H5) over a period of three consecutive seasons (2007-2009). Four commercial farms were identified in each area, and fruit with similar fruit diameter were sampled from the same trees. 240 Fruit were harvested randomly at shoulder height around the tree into a fruit picking bag at each harvest date. All except 20 fruit were stored according to commercial packaging practice at -0.5°C for further analysis (Chapter 3 and 4). Harvested fruit were placed on pear pulp trays and then packed into cartons lined with a polyethylene bag (37.5 μ m), which was then folded over to cover the fruit completely. The 20 fruit were then used for maturity indexing as described in section 2.2. In this study, the industry norm (20 fruit per orchard evaluated for maturity to aid in deciding on release dates, based on optimum levels of the maturity variables) was implemented per harvest in each season in order to determine optimum harvest point for each area based on the assessed maturity (ground colour index ≥ 2.5 ; fruit firmness ≤ 6.4 ; TSS ≥ 14.6 ; titrable acidity (TA) \leq 0.27).

2.2 Fruit maturity indexing

Individual fruit from a random sample of 20 fruit from each site were numbered to maintain identity of quality attributes per fruit. Fruit were evaluated as follows within 24 hours after harvest:

2.2.1 Fruit mass, diameter and flesh firmness

The fruit mass and diameter were determined by using an electronic balance and Cranston gauge, respectively, that were both attached to a fruit texture analyser. Flesh firmness was measured using an electronic fruit texture analyzer (FTA 2007, Güss, Strand, South Africa) fitted with an 8.0 mm diameter plunger. Two readings were taken on pared opposite sides of each fruit.

2.2.2 Fruit ground colour

This refers to the change from green to a yellow ground colour, not the conspicuous red colour development on the fruit. The colour chart developed for apples and pears by Unifruco Research Services (URS) with a scale of 0.5 to 5 (where 0.5 = dark green, and 5 = deep yellow) was used to evaluate ground colour.

2.2.3 TSS and TA

A pooled juice sample extracted from fruit slices $(\pm 1/9^{\text{th}} \text{ of a fruit dissected across the endocarp})$ of all 20 fruit per site was used to determine TSS % with a digital refractometer (PR-32, Atago, TSS 0-32%, Palette, Tokyo, Japan). TA was measured by titrating 10 g of the pooled juice with 0.1N NaOH to a pH of 8.2 and malic acid content calculated per 100 g of

juice using an automated titrator (Tritino 760 Sample Changer, Metrohm Ltd, Herisau, Switzerland).

2.2.4 Starch breakdown

The degree of starch breakdown (percentage starch breakdown) was determined on the calyx-end half of the fruit using the iodine test. The cut surface of each fruit was covered with iodine solution (50 g KI and 10 g of I₂ dissolved in 1 L distilled water) applied with a brush and then allowed to dry for 15 min. The percentage of the unstained area on each fruit was scored using a starch conversion chart for pome fruit developed by URS, South Africa, with a scale of 0% - 100%, where 0% is equivalent to totally stained surface and 100% equivalent to completely unstained surface.

2.2.5 Ethylene production

Pre-harvest analysis for ethylene production was only carried out during the 2008 and 2009 season. Three replicates of five fruit were put into 5 L air tight plastic jars and placed at room temperature for 30 min. After the 30 min. had elapsed, gas samples were taken using gas tight 10 mL syringes, which were then injected into a gas chromatograph (Model N6980, Agilent technologies, Wilmington, U.S.A) with a PorapakQ and Molsieve packed column and flame ionization and thermal conductivity detectors. The total fruit mass and volume of free space in the jar were used to calculate the ethylene production rates.

2.2.6 Statistical analysis

Each maturity parameter was plotted against DAFB for all the sites per area per season using Microsoft Office Excel, 2007. Then a linear regression equation of the form $y = \alpha + \beta x$ was fitted to the data to determine the rate of change (slope- β), and adjusted R² value. These were then analysed using the General Linear Models (GLM) procedure in the Statistical Analysis System (SAS) program (SAS Institute Inc, Cary, North Carolina, 1990). Mean separation was done using the least significant difference (LSD) at 5%.

3. Results

In Tables 1a, 1b and 1c, averages of each maturity variable determined from the sample of 20 fruit are presented. The harvest dates at which the optimum maturity standards (ground colour index ≥ 2.5 ; fruit firmness ≤ 6.4 ; TSS ≥ 14.6 ; TA ≤ 0.27) were reached differed between the areas and seasons (Tables 1a, 1b and 1c). In 2007, for instance, 'Forelle' from the Elgin area reached optimum ground colour index (2.5) two weeks earlier than fruit from WBV and KBV (Table 1a). The desired optimum maturity indices were not reached simultaneously over the growing season. In 2008, fruit from WBV reached optimum ground colour at commercial harvest time, then optimum firmness and TSS was observed four weeks later (Table 1b). Optimum TA (0.27) was observed earlier (before week 5) in the 2007 season for all the areas, while in 2008 and 2009, TA was reached after week five (1a, 1b and 1c). This variation in maturity was probably due to, amongst others, differences in full bloom dates between the areas and seasons.

3.1 Ground colour

A significant (P < 0.0001) interaction was found between harvest time and season on ground colour (Fig. 1A). A gradual linear progression of colour change from green (> 1.0) to slightly yellow (> 2.5) was observed over time of harvest in all seasons, but at different rates (Fig. 1A and Table 2). At initial harvest (H1), fruit from the 2008 season had a lower colour index (1.2) compared to the 2007 and 2009 seasons. Furthermore, the 2008 season experienced the highest rate of change (0.033 colour index unit day⁻¹), although not significantly higher than 2007. By the final harvest (H5), the ground colour index had increased by 1.7 units in the 2008 season, and it was statistically similar to fruit from the 2009 season (Fig. 1A). 'Forelle' harvested from the Elgin area were significantly advanced (2.5) in ground colour compared to those from the WBV (2.3) and KBV (2.3) areas (Fig. 1B).

3.2 Flesh firmness and fruit diameter (size)

Flesh firmness declined with time of harvest and highly significant (P < 0.0001) differences were found between harvests, seasons and areas (Fig. 2A, 2B and 2C). At initial harvest (H1), fruit were significantly firmer with an average firmness of more than 8.0 kg. By the final harvest, firmness had dropped by more than 1.8 kg. Fruit harvested in the 2007 season had an average firmness of 6.6 kg, that was the lowest (P < 0.0001) compared to that of fruit from the 2008 and 2009 seasons, respectively. Fruit from Elgin were significantly less firm (> 6.8 kg) on average than fruit from WBV and KBV (Fig. 2C).

The seasons differed significantly (p < 0.0001) in their rates of change in firmness (Table 2). The 2007 season had a significantly slower softening rate (-0.034 kg day⁻¹) compared to the 2008 (-0.041 kg day⁻¹) and 2009 (-0.044 kg day⁻¹) seasons. Fruit harvested in WBV had a higher softening rate of -0.044 kg day⁻¹ (non-significant) and the highest percentage variance of 95.6 compared to other areas. Flesh firmness further displayed a close

association (r = -0.657) with fruit diameter (Fig. 3). Small sized (50-60mm) fruit were firmer (> 7.0 kg) (Fig. 3).

3.3 TSS and TA

There were differences between seasonal rates of change in TSS and TA (Table 2). TSS increased significantly (P < 0.0001) with time of harvest (Fig. 4A). No significant differences were observed between areas and seasons in TSS (Fig. 4B and 4C). TSS determined from the 2007 season fruit changed at a rate of 0.040% day⁻¹, and differed significantly (P < 0.05) from the 2008 and 2009 seasons. A similar pattern of seasonal differences was observed with TA (Table 2). TSS and TA also displayed a linear relationship with DAFB (P < 0.05), however, less than 70% was explained by the variation in the linear model ($y = \alpha + \beta x$), whereas with ground colour and flesh firmness more than 73% and 90%, respectively could be accounted for by the model.

Areas and seasons interacted significantly (P = 0.0077) in TA (Fig. 5A). Fruit harvested in the 2008 season from KBV had the highest average TA (0.297) compared to all other treatment combinations. No statistical differences between seasons were observed in TA levels for fruit harvested from WBV (Fig. 5A). WBV had the lowest average TA (< 0.23) in all seasons. The low TA observed in WBV was not different to that of fruit harvested from Elgin and KBV in the 2007 and 2009 seasons (Fig. 5A). TA decreased significantly (P < 0.0001) with time of harvest (Fig. 5B). However, no significant differences were observed in TA levels between early harvested fruit (\pm 0.27) (H1 and H2) or between late harvested fruit (\pm 0.18) (H4 and H5) (Fig. 5B).

3.4 Starch breakdown (%) and ethylene production ($\mu L^{*}kg^{-1}h^{-1}$)

Erratic data were obtained from the starch test and this resulted in a percentage variance (R^2_{adj}) of less than 5% in the linear regression analysis. The pattern of starch breakdown was inconsistent and unreliable to use in predicting maturity as it varied considerably within samples, sites and seasons (data not shown). Furthermore, no ethylene was detected at harvest (data not shown), thus making this parameter unsuitable for maturity indexing of 'Forelle'.

3.5 Distribution of optimum harvest maturity over growing season (DAFB)

Optimum harvest maturity based on evaluated maturity variables did not express a similar behaviour in their distribution over seasons (Fig. 6). The optimum values of these maturity variables were based on the industry standards for release dates as earlier shown in Tables 1a, 1b and 1c. A high percentage of fruit (34.3%) were at optimum ground colour (2.5) at 150-159 DAFB. At 150-159 DAFB only 8.6% of fruit had reached optimum firmness (6.4 kg). A fairly high percentage of fruit (37.1%) reached optimum firmness at 160-169 DAFB. In addition, a higher percentage of fruit (31.4%) reached optimum TSS (14.6) at a similar length of growing season to optimum firmness (Fig. 6). A higher percentage of fruit (29.4%) reached optimum TA (0.27) earlier in the season (140-149 DAFB) than all other variables.

4. Discussion

Season significantly influenced the rates of change of the evaluated maturity variables (Table 2). This was a clear indication that seasonal differences have a direct effect on fruit quality, which confirms previous results (Wang et al., 1971; Mellenthin and Wang, 1976;

Frick, 1995). This also reflected a variation in the physiological condition of the fruit within the seasons. For instance, the 2007 season expressed a significantly different behaviour in the rates of change for firmness, TSS and TA compared to the 2008 and 2009 seasons, while the latter two behaved similarly. A higher rate of change in TSS was associated with a less rapid decline in TA and less rapid decline in firmness. A drastic decrease in TA was noted from the second harvest to the third harvest in all areas (Table 1a, 1b and 1c). This possibly marked the first maturation phase as earlier identified in apples (Truter et al., 1985). Moreover, this also explained variation in fruit maturation between seasons, as the 2007 season further expressed considerably lower average flesh firmness (< 6.8 kg) than the 2008 and 2009 seasons (Fig. 2B).

Comparisons across variables demonstrated clear differences in the rates of change of the evaluated maturity indices. Flesh firmness was changing at a higher rate than all the other parameters, and this was comparable to rates of change in ground colour. Contrary to this, TA changed slower in all seasons. This confirmed that the different maturity variables do not behave in a similar pattern during maturation, with changes in fruit firmness and ground colour being more drastic compared to changes of TA and TSS. Thus more noticeable changes such as firmness and ground colour could be simpler and more accurate to use in prediction of harvest maturity.

Flesh firmness also had a strong linear relationship with DAFB, and this was illustrated by the high R^2_{adj} values in the linear regression analysis that was consistently more than 90% in all three areas and seasons. This conformed to earlier research (Marcos et al., 2008). In agreement with Lötze and Bergh (2005) and De Salvador et al. (2006), firmness displayed an inverse relationship to fruit diameter; lower flesh firmness was linked with larger sized fruit. This is related to a higher proportion of intercellular airspace in larger fruit, and such fruit are therefore generally softer (Volz et al., 2004).

Optimum harvest maturity for the different maturity indices occurred at different times during the growing season (DAFB) (Fig. 6). The distribution of optimum flesh firmness over the season reached a higher percentage of fruit between 160 and 169 DAFB. The same trend was observed in optimum TSS. In concurrence with what Van Rensburg (1995) found in apples, the average length of the season was shortest when TA was used as a maturity variable, whereas if TSS and firmness were used the season was longer. However, this implied that TSS and flesh firmness are most likely to reach their optimum after TA. Thus, fruit possibly reach physiological maturity at lower TA levels than the industry optimum (0.27), when fruit size was relatively small. These findings further suggested that maturity indices do not reach the desired optimum standard synchronously which concurs with previous studies on pears (Frick, 1995) and apples (Van Rensburg, 1995).

The number of DAFB until optimum maturity for the various maturity variables differed between areas. Fruit from the Elgin area consistently reached optimum firmness earlier than WBV and KBV in all the seasons. Furthermore, the Elgin area was earliest to attain optimum TSS and ground colour in 2007. TSS was reached at 152 DAFB while optimum ground colour was achieved after 138 DAFB. This may suggest that fruit maturation occurred at a much faster rate in this region. This could be attributable to differences in prevailing climatic conditions within the three areas, since high spring temperatures cause a rapid drop in flesh firmness (Lötze and Bergh, 2005).

An unexpected decline was observed in TSS in the last harvest for the 2007 and 2009 seasons. This may be due to inadequate fruit sampling typically experienced after commercial harvest, as fruit size from Platvlei (2009 season) and Kentucky (2007 season) farms varied widely during the final harvest (week 13) (data not shown). It seems likely that some of the fruit were immature as low levels of TSS are typical of small sized fruit. Similarly, with ground colour in the 2009 season, a sudden drop in colour index for all the

areas was recorded at commercial harvest week (H3) and this can probably be ascribed to a sampling error. It is possible that most of the fruit were harvested from the inside of the canopy, due to commercial harvesting having removed most of the outer canopy fruit, as mentioned previously.

Unlike other maturity indices, starch breakdown proved to be unsatisfactory in assessing harvest maturity of 'Forelle' pears. Although in apples, starch is a good maturity indicator (Van Rensburg, 1995), as it is positively correlated with internal ethylene concentration (Lau, 1988; Walsh and Altman, 1993; Tomala, 1999), this was not the case for 'Forelle'. We found inconsistency and a wide variability between samples, seasons and sites in the starch breakdown as did Lötze and Bergh (2005). This made it unreliable to use in a prediction model for 'Forelle'. Furthermore, the study confirmed that postharvest cold treatment is a prerequisite for 'Forelle' pears in order to allow accumulation of 1-aminocyclopropane-1-carboxylic acid for even fruit ripening (Wang et al., 1985; Martin, 2002), since no ethylene was detected at any of the harvest dates.

5. Conclusion

Flesh firmness and ground colour were the most reliable variables that could be fitted in a linear model of the equation $y = \alpha + \beta x$ used to determine harvest maturity of 'Forelle' pears. Both variables displayed a strong linear relationship to DAFB as explained by the high R^2_{adj} values, and behaved consistently over the season. However, due to the subjectivity of assessing ground colour using a colour chart, it may be proposed that a more objective measurement (e.g. hue angle) is considered, and this variable could be used concurrently with the firmness variable that is presently used by the industry. Moreover, TA and TSS cannot be ruled out as they are important fruit quality attributes hence, these will remain useful aids if coupled with other maturity variables.

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Area	DAFB	Harvest ^x week	Ground ^y colour	Firmness (kg)	Total Soluble Solids (%)	Titratable acid (%Malic)
WBV	135	5	1.53	7.81	13.15	0.21 ^z
	149	7	2.14	7.21	12.63	0.22
	163	9	2.77 ^z	6.63	14.08	0.20
	177	11	2.68	6.19 ^z	14.63 ^z	0.18
	191	13	3.32	5.60	13.50	0.15
Elgin	126	5	1.60	7.47	12.68	0.23 ^z
	138	7	2.59 ^z	6.74	13.95	0.27
	152	9	3.14	6.14 ^z	14.60 ^z	0.21
	166	11	2.98	6.10	14.55	0.19
	180	13	3.55	5.45	14.18	0.18
KBV	143	5	1.63	7.91	13.63	0.24 ^z
	157	7	2.36	7.21	13.50	0.26
	171	9	2.86 ^z	6.81	14.33	0.23
	185	11	2.69	6.31 ^z	15.63 ^z	0.21
	199	13	3.36	6.17	15.68	0.21

Table 1a. Average maturity indices of 'Forelle' pears harvested biweekly at various harvest maturity from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in the 2007 season.

^x Commercial harvest week = week 9

^y Colour index of 0.5 = dark green and 5 = deep yellow.

^z Optimum commercial harvest maturity according to parameter: colour index \geq 2.5; fruit firmness \leq 6.4; TSS \geq 14.6; TA \leq 0.27.

Area	DAFB	Harvest ^x week	Ground ^y colour	Firmness (kg)	Total Soluble Solids (%)	Titratable acid (%Malic)
WBV	129	5	1.05	8.48	13.45	0.28
	143	7	1.70	7.81	13.70	0.26 ^z
	157	9	2.71 ^z	6.99	13.70	0.20
	171	11	2.55	6.47	14.40	0.18
	185	13	2.98	6.01 ^z	14.67 ^z	0.18
Elgin	129	5	1.34	7.90	13.60	0.32
	143	7	1.49	7.03	13.60	0.31
	157	9	2.69 ^z	6.58	14.10	0.24 ^z
	171	11	2.90	6.19 ^z	14.70 ^z	0.21
	185	13	3.13	5.71	15.05	0.16
KBV	126	5	1.33	8.22	14.13	0.35
	140	7	1.37	7.44	14.63 ^z	0.36
	154	9	2.44	7.07	14.87	0.29
	168	11	2.45	6.43	14.77	0.24 ^z
	182	13	2.68 ^z	5.98 ^z	14.80	0.23

Table 1b. Average maturity indices of 'Forelle' pears harvested biweekly at various harvest maturity from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in the 2008 season.

^x Commercial harvest week = week 9

^y Colour index of 0.5 = dark green and 5 = deep yellow.

^zOptimum commercial harvest maturity according to parameter: colour index \geq 2.5; fruit firmness \leq 6.4; TSS \geq 14.6; TA \leq 0.27.

Area	DAFB	Harvest ^x week	Ground colour ^y	Firmness (kg)	Total Soluble Solids (%)	Titratable acid (%Malic)
WBV	130	5	1.76	8.80	13.25	0.25
	144	7	2.26	7.69	13.90	0.27^{z}
	158	9	1.63	6.99	14.48	0.20
	172	11	2.85 ^z	6.65	14.75 ^z	0.17
	186	13	2.99	6.01 ^z	11.15	0.16
Elgin	134	5	1.71	8.17	12.75	0.29
	148	7	2.28	7.41	13.73	0.33
	162	9	1.96	6.48	13.56	0.22^{z}
	176	11	2.93 ^z	6.11 ^z	14.08	0.19
	190	13	3.10	5.89	13.73	0.16
KBV	129	5	1.65	8.78	12.95	0.24
	143	7	2.00	7.70	13.65	0.29
	157	9	1.53	6.99	13.93	0.20 ^z
	171	11	2.65 ^z	6.50	14.20	0.18
	185	13	2.81	6.30 ^z	14.05	0.16

Table 1c. Average maturity indices of 'Forelle' pears harvested biweekly at various harvest maturity from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in the 2009 season.

^x Commercial harvest week = week 9

^y Colour index of 0.5 = dark green and 5 = deep yellow

^z Optimum commercial harvest maturity according to parameter: colour index \geq 2.5; fruit firmness \leq 6.4; TSS \geq 14.6; TA \leq 0.27.

Treatment	Ground col	our index ^y	Firmness		Total Solul	ole Solids	Titratable A	cid
	β	R^2_{adj}	β	R^2_{adj}	β	R^2_{adj}	β	R^2_{adj}
Area								
WBV	0.027ns	0.743(<0.0001)	-0.044ns	0.956 (<0.0001)	0.027ns	0.503(<0.0001)	-0.0017ns	0.683(<0.0001)
Elgin	0.030	0.810(<0.0001)	-0.037	0.927 (<0.0001)	0.030	0.627(<0.0001)	-0.0024	0.690(<0.0001)
KBV	0.025	0.740(<0.0001)	-0.038	0.917 (<0.0001)	0.027	0.503(<0.0001)	-0.0017	0.547(<0.0001)
Season								
2007	0.029a	0.792(<0.0001)	-0.034a	0.942(<0.0001)	0.040a	0.616(<0.0001)	-0.0012a	0.472(<0.0001)
2008	0.033a	0.842(<0.0001)	-0.041b	0.941(<0.0001)	0.021b	0.508(<0.0001)	-0.0025b	0.774(<0.0001)
2009	0.021b	0.662(<0.0001)	-0.044b	0.921(<0.0001)	0.024b	0.523(<0.0001)	-0.0022b	0.674(<0.0001)
Significance leve	el: $Pr > F$							
Area	0.1695		0.1298		0.8805		0.0969	
Season	0.0009		<0.0001		0.0140		0.0015	
Area*Season	0.5555		0.9362		0.1317		0.6984	

Table 2. Pre-harvest rates of change (β) and R^2_{adj} values (with significance levels) of ground colour index, firmness, total soluble solids, and titratable acid of 'Forelle' pears harvested from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in the 2007, 2008 and 2009.

Means with the same letter are not different at 5% significant level.

^y Colour index of 0.5 = dark green and 5 = deep yellow.

ns : No significant difference between treatments.

Year	Area	Chill units ^x	Heat units ^x	
2006				
	WBV	1093	10008	
	Elgin	821	18523	
	KBV	1480	17067	
2007				
	WBV	990	19632	
	Elgin	835	17995	
	KBV	1385	16115	
2008				
	WBV	939	20336	
	Elgin	648	17117	
	KBV	1567	9904	

Table 3. Accumulated daily positive chill units with accumulated heat units (for 24 hours with base 10°C and upper limit 30°C) for each season (2007 - 2009) in the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) areas (DFPT climate data base).

* Accumulated Daily Positive Chill units were recorded from May, 1 to August, 31; while heat units were recorded from September, 1 to December, 31 of each year from an automatic industry weather station in the region.



Fig. 1. Average ground colour index of 'Forelle' pears harvested biweekly for three consecutive seasons (2007-2009) (A) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) (B). H: Represent harvest date at two weeks interval. (A colour index of 0.5 = dark green and 5 = deep yellow).



Fig. 2. Average firmness of 'Forelle' pears harvested biweekly (A) for three consecutive seasons (2007-2009) (B) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) (C). H: Represent harvest date at two weeks interval.



Fruit diameter (mm)

Fig. 3. Correlations between flesh firmness and fruit diameter of 'Forelle' pears harvested biweekly from Warm Bokkeveld, Elgin and Koue Bokkeveld on three consecutive seasons (2007-2009).



Fig. 4. Average total soluble solids (TSS) of 'Forelle' pears harvested biweekly (A) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) (B) for three consecutive seasons (2007-2009) (C). H: Represent harvest date at two weeks interval.



Significance level					
Treatment	Pr > F				
Area (A)	0.0008				
Harvest time (H)	< 0.0001				
Season (S)	< 0.0001				
A*H	0.6937				
A*S	0.0077				
S*H	0.0549				
A*S*H	0.9995				

Fig. 5. Average titratable acidity (TA) of 'Forelle' pears harvested from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) for three consecutive seasons (2007-2009). H: Represent harvest date at two weeks interval.



Fig. 6. Histogram showing the distribution of days after full bloom (DAFB), at which optimum harvest maturity was reached for each maturity variable (2007-2009).

Chapter 3: Paper 2

Influence of cold storage duration and harvest maturity on ripening potential of 'Forelle' pears

Abstract

The study was conducted to determine the ripening potential of 'Forelle' pears as influenced by growing area, harvest maturity and cold storage duration. Fruit were sourced from three climatically diverse areas: Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Harvesting was done biweekly on five harvest dates over three successive seasons (2007-2009). Fruit were stored under regular atmosphere at -0.5°C. In 2007, fruit were stored for eight, 10 and 12 weeks, while in 2008 and 2009, fruit were stored for eight, 12 and 16 weeks. Thereafter, fruit were ripened at 15°C for seven and 11 days. In the 2007 and 2009 seasons, fruit harvested before commercial harvest time (pre-optimum) failed to ripen to an "edible firmness" (3.5 kg) when stored for eight weeks at -0.5°C plus 11 days at 15°C. In 2008, pre-optimum harvested fruit managed to ripen after the eight week storage period. The development of ripening potential in the 2008 season on earlier harvested fruit, stored for eight weeks at -0.5°C corresponded with a higher rate of change $(3.15 \ \mu L^{+}kg^{-1}h^{-1}day^{-1})$ in ethylene production at 15°C compared to the 2007 (1.98 µLkg⁻¹.h⁻¹.day⁻¹) and 2009 $(1.87 \ \mu L^{-}kg^{-1}h^{-1}day^{-1})$ seasons. In all three seasons, in fruit harvested at commercial harvest time and later (optimum and post-optimum), an eight week period at -0.5°C was sufficient to induce ripening. However, the eight week storage period resulted in more rapid softening at 15°C than the 10, 12 and 16 weeks duration, and fruit were much yellower. In 2008, firmness of late harvested fruit, stored for eight weeks at -0.5° C was ≈ 1.0 kg lower than that of fruit stored for 12 and 16 weeks. WBV fruit softened faster in the 2008 season than fruit from

Elgin and KBV. This was associated with higher accumulated heat units in the WBV area compared to Elgin and KBV. In conclusion, the eight week storage period (instead of the mandatory 12 weeks) at -0.5°C was sufficient to induce ripening of 'Forelle' harvested at optimum harvest time and later, but fruit shelf life was shorter.

Keywords: Ethylene; Firmness; Ground colour; Pyrus communis L.; Rates of change

1. Introduction

'Forelle' (*Pyrus communis* L.) is South Africa's most valuable red blush pear cultivar accounting for 15.7% of total pear exports. It is the third most important pear cultivar planted and occupies 25% of the area under pear production (Deciduous Fruit Producer's Trust (DFPT), 2009). 'Forelle' is a late harvested cultivar, and marketed after the other blush cultivars 'Rosemarie' and 'Flamingo'. The minimum cold storage duration of 12 weeks at - 0.5°C is mandatory to producers. This prevents access to earlier markets, which might offer premium prices (De Vries and Hurndall, 1993). This minimum cold storage duration is, however, applied to ensure that fruit ripen evenly, since in the past years export reports have shown that 'Forelle' marketed before the minimum 12 weeks of cold storage could either be mealy or astringent (Martin, 2002; Hurndall, 2008; Crouch and Bergman, 2010).

'Forelle' is a winter pear requiring a lengthy cold treatment before even ripening occurs. The cold treatment induces accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), which is a close precursor to ethylene, to a degree that ripening resistance declines (Wang et al., 1985, Martin, 2002). The ACC is then oxidised to ethylene by ACC oxidase, which is active after fruit are transferred to room temperature. The autocatalytic ethylene is then expressed, thus resulting in normal and even ripening.

The duration needed for pears to fully soften to an "edible firmness" and develop a full flavour and buttery juicy texture, varies depending on the duration of cold storage prior to ripening, as well as temperature at ripening (Agar et al., 2000; Villalobos-Acuña and Mitcham, 2008). Agar et al. (2000) found that when the storage period for 'Bartlett' pears was prolonged from two to 12 weeks, the levels of ethylene produced increased significantly upon transfer to 20°C, and ripening occurred at a faster rate. However, the rate of firmness loss was similar when 'Bartlett' pears were stored for six or 12 weeks. This implies that ripening was fully induced within six weeks of cold storage and extending the length of cold storage did not have any further effect.

The length of cold storage after harvest is closely related to ethylene biosynthesis, and the chilling period required for even ripening varies with harvest maturity (Wang et al., 1971). Normally, fruit harvested at an advanced stage of maturity (late harvest) will require a shorter storage period compared to earlier harvested fruit. This has been demonstrated in 'd'Anjou' pears, where Chen and Mellenthin (1981) observed earlier development of ripening capacity in fruit harvested after optimum harvest, due to slightly higher ACC synthase (ACS) and ACC oxidase (ACO) activity (Agar et al., 2000). Despite this benefit, that may appear to compensate for the length of cold storage, late harvested fruit tend to have a short shelf life and develop a coarse texture (Hansen and Mellenthin, 1979), compared to early harvested fruit.

Harvest maturity has a great influence on postharvest behaviour and the ultimate organoleptic quality of pears (Chen and Mellenthin, 1981). Studies on 'Red d' Anjou' pears revealed that harvest maturity impacts greatly on the ripening behaviour during storage (Chen et al., 1994; Chen et al., 1997). Fruit harvested at different firmness levels presented distinct behaviours during ripening following storage in regular atmosphere at -1°C. Fruit that were harvested between a firmness of 5.4 and 6.3 kg did not develop the capacity to ripen evenly

after three months of cold storage followed by eight days at 20°C, whereas those harvested at a firmness less than 5.4 kg began ripening after a month of cold storage. This behaviour is likely associated with later harvested fruit requiring a shorter time in cold storage in order to stimulate ripening activity.

Although previous research has generally emphasized harvest maturity as a prime factor regarding postharvest behaviour of pears (Lau, 1998; Kader, 1999), climatic conditions before harvest also play a crucial role in the ultimate quality. 'Bartlett' pears grown in cooler districts matured earlier in the season than those grown in warmer districts, and could be harvested at a slightly higher firmness to overcome the effect of advanced ripening (Mellenthin and Wang, 1977). Furthermore, pre-harvest day and night temperatures of 21.1°C and 7.2°C, respectively occurring four weeks prior to harvest date accelerate maturation and ripening (Mellenthin and Wang, 1977).

In South Africa, there are three major 'Forelle' growing areas viz. Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). These areas exhibit considerable climatic differences in terms of annual accumulated heat and chill units. The KBV usually experiences lower daily and seasonally minimum temperatures compared to WBV, situated at a lower altitude (Wand et al., 2008). Thus such climatic diversity between these production areas may influence the ripening capacity of 'Forelle'. Higher ethylene production rates from other pear cultivars were associated with growing districts experiencing cooler pre-harvest temperatures (Mellenthin and Wang, 1977; Agar et al., 1999). This could suggest that differences in ripening behaviour might occur in fruit of the same cultivar grown under varying climatic conditions.

Commercially, optimum harvest maturity of 'Forelle' pears is defined by an average flesh firmness of 6.4 kg (DFPT technical service, 2010), regardless of growing location or season. This is assumed to be the stage in development at which, once the fruit has been

detached from the tree, fruit will be able to ripen to an 'acceptable' eating quality subsequent to low temperature exposure for 12 weeks, followed by room temperature. However, firmness (\leq 6.4 kg) alone does not indicate ripening potential during storage, since previous research on 'Forelle' indicated that time required for normal ripening per season would vary from six to 12 weeks at -0.5°C (Martin, 2002). Therefore, our objective in this study was to determine the ripening potential of 'Forelle' pears, as influenced by cold storage periods and harvest maturity in the three climatically diverse growing locations.

2. Materials and methods

2.1 Plant material and treatment

The experimental layout is summarised in Table 1. 'Forelle' pears were studied over three successive seasons (2007 to 2009). Fruit were sourced from the three main producing areas; WBV (33°15'S; 19°15'E), Elgin (33° 54'S; 19°4'E) and KBV (33°8'S; 19°23'E), located in the Western Cape, South Africa. In each area, four commercial farms were identified for the trial, and were used as blocks. Fruit were harvested at two week intervals i.e. four and two weeks prior to commercial harvest (week five and seven; H1 and H2, respectively), at commercial harvest (week nine; H3), and two and four weeks after commercial harvest (weeks 11 and 13; H4 and H5, respectively).

At each harvest date, a uniform fruit size was sampled randomly at shoulder height from all sides of the tree canopy to eliminate possible influences of fruit position. 240 Fruit were sampled from every block at each harvest date. 20 Fruit were used for maturity indexing at harvest.

2.2 Storage and ripening

The remaining 220 fruit from each block were stored under regular atmosphere (RA) at -0.5°C, packed in polyethylene-lined (37.5 μ m) cartons (to overcome shrivelling during storage and to simulate industry practice). Three storage durations were used per season. In 2007, fruit were stored for eight, 10 and 12 weeks, while in 2008 and 2009, fruit were stored for eight, 12 and 16 weeks (Table 1). After each cold storage treatment, a batch of 20 fruit per block was removed and placed at room temperature for maturity indexing within 24 hours (fruit were allowed to reach room temperature). The remaining fruit for that particular storage duration were transferred to 15°C to allow ripening. Two ripening periods were used; seven and 11 days, to simulate shelf life. Subsequent to each ripening period, maturity and quality analysis were performed; a similar procedure as after storage.

2.3 Maturity indexing

The batch of 20 fruit used on each evaluation date was numerically labelled in order to maintain identity of quality attributes per fruit. Ground colour change from green to yellow was scored using a colour chart for apples and pears (developed by Unifruco Research Services (URS), South Africa) with a scale of 0.5 to 5.0 (where 0.5 = dark green, and 5.0 = deep yellow). Two flesh firmness readings were taken on pared, opposite sides of each fruit using an electronic fruit texture analyser (FTA 2007, Güss, Strand, South Africa), fitted with a 8.0 mm diameter plunger.

2.4 Ethylene production

Ethylene production was measured after storage at -0.5°C and again after ripening at 15°C. Three replicates of five fruit each (individually labelled) were used from the 20 fruit sample. These were placed in 5 L air tight plastic jars at room temperature. After 30 min, a 10 mL gas tight syringe was used to withdraw headspace gas from the jar. This was injected into a gas chromatograph (Model N6980, Agilent technologies, Wilmington, U.S.A), with PorapakQ and Molsieve packed columns and flame ionization and thermal conductivity detectors. Total fruit mass and free space volume of the jar were then used to calculate the ethylene production rates per replicate.

2.5 Ripening potential

Ripening potential was assessed by determining the rate of change (β) in firmness, ground colour and ethylene production during ripening at 15°C. This was done per harvest date after each storage period used, for all the blocks per area per season. Rates of change were calculated by plotting each variable against days of ripening using Microsoft Office Excel, 2007. A linear regression equation of the form $y = \alpha + \beta x$ was then fitted to the data, to determine the slope / gradient (β), the response value (intercept- α) and adjusted R² value. The number of days to an "edible firmness" (3.5 kg) during ripening was also considered.

2.6 Data analysis

The gradient (β) and R² values for firmness, ground colour index and ethylene production were analysed per season using the General Linear Means (GLM) procedure in Statistical Analysis System (SAS) program (SAS Institute Inc. Cary, North Carolina, 1990). Significant differences between treatment means were separated using the least significant difference (LSD) at 5%. Further analysis using the same procedure in SAS was done on the values of each maturity variable.

3. Results

3.1 Firmness changes after storage (-0.5°C) and during ripening ($15^{\circ}C$)

3.1.1 2007 season

3.1.1.1 Immediately after storage at $-0.5^{\circ}C$

There were no significant interactions between storage duration, harvest time and area on firmness immediately after removal from -0.5°C, therefore data are presented as main effects (Fig. 1). 'Forelle' from KBV were significantly (P < 0.0001) firmer (6.4 kg) than fruit from WBV and Elgin (6.2 kg and 6.0 kg, respectively) (Fig.1A). Storage durations showed no statistical differences (P = 0.1535) (Fig. 1B). Flesh firmness decreased linearly (P < 0.0001) with time of harvest after storage at -0.5°C. After storage at -0.5°C the flesh firmness was \approx 0.5 kg lower than at harvest, for all harvest dates (Fig. 1C).

3.1.1.2 Ripening at $15^{\circ}C$

Harvest time and storage duration interacted significantly on flesh firmness rates of change during ripening at 15°C (Table 2). Fruit that were harvested four weeks prior to commercial harvest (H1) and stored for eight weeks at -0.5°C softened at a significantly lower rate of -0.287 kg day⁻¹, than fruit stored for 10 and 12 weeks (Table 2). Such fruit failed to attain "edible firmness" (3.5 kg) within 11 days at 15°C (Fig. 2A and 2B). This was
typical of fruit mainly from Buchuland farm in WBV, Graymead and Riveria farms in Elgin, Koelfontein and Parys located in KBV (data not shown). Fruit of the same harvest date from other farms (Achtertuin, Doornkraal and Platvlei = WBV; Kentucky and Molteno = Elgin; Molenrivier and Remhoogte = KBV), managed to attain "edible firmness" after seven days at 15° C (data not shown).

'Forelle' harvested after week five (H2, H3, H4 and H5), stored for eight weeks at -0.5° C had a lower firmness after seven and 11 days (< 3.0 and < 2.0 kg, respectively) at 15°C (Fig. 2A and 2B), compared to fruit stored for 10 and 12 weeks and ripened for seven and 11 days. Furthermore, these fruit showed a slightly higher softening rate during ripening (> 0.3 kg day⁻¹) (Table 2). On day seven at 15°C, H3 fruit stored for 12 weeks had a higher firmness (3.4 kg) than fruit stored for eight and 10 weeks. However, this was comparable to that of H1 fruit, previously stored for 12 weeks (Fig. 2A). Firmness of fruit harvested at H2, H4 and H5 was more or less 0.8 kg lower than an "edible firmness" (3.5 kg) after 12 weeks at -0.5°C plus seven days at 15°C (Fig. 2A). Late season fruit (H4 and H5) displayed a similar softening behaviour (> 0.35 and > 0.30 kg day⁻¹, respectively) at 15°C for all storage periods (Table 2 and Fig. 2B). Elgin fruit softened at a significantly slower rate (-0.366 kg day⁻¹) compared to fruit from WBV (-0.396 kg day⁻¹) and KBV (-0.392 kg day⁻¹) (data not shown).

3.1.2 2008 season

3.1.2.1 Immediately after storage at $-0.5^{\circ}C$

A significant interaction (P = 0.0106) between harvest time and storage period was observed just after fruit removal from -0.5° C (0 days at 15° C) (Fig. 3A) in the 2008 season. Flesh firmness declined linearly with harvest time for all atorage periods (Fig. 3A). The

earlier the harvest the longer the cold storage needed for fruit to soften. However, from the optimum harvest to the last harvest (H3 to H5), storage duration did not differ significantly due to ripening already being initiated. Only a slight drop in firmness (0.4 kg) was observed after eight weeks at -0.5°C on 'Forelle' harvested at commercial harvest and earlier. Late harvested fruit (H4 and H5) nearly maintained the firmness they had at harvest (Fig. 3A). Extending the length of storage at -0.5°C significantly reduced firmness, particularly on fruit harvested before commercial harvest (H1 and H2). Firmness of H1 and H2 fruit, stored for 16 weeks was 1.1 kg and 0.5 kg lower than that of fruit stored for eight weeks (Fig. 3A). However, storage durations displayed no significant effect on firmness of 'Forelle, harvested at commercial harvest and later (Fig. 3A). Elgin fruit had a significantly lower firmness (6.1 kg) compared to fruit from WBV (6.6 kg) and KBV (6.4 kg) (Fig. 3B).

3.1.2.2 Ripening at 15°C

Contrary to 2007, H1 fruit from all farms harvested in 2008 managed to soften to an "edible firmness" (3.5 kg) within 11 days (15° C), when stored for eight weeks at -0.5°C (data not shown). By day seven at 15° C, firmness was 4.0 kg lower than that determined directly after eight weeks (0 days at 15° C) (Fig. 4A). H1 fruit softened at a rate of -0.494 kg day⁻¹, during the ripening period up to 11 days (Table 2). On all harvest dates, firmness rate of change at 15° C appeared to decrease with increased time in storage (-0.5°C) (Table 2). Fruit stored for eight weeks softened at an advanced rate at 15° C (Table 2), compared to 12 and 16 weeks storage duration. Consequently, such fruit were the softest (< 2.5 kg) after 11 days at 15° C, particularly the optimum and late harvested fruit (H3, H4 and H5) (Fig. 4B).

Eight and 12 week periods showed no significant differences in firmness, at day seven of ripening for the H1, H2, H3 and H5 fruit (Fig. 4A). However, fruit harvested two weeks

after commercial harvest (H4), stored longer than eight weeks (-0.5° C) displayed a significantly higher firmness at 15°C (Fig. 4A). Firmness was 0.7 kg and 1.0 kg higher on fruit previously stored for 12 and 16 weeks, respectively compared to the H4 fruit stored for eight weeks (Fig. 4A). The rates of change during ripening of H4 fruit, stored for 12 and 16 weeks were -0.359 and -0.304 kg day⁻¹, respectively (Table 2).

Only fruit harvested in 2008 showed a significant interaction (P = 0.0040) between harvest time and growing area in rates of change of firmness at 15°C (Table 3). Softening rates decreased in sequence with time of harvest in all the areas during ripening at 15°C. Fruit harvested early (H1) in the season, which initially had higher firmness, had the highest softening rates (Table 3), and the later the harvest the lower was the rate of change. Softening behaviour was similar for fruit from H1 and H2 in WBV, and firmness decreased at a rate of -0.504 and -0.491 kg/day⁻¹, respectively. Firmness of fruit from the Elgin area decreased at a similar rate when harvested at both commercial harvest (H3) and two weeks after commercial harvest (H4) (Table 3). In KBV, fruit harvested at commercial harvest softened at a rate of -0.362 kg/day⁻¹. Such behaviour did not differ significantly from that of fruit from Elgin harvested at commercial harvest (H3) and two weeks after (H4).

Growing area interacted significantly (P < 0.05) with storage period during ripening at 15° C (Table 4, Fig 5A and 5B). 'Forelle' previously stored for a shorter duration at -0.5° C softened rapidly during ripening (Table 4). Fruit from the WBV softened at a faster rate on any given length in storage compared to Elgin and KBV, and were the softest after 11 days at 15° C (Table 4 and Fig. 5B). Flesh firmness for the three areas did not differ significantly when fruit were stored for eight weeks at -0.5° C and held for seven and 11 days at 15° C (Fig. 5A and 5B). In all areas, 'Forelle' stored for 12 and 16 weeks of cold storage showed no differences in firmness after seven days at 15° C (Fig. 5A). On day 11 at 15° C, fruit from

KBV, stored for 12 and 16 weeks maintained a higher firmness (2.5 kg and 2.7 kg, respectively), compared to the WBV and Elgin fruit (Fig. 5B).

3.1.3 2009 season

3.1.3.1 Immediately after storage at $-0.5^{\circ}C$

Immediately after storage (0 days at 15° C), a significant (P = 0.0189) interaction between growing area and harvest time was observed for fruit firmness (Fig. 6A). Similar to firmness behaviour at harvest (Fig. 6B) (not a significant interaction – indicated for differences between harvest and after storage), firmness decreased with harvest time in all areas at -0.5°C (Fig. 6A). The H1 and H5 fruit from WBV and KBV, had their firmness reduced by less than 0.7 kg from initial harvest to the time when fruit were removed from storage (Fig. 6A). While those from Elgin, only H1 and H2 fruit expressed a decline in firmness (0.4 kg and 0.6 kg, respectively) after storage (Fig. 6A). For the remaining harvests in these areas fruit retained their initial firmness, after storage at -0.5°C. The Elgin fruit displayed a lower firmness trend than fruit from WBV and KBV (Fig. 6A).

3.1.3.2 Ripening at $15^{\circ}C$

During ripening at 15°C, firmness declined significantly with increased time in storage of fruit harvested before commercial harvest (H1 and H2) (Fig. 7Ai). Fruit from KBV were significantly firmer (4.1 kg) compared to fruit from WBV and Elgin after seven days at 15°C (Fig. 7Aii). The rate of softening at 15°C for H1 and H2 was significantly lower (-0.150 and -0.245 kg'day⁻¹) on fruit exposed to chilling (-0.5°C) for only eight weeks, hence these fruit did not attain an "edible firmness" (3.5 kg) (Table 2, Fig. 7Ai and 7B). After

seven days at 15°C, firmness was only 0.2 kg (H1) and 1.6 kg (H2) lower than initial firmness at harvest. When these fruit were stored for up to 12 weeks at -0.5°C, firmness dropped drastically by approximately 50% at a rate of -0.493 and -0.464 kg day⁻¹, respectively. The H1 fruit, however, did not reach an "edible firmness" whereas H2 did (Fig. 7Ai).

Excluding H3 fruit, all other harvest dates resulted in a similar, low firmness, when 'Forelle' was stored (-0.5°C) for 12 and 16 weeks followed by seven days at 15°C (Fig. 7Ai). After 11 days at 15°C, firmness for all three storage periods did not differ significantly in fruit harvested at commercial harvest and later (Fig. 7B), since fruit were very ripe and soft.

3.2 Ethylene behaviour after storage $(-0.5^{\circ}C)$ and ripening $(15^{\circ}C)$

3.2.1 2007 season

3.2.1.1 Immediately after storage at $-0.5^{\circ}C$

Prolonged storage at -0.5°C significantly increased ethylene production of fruit harvested at commercial harvest or earlier (H3, H2 and H1) (Fig. 8Ai). When storage was extended from 10 to 12 weeks, ethylene increased seven-fold ($32.2 \ \mu L^{+}kg^{-1}h^{-1}$) and two-fold (77.9 $\ \mu L^{+}kg^{-1}h^{-1}$) on H1 and H2 (pre-optimum), respectively (Fig. 8Ai). After commercial harvest (H4 and H5) (post-optimum), no differences were observed between ethylene produced after eight, 10 and 12 weeks at -0.5°C (Fig. 8Ai). The WBV and Elgin fruit produced lower ethylene levels (34.1 and 34.8 $\ \mu L^{+}kg^{-1}h^{-1}$, respectively) than fruit from the KBV (42.9 $\ \mu L^{+}kg^{-1}h^{-1}$) (Fig. 8Aii).

3.2.1.2 Ripening at 15°C

Typical of ethylene production in climacteric fruit during ripening, this variable either increased or decreased at 15°C (Table 5 and 6). After eight weeks of storage at -0.5°C fruit displayed an increase in ethylene production during ripening at 15°C on all other harvest dates, besides the last harvest (Table 5). Comparison between harvest dates for the eight week period at -0.5°C showed that the rate of change in ethylene production at 15°C increased the fastest ($3.355 \ \mu L \ kg^{-1} \ h^{-1} \ day^{-1}$) on the H2 fruit (Table 5). By day seven at 15°C, the rate of ethylene production was 28.3 $\ \mu L \ kg^{-1} \ h^{-1}$ higher than when fruit were removed from -0.5°C (Fig. 9A). On day 11 at 15°C, ethylene production was 36 times higher than when H1 fruit were removed from cold storage (eight weeks) (Fig. 9Bi). The H1 fruit, stored for eight weeks, demonstrated similar rates of change to commercially harvested fruit (H3) stored for eight and 12 weeks (Table 5).

In 'Forelle' pears stored for 10 weeks, ethylene production appeared to be decreasing (post-climacteric) only in fruit harvested four weeks after commercial harvest (H5). Ethylene production decreased at a slope of -0.364 μ L'kg⁻¹.h⁻¹.day⁻¹ (Table 5). On other harvest dates, ethylene production increased during ripening at 15°C (Table 5). The H1 fruit produced 23.9 and 42.7 μ L'kg⁻¹.h⁻¹ of ethylene after seven and 11 days respectively (Fig. 9A and 9B). Rates of change for the H2, H3 and H4 fruit did not differ significantly on the 10 week period at 15°C (Table 5).

After 12 weeks at -0.5°C, the rate of change in ethylene production at 15°C did not differ significantly for the H2, H3, H4 and H5 fruit, however, the H2 and H3 still increased while H4 decreased (Table 5). In H1 fruit, however, the ethylene production increased at a significantly high rate of 2.067 μ L·kg⁻¹·h⁻¹·day⁻¹ (Table 5). By day 11 at 15°C, ethylene production was 22.2 μ L·kg⁻¹·h⁻¹ higher than when the H1 fruit were removed from cold storage (-0.5°C) (Fig. 9Bi). Rate of change for ethylene production in WBV fruit was significantly higher (1.766 μ L·kg⁻¹·h⁻¹·day⁻¹) than in Elgin and KBV (0.961 and 0.709)

 μ L'kg⁻¹·h⁻¹·day⁻¹, respectively) fruit, that did not differ significantly (data not shown). Consequently, the WBV fruit produced the highest (53.6 μ L'kg⁻¹·h⁻¹) amount of ethylene after 11 days at 15°C, (Fig. 9Bii).

3.2.2 2008 season

3.2.2.1 Immediately after storage at $-0.5^{\circ}C$

Immediately after removal from cold storage (-0.5°C), harvest time interacted significantly (P < 0.05) with growing area (Fig. 10A) and storage duration (Fig. 10B). At all harvests except for H5, ethylene production did not differ significantly for fruit sourced from the Elgin area (Fig. 10A). In early season fruit (H1), 'Forelle' from Elgin produced significantly lower levels of ethylene (43.8 μ L'kg⁻¹·h⁻¹) compared to fruit from WBV (64.4 μ L'kg⁻¹·h⁻¹) and KBV (68.7 μ L'kg⁻¹·h⁻¹) (Fig. 10A). WBV and KBV did not differ significantly in their ethylene production levels on all the harvest dates (Fig. 10A).

Ethylene appeared to increase with prolonged periods at -0.5°C in fruit harvested at H4 and earlier (Fig. 10B), even though firmness was similar at all storage periods for the H3 fruit (Fig. 3A). In late season fruit (H5), the ethylene production decreased with time at -0.5°C (Fig. 10B). Ethylene increased with harvest maturity after the eight week storage period (Fig. 10B). Apart from the H5 fruit stored for 12 weeks, the production of ethylene seemed to decline with maturity, when fruit was stored for 12 and 16 weeks at -0.5°C (Fig. 10B). Extending the cold storage period from 12 to 16 weeks at -0.5°C did not show a significant increase in ethylene production in fruit harvested from H1, H2, H3, and H4. Ethylene produced by the H5 fruit after eight weeks of storage was similar (\geq 59.6 µL·kg⁻¹·h⁻¹) to H2 fruit stored for 12 and 16 weeks, and the H3 fruit stored for 16 weeks (Fig. 10B).

3.2.2.2 Ripening at $15^{\circ}C$

There was a significant interaction of harvest time by storage duration (P < 0.0001) and harvest time by growing area (P = 0.0433) on the rates of change of ethylene production during ripening at 15°C (Table 5 and 6). However, no interactions were observed on the levels of ethylene produced after seven and 11 days at 15°C (Fig. 11A, 11B, 11C, 12A, 12B and 12C).

After seven days of ripening, fruit from the WBV produced higher (not significant) levels (49.1 μ L·kg⁻¹·h⁻¹) of ethylene than the Elgin (44.8 μ L·kg⁻¹·h⁻¹) and KBV (42.6 μ L·kg⁻¹·h⁻¹) areas (Fig. 11A). Ethylene production increased significantly with extended time in storage (Fig. 11B and 12B). Harvest dates did not differ in ethylene production during day seven of ripening (Fig. 11C) but, after 11 days at 15°C clear differences were observed (Fig. 12C).

Apart from the late season fruit (H5), after eight weeks of cold storage (-0.5°C), ethylene increased with days during ripening at 15°C (Table 5). The rate of change was significantly higher in early season's fruit (H1 and H2) (3.148 and 2.678 μ L'kg⁻¹h⁻¹day⁻¹, respectively) previously stored for eight weeks at -0.5°C. These production rates were comparable to H4 and H5 fruit stored for 16 weeks (Table 5). 'Forelle' stored for up to 12 weeks experienced a decrease in ethylene production at 15°C when harvested at H1, H2 and H5 (Table 5). After 16 weeks of storage ethylene production declined only when fruit were harvested in week five, otherwise fruit for other harvest dates expressed an increase in ethylene production during ripening at 15°C (Table 5). Also, fruit from areas that initially produced more than 53.0 μ L.kg⁻¹h⁻¹ of ethylene just after storage (0 days at 15°C) (Fig. 10A), experienced a decrease in ethylene production at 15°C (Table 6).

3.2.3 2009 season

3.2.3.1 Immediately after storage at $-0.5^{\circ}C$

Upon removal from -0.5°C, ethylene production appeared to increase with maturity on fruit stored for eight and 12 weeks, but when stored for up to 16 weeks ethylene declined gradually with maturity (Fig. 13). Increasing the duration of cold storage at -0.5°C, increased ethylene production significantly at all the harvest dates (Fig. 13). 'Forelle' harvested at commercial harvest time and earlier (H3, H2 and H1) produced small concentrations of ethylene (< $1.2 \ \mu L \ kg^{-1} \ h^{-1}$) after eight weeks at -0.5°C, whereas H4 and H5 produced 5.8 and 17.8 $\mu L \ kg^{-1} \ h^{-1}$, respectively (Fig. 13). For late season fruit (H5), extending the storage period from 12 to 16 weeks showed no significant effect on ethylene production (Fig. 13), since at 16 weeks ethylene production was decreasing already. In other harvest dates (H1, H2, H3, and H4), all three storage periods differed significantly. In commercial harvested fruit, for example, after 12 weeks at -0.5°C, ethylene production was 26 times higher than in fruit previously stored for eight weeks (Fig. 13).

3.2.3.2 Ripening at $15^{\circ}C$

Ethylene production increased linearly with days at 15° C (Table 5). Although the rates of change did not differ significantly in the 2009 season, H1 fruit expressed an exceptionally high rate of change (3.169 μ L·kg⁻¹·h⁻¹·day⁻¹) at 15°C, in fruit previously stored for 12 weeks (Table. 5). After seven and 11 days at 15°C, harvest time and storage duration interacted significantly. Also the areas had a significant effect on the ethylene produced after ripening (Fig. 14Ai, 14Aii, 14Bi, 14Bii). Fruit harvested before commercial harvest (H1 and

H2), stored for 16 weeks at -0.5°C produced high ethylene levels (> 80.0 μ L·kg⁻¹·h⁻¹) on day 11 at 15°C. While, those harvested at commercial harvest and later (H3, H4 and H5) produced 74.9, 63.1 and 71.1 μ L·kg⁻¹·h⁻¹, respectively (Fig. 14Bi).

3.3 Ground colour changes after storage $(-0.5^{\circ}C)$ and during ripening $(15^{\circ}C)$

3.3.1 2007 season

3.3.1.1 Immediately after storage at $-0.5^{\circ}C$

Harvest time and storage duration interacted significantly (P = 0.0010) on ground colour of 'Forelle' (Fig. 15A). Colour change from green to yellow progressed gradually over time of harvest (Fig. 15A). Earlier harvested fruit (H1) were green (\approx 2.5) and late harvested (H5) fruit slightly yellow (\approx 3.5). Ground colour yellowing also slowly advanced with cold storage duration at -0.5°C (Fig. 15A), but this difference was not significant in H1, and H5 fruit. Fruit from commercial harvest (H3), stored for eight and 10 weeks retained their initial colour index (2.9) determined at harvest. However, when stored up to 12 weeks, colour advanced by 0.7 colour index units. The 10 and 12 week storage periods did not differ significantly on ground colour (±3.6), when 'Forelle' was harvested two weeks after commercial harvest (H4) (Fig. 15A). Elgin fruit were more advanced in ground colour (3.3) compared to WBV (2.9) and KBV (3.1), when fruit were removed from -0.5°C (Fig. 15B).

3.3.1.2 Ripening at 15°C

Figures 16 and 17 show changes in 'Forelle' ground colour after seven and 11 days at 15° C. At day seven of ripening, no interaction (P > 0.05) was observed between factors (Fig.

16). 'Forelle' from the Elgin area were significantly advanced (3.6) in ground colour after seven days at 15°C compared to the WBV (3.4) and KBV (3.5) (Fig. 16A). Ground colour increased significantly with storage time (Fig. 16B) and harvest time (Fig. 16C).

After 11 days at 15°C, harvest time and storage period interacted significantly (P = 0.0003) and growing area had a significant effect on ground colour (Fig. 17). Colour progression from green (< 2.5) to yellow (> 3.5) increased linearly during ripening at 15° C in all treatment combinations (Table 7). The rate of change of this parameter appeared to decline with maturity (Table 7). Progression from green to yellow was the slowest (<0.035 colour index unit day⁻¹) for late harvested (H5) fruit, compared to fruit harvested earlier (Table 7), as fruit from later harvests are already yellow. Also, for early season fruit (H1) the rate of colour progression at 15°C increased with prolonged storage at -0.5°C (Table 7 and Fig. 17A). The rate of change in H1 fruit stored for 12 weeks was 0.117 colour index unit day⁻¹ (Table 7), and by day 11 at 15°C, ground colour index in the H1 fruit was 3.9 (yellow). For harvests after H1, the rate of ground colour change was higher when fruit were stored for eight weeks (Table 7). All three storage periods resulted in the same ground colour (4.1) on day 11 at 15°C for H2 and H5, while other harvests showed differences (Fig. 17A). The ground colour rate of change was highest (0.088 colour index unit day⁻¹) in the WBV at 15°C than in Elgin and KBV fruit (data not shown). After 11 days at 15°C the ground colour index for the KBV was at 4.0 and did not differ significantly to that of WBV (3.9) and Elgin (4.1) fruit (Fig. 17B).

3.3.2 2008 season

3.3.2.1 Immediately after storage at $-0.5^{\circ}C$

Upon removal from cold storage (-0.5°C) the 'Forelle' ground colour index increased with storage period and harvest maturity (Fig. 18A). Fruit stored for up to 16 weeks at -0.5°C showed a more advanced colour index than fruit stored for eight and 12 weeks (-0.5°C), respectively (Fig. 18A). The 12 and 16 weeks at -0.5°C did not differ significantly in ground colour of 'Forelle' harvested two weeks after commercial harvest (H4) (Fig. 18A). Also, at commercial harvest fruit retained the same colour (2.6) observed at harvest when stored for eight weeks, but when stored for 12 and 16 weeks, ground colour index increased to 3.2 and 3.4, respectively. Similar to the 2007 season, fruit from the Elgin area were more advanced in ground colour index (3.2) than the WBV (2.8) and KBV (3.0) upon removal from -0.5°C (Fig. 18B).

3.3.2.2 Ripening at 15°C

In early season fruit (H1 and H2), ground colour increased at a relatively similar rate (±0.110 colour index unit day⁻¹) (Table 7) for fruit of all treatment combinations (harvest time by storage duration), except in H2 fruit stored for 16 weeks. There was a significant interaction between harvest time and storage duration on day seven of ripening (Fig. 19Ai). 'Forelle' fruit from the Elgin area had a significantly higher colour index (3.8) than fruit from the WBV (3.4) and KBV (3.6) (Fig. 19Aii) on day seven at 15°C. Both after seven and 11 days at 15°C, ground colour for H2 fruit did not differ significantly when fruit were stored for 12 and 16 weeks (Fig. 19Ai and 19Bi).

'Forelle' harvested at commercial harvest and stored for the mandatory 12 weeks showed a similar rate of change (± 0.096 colour index unit day⁻¹) to fruit stored for eight and 16 weeks at -0.5°C (Table 7). However, when harvested after commercial harvest (H4 and H5), the ground colour rate of change at 15°C decreased at -0.5°C (Table 7). As a result, fruit that were stored for 16 weeks showed a lower trend in colour index than fruit stored for eight and 12 weeks on day 11 of ripening (Fig. 19Bi).

Figure 19Bii shows the growing area by harvest time interaction (P = 0.0347), observed on day 11 at 15°C. Fruit from the three areas expressed the same ground colour index (4.2) on day 11 at 15°C, for the H2 fruit. The Elgin fruit showed a more advanced ground colour (> 4.3) compared to the WBV and KBV, respectively when harvested at commercial harvest time and later (Fig. 19Bii).

3.3.3 2009 season

3.3.3.1 Immediately after storage at $-0.5^{\circ}C$

There was no treatment interactions observed directly after cold storage (-0.5°C) (Fig. 20) in this season. Growing areas showed no effect (P = 0.2707) (Fig. 20A), but storage duration at -0.5°C and harvest time affected 'Forelle' ground colour significantly (P < 0.05) (Fig. 20B and 20C). Colour progression from green to yellow increased with storage and harvest time (Fig. 20B and 20C). Fruit that were stored for eight weeks were 0.3 units greener in colour index than those stored for 12 weeks (Fig. 20B). H1 fruit had advanced by 0.9 units from the time of harvest to when fruit were removed from cold storage (-0.5°C) (Fig. 20C).

3.3.3.2 Ripening at 15°C

Similarly to the 2007 and 2008 seasons, harvest time interacted significantly (P < 0.05) with storage period on ground colour during ripening at 15° C (Table 7, Fig. 21). Ground colour index also progressed linearly during ripening at 15° C (Table 7). For H2 fruit,

the ground colour rate of change at 15°C increased with extended time in storage (-0.5°C). However, in 'Forelle' harvested at commercial harvest and later, the rate of change was slowest when fruit were stored for 16 weeks at -0.5°C (Table 7). Between the three storage periods (8, 12 and 16 weeks) on day seven at 15°C, ground colour progressed rapidly with harvest time for fruit stored for eight weeks (-0.5°C) (Fig. 21Ai). WBV and Elgin fruit had a significantly higher (3.3) ground colour index after seven days at 15°C compared to the KBV (3.1) fruit (Fig. 21Aii). By day 11 at 15°C, late harvested fruit were more yellow (3.9) after eight weeks storage as opposed to 12 and 16 weeks (Fig. 21Bi), and this was associated with lower firmness (Fig. 7B).

Little change in ground colour was observed during ripening $(15^{\circ}C)$ in the H1 fruit stored for eight weeks at -0.5°C. Ground colour changed at a rate of 0.024 colour index unit day⁻¹ (Table 7), and by day 11 at 15°C fruit were still green (2.8) (Fig. 21Bi). Also, lengthening the storage duration to 12 weeks significantly improved ground colour by 1.0 colour index unit (light yellow = 3.8) after 11 days at 15°C (Fig. 21Bi). Storing fruit longer than 12 weeks at -0.5°C did not show any further effect on ground colour at 15°C on day 11 for the H1 fruit (Fig. 21Bi).

The H2 fruit stored for 12 and 16 weeks changed to the same ground colour (slightly yellow = 3.2) after seven days (15° C) (Fig. 21Ai), at rates of 0.058 and 0.078 colour index day⁻¹, respectively (Table 7). On day 11 at 15° C 'Forelle' harvested at commercial harvest (H3) and two weeks later (H4), also stored for eight and 12 weeks at -0.5°C were more than 0.1 unit advanced in ground colour index than those stored for 16 weeks (Fig. 21Bi). The rate of change in ground colour was significantly higher (0.066 colour index unit day⁻¹) in WBV and Elgin fruit compared to KBV (0.050 colour index unit day⁻¹) fruit (data not shown). After ripening for 11 days, WBV and Elgin fruit still

maintained a significantly higher ground colour (3.8 and 3.7, respectively) than the KBV fruit (3.5) (Fig. 21Bii).

4. Discussion

Ripening of pears is closely associated with loss of firmness, gradual yellowing of skin colour as well as a climacteric rise in ethylene production (Chen and Mellenthin, 1981; Chen et al., 1983; Martin, 2002). In all these changes, harvest maturity, storage duration and growing area played a significant role in this study. Firmness and ground colour at 15°C showed a consistent pattern in all three seasons. Firmness decreased with increased ripening time at 15°C (Table 2, 3, and 4), while ground colour increased. Ethylene on the other hand, either increased or decreased during ripening at 15°C depending on the developmental stage of fruit relative to their climacteric. However, the behaviour of these variables at 15°C was influenced significantly mainly by the interaction of these factors.

In agreement with Chen and Mellenthin (1981), earlier harvested fruit (H1, H2) showed the lowest ripening potential, when stored for a short period (8 weeks) at -0.5°C compared to the late harvests (H3, H4 and H5). In 2007, fruit harvested in week five (H1) and stored up to eight weeks did not reach an "edible firmness" (3.5 kg) by day 11 at 15°C. This was also observed in 2009, when fruit were harvested at week five and seven (H1 and H2) and stored for eight weeks.

Furthermore, the H1 fruit of the 2007 and 2009 seasons had extremely low rates of ethylene production ($<0.5 \ \mu L^{1}kg^{-1}h^{-1}$) compared to fruit stored for more than eight weeks at -0.5°C. This indicated that in earlier season fruit, an eight week period at -0.5°C is not sufficient to induce substantial amounts of ethylene to promote ripening changes. Our findings conformed to those of 'Bartlett' pears (Puig et al., 1996), where less mature fruit

lacked the ability to start autocatalytic ethylene production at ripening. Mellenthin and Wang (1977) discovered that pre-harvest day and night temperatures of 21.1°C and 7.2°C, respectively, prevailing four weeks prior to harvest would cause accelerated ripening. Also, in South Africa, Frick (1995) found that an accelerated decline in 'Bon Chretien' flesh firmness was associated with a cool summer. This may be the case for the H1 and H2 fruit in the 2008 season.

Loss of firmness at 15°C tended to increase with increased time in storage (-0.5°C) on harvest dates before commercial harvest (pre-optimum). However, if fruit were harvested at commercial harvest and later (optimum and post-optimum), reduced periods (< 12 weeks) at -0.5°C resulted in rapid softening at 15°C, compared to longer periods. Extending the cold period seemed to influence cell wall degradation; the longer the storage duration probably the less rapid was the cell wall degradation, hence fruit were firmer. 'Forelle' (H3, H4 and H5) stored for eight weeks also expressed a more pronounced yellow ground colour at 15°C. This was a common observation in all three seasons. Murayama et al. (2002) associated rapid softening in 'Marguerite Marillat' and 'La France' pears with extensive degradation of cell wall polysaccharides, particularly the alkali-soluble polyuronides, hence resulting in abnormal ripening. This could possibly relate to 'Forelle' stored for a short period at -0.5°C, since the fruit from the eight week storage duration (-0.5°C) were generally associated with mealiness after ripening, particularly in the late harvested fruit. Also, this may further suggest that extended storage causes cell wall enzymes to become less active during ripening, as those fruit stored for 10, 12 and 16 weeks were firmer than those stored for eight weeks.

However, in 2009 H4 fruit, cold stored for 12 and 16 weeks, yielded the same firmness (± 3.2 kg by day 7 at 15°C) as fruit stored for eight weeks. In 2008, at the same storage periods as 2009, clear firmness differences were observed between storage periods at 15°C (Fig. 4). This could possibly be related to increased maturation observed pre-harvest on

the 2009 fruit, where the firmness decreased at a higher rate (-0.044 kg day⁻¹) compared to 2008.

Unlike firmness and ground colour that expressed a uniform pattern during ripening at 15°C, ethylene behaved differently, as it was either increasing or decreasing at 15°C. It is typical of this parameter to behave in such manner, as 'Forelle' is a climacteric fruit. However, we could not deduce a clear pattern as to whether the rising and declining of ethylene production is a response associated with maturity, or a specific threshold in ethylene production after storage. For example in 2007, H4 fruit stored for 12 weeks at -0.5°C, initially produced 50.0 μ L·kg⁻¹·h⁻¹ (0 days at 15°C) of ethylene, and this declined at a rate of -0.105 μ L·kg⁻¹·h⁻¹·day⁻¹ at 15°C. At a more or less equivalent rate of ethylene (51.8 μ L·kg⁻¹·h⁻¹) produced after 12 weeks (-0.5°C), in the H5 fruit, ethylene production was increasing at a rate of change of 1.193 μ L·kg⁻¹·h⁻¹·day⁻¹ at 15°C. In addition, H2 fruit in the 2008 season, stored for 12 and 16 weeks, respectively, produced similar amounts of ethylene (63.0 μ L·kg⁻¹·h⁻¹) directly after removal from -0.5°C, but their behaviour at 15°C differed. The 12 week period expressed a declining pattern, while for the 16 week period, ethylene continued to increase at 15°C. In 2009, the rate of change in ethylene production was increasing in all treatment combinations at 15°C.

Therefore, the behaviour of ethylene during ripening (15°C) of 'Forelle' merits further investigation, in order to have a clear understanding of the actual mechanism and related factors. More fruit per replicate is recommended, since 'Forelle' maturity and therefore ripening was very variable at times. Among other factors, latent infections (eg Penicillium) in the calyx may occur on later stored fruit, and this may increase the ethylene production rates abnormally.

Ground colour yellowing was slowly increased with prolonged cold storage at -0.5° C in all seasons. The trend of ground colour progression over prolonged storage at -0.5° C was

more or less similar in all the seasons; the longer the storage period at -0.5°C, the more advanced was the ground colour index at removal from -0.5°C. Ripening fruit at 15°C for seven and 11 days subsequent to cold storage (-0.5°C), significantly increased yellowing of the ground colour. The H4 and H5 fruit at 15°C expressed a more pronounced yellow ground colour following shorter storage duration. This could possibly be associated with the rapid softening earlier observed on fruit stored for eight weeks, as firmness is highly correlated with ground colour (Eccher Zerbini, 2002).

Over harvest time, fruit stored for eight weeks showed a steeper slope in firmness compared to longer storage periods, while for ground colour the trend was more similar between the storage durations. This suggested that for ground colour, storage periods show a similar progression over time of harvest. The only distinction is that longer stored fruit show a more advanced colour directly after removal from -0.5°C. However, after days (7 and 11) at 15°C, the relationship was more curvelinear. The first slope from H1 to H3 being much steeper on 'Forelle' stored for the eight week storage period than longer periods. Later for the H3 to H5, the slope increased gradually in a steady pattern. This implied that from H3 to H5, little change in firmness and ground colour could be observed since the fruit is already ripe.

Of the three 'Forelle' growing areas studied, distinct ripening patterns were mainly evident in 2008 (Table 3, 4, 6). In 2008 fruit from WBV softened faster in response to a period in storage than fruit from Elgin and KBV (Table 4). The differences between the growing areas are most likely related to the rate of maturation (firmness rates of change), that was due to the differences in maturity as influenced by days after full bloom (DAFB). The WBV fruit matured at a faster rate (-0.044 kg'day⁻¹) when compared to Elgin and KBV. Furthermore, the WBV area accumulated more heat units (HU) (19632 HU) before harvest than did Elgin (17995 HU) and the KBV (16115 HU). This may explain the differences in

ripening pattern between these areas, since high spring temperatures cause a rapid drop in firmness (Lötze and Bergh, 2005).

5. Conclusion

The duration of cold storage (-0.5°C) necessary to induce ripening potential in 'Forelle' is influenced by harvest maturity. Early season fruit would require more than eight weeks of cold storage at -0.5°C to sufficiently induce ripening, but this varies between seasons, possibly because of different prevailing climatic conditions. For fruit harvested at commercial harvest and later, an eight week storage period instead of the mandatory 12 week used by the industry, was sufficient to induce ripening. However, it appeared that fruit stored for eight weeks will soften rapidly at 15°C, thus indicating a shorter shelf life, compared to fruit stored for longer. Therefore, these findings may not change the current mandatory 12 week period, based only on ripening potential without considering mealiness and astringency (Chapter 4).

Of the three variables used to determine ripening potential, firmness and ground colour showed a more uniform and consistent behaviour at 15°C, as opposed to ethylene production. Although, ethylene is a prime indicator of ripening, particularly in climacteric fruit, its behaviour during 'Forelle' ripening was unpredictable. Ethylene increased and declined, with no defined pattern related to the different factors (harvest maturity, storage duration and growing area) used in the study. This may have been due to the limitation of our study, since it was measured on a rather too wide interval (0, 7 and 11 days), considering that ethylene is a sensitive measurement. Henceforth, for such a variable to be reliably used in a prediction model, one has to consider evaluating it at shorter intervals e.g. daily, possibly

on seven consecutive occasions. This may help get a clearly defined pattern between the three phases i.e. pre-climacteric, climacteric and post-climacteric.

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-0.5 C ai	na mpennig per	ious at 15 C.		
Seasons	Areas ^x	Harvests ^y	Storage weeks	Ripening days
2007	A_1, A_2, A_3	H1, H2, H3, H4, H5	8, 10, 12	7, 11
2008	A ₁ , A ₂ , A ₃	H1, H2, H3, H4, H5	8, 12, 16	7, 11
2009	A_1, A_2, A_3	H1, H2, H3, H4, H5	8, 12, 16	7, 11

Table 1. Overview of experimental layout: Seasons, areas, time of harvest, storage periods at <u>-0.5°C and ripening periods at 15°C.</u>

^x A_1 = Warm Bokkeveld (WBV), A_2 = Elgin, A_3 = Koue Bokkeveld (KBV). ^y Harvests based on calendar dates: H1 = week 5, H2 = week 7, H3 = week 9, H4 = week 11, H5 = week 13.

Treatments	1 0	2007		2008		2009	
Harvest	Storage ^x	β ^y	R^2	β ^y	R^2	β^{y}	R^2
time	period			•		•	
Week 5 (H1)	\mathbf{S}_1	-0.287a	0.859(<0.0001)	-0.494g	0.979(<0.0001)	-0.150a	0.575(<0.0001)
	S_2	-0.457i	0.983(<0.0001)	-0.458f	0.968(<0.0001)	-0.493ef	0.986(<0.0001)
	S_3	-0.460i	0.968(<0.0001)	-0.425d	0.954(<0.0001)	-0.463e	0.983(<0.0001)
Week 7 (H2)	\mathbf{S}_1	-0.448hi	0.954(<0.0001)	-0.475f	0.965(<0.0001)	-0.245b	0.917(<0.0001)
	S_2	-0.453i	0.972(<0.0001)	-0.442e	0.966(<0.0001)	-0.464ef	0.978(<0.0001)
	S_3	-0.418ghi	0.960(<0.0001)	-0.396d	0.977(<0.0001)	-0.451e	0.987(<0.0001)
Week 9 (H3)	\mathbf{S}_1	-0.425ghi	0.946(<0.0001)	-0.428e	0.963(<0.0001)	-0.412de	0.991(<0.0001)
	S_2	-0.402fg	0.956(<0.0001)	-0.381cd	0.963(<0.0001)	-0.427e	0.964(<0.0001)
	S_3	-0.352cde	0.987(<0.0001)	-0.358b	0.967(<0.0001)	-0.391d	0.980(<0.0001)
Week 11 (H4)	\mathbf{S}_1	-0.394efg	0.965(<0.0001)	-0.428e	0.959(<0.0001)	-0.408d	0.972(<0.0001)
	S_2	-0.362def	0.985(<0.0001)	-0.359bc	0.992(<0.0001)	-0.396d	0.983(<0.0001)
	S_3	-0.352cde	0.806(<0.0001)	-0.304a	0.975(<0.0001)	-0.362cd	0.980(<0.0001)
Week 13 (H5)	\mathbf{S}_1	-0.314abc	0.958(<0.0001)	-0.366bcd	0.949(<0.0001)	-0.395d	0.959(<0.0001)
	S_2	-0.342bcd	0.975(<0.0001)	-0.338b	0.951(<0.0001)	-0.339c	0.975(<0.0001)
	S_3	-0.307ab	0.973(<0.0001)	-0.283a	0.963(<0.0001)	-0.308c	0.992(<0.0001)
Significance leve	el: Pr >F						
Area (A)		0.0105		<0.0001		0.1862	
Harvest time H)		<0.0001		<0.0001		<0.0001	
Storage period (S	S)	0.0091		<0.0001		<0.0001	
$H^*\!A$		0.2200		0.0004		0.6684	
A*S		0.5193		0.0043		0.3619	
H^*S		<0.0001		0.0372		<0.0001	
A*H*S		0.03085		0.5200		0.4487	

Table 2. Effect of harvest time and storage period (S) at -0.5°C on the rates of change.day⁻¹ (β) and R² (with significance levels at 5%) of firmness during ripening of 'Forelle' pears at 15°C in 2007, 2008 and 2009.

^x 2007 season: $S_1 = 8$ weeks, $S_2 = 10$ weeks, $S_3 = 12$ weeks. 2008 and 2009 season: $S_1 = 8$ weeks, $S_2 = 12$ weeks, $S_3 = 16$ weeks

^yMeans followed by the same letter do not differ significantly at P = 0.05.

Treatr	nents	,	2007		2008	0	2009
Harvest time	Area ^x	β	R^2	β ^y	R^2	β	R^2
Week 5 (H1)	WBV	-0.430ns	0.951(<0.0001)	-0.504f	0.967(<0.0001)	-0.404ns	0.840(<0.0001)
	Elgin	-0.350	0.906(<0.0001)	-0.435e	0.963(<0.0001)	-0.350	0.822(<0.0001)
	KBV	-0.419	0.948(<0.0001)	-0.432e	0.971(<0.0001)	-0.368	0.903(<0.0001)
Week 7 (H2)	WBV	-0.458	0.964(<0.0001)	-0.491f	0.966(<0.0001)	-0.428	0.937(<0.0001)
	Elgin	-0.425	0.964(<0.0001)	-0.407d	0.970(<0.0001)	-0.334	0.957(<0.0001)
	KBV	-0.436	0.957(<0.0001)	-0.407de	0.973(<0.0001)	-0.398	0.990(<0.0001)
Week 9 (H3)	WBV	-0.397	0.965(<0.0001)	-0.428de	0.959(<0.0001)	-0.439	0.977(<0.0001)
	Elgin	-0.377	0.959(<0.0001)	-0.370bc	0.961(<0.0001)	-0.392	0.973(<0.0001)
	KBV	-0.405	0.966(<0.0001)	-0.362bc	0.976(<0.0001)	-0.400	0.985(<0.0001)
Week 11 (H4)	WBV	-0.368	0.810(<0.0001)	-0.382c	0.968(<0.0001)	-0.418	0.975(<0.0001)
	Elgin	-0.369	0.980(<0.0001)	-0.362bc	0.979(<0.0001)	-0.374	0.980(<0.0001)
	KBV	-0.372	0.981(<0.0001)	-0.342ab	0.981(<0.0001)	-0.373	0.980(<0.0001)
Week 13 (H5)	WBV	-0.317	0.961(<0.0001)	-0.339ab	0.963(<0.0001)	-0.377	0.976(<0.0001)
	Elgin	-0.317	0.971(<0.0001)	-0.324a	0.957(<0.0001)	-0.328	0.979(<0.0001)
	KBV	-0.329	0.972(<0.0001)	-0.326a	0.942(<0.0001)	-0.345	0.973(<0.0001)
Significance level	l: Pr >F						
Area (A)		0.0105		<0.0001		0.1862	
Harvest time (H)		<0.0001		<0.0001		<0.0001	
Storage period (S))	0.0091		<0.0001		<0.0001	
H*A		0.2200		0.0004		0.6684	
A*S		0.5193		0.0043		0.3619	
H^*S		<0.0001		0.0372		<0.0001	
A*H*S		0.03085		0.5200		0.4487	

Table 3. Influence of harvest time and growing area on the rates of change.day⁻¹ (β) and R² (with significance levels at 5%) on firmness during ripening at 15°C of 'Forelle' pears, that were stored for weeks at -0.5°C during 2007, 2008 and 2009 cropping season.

^x WBV = Warm Bokkeveld, KBV = Koue Bokkeveld. ^y Means followed by the same letter do not differ significantly at P = 0.05.

Table 4. Effect of growing area and storage period (S) at -0.5° C on rates of change.day⁻¹ (β) and R² (with significance levels at 5%) on firmness during ripening at 15°C of 'Forelle' pears. Fruit were harvested in three consecutive seasons from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV).

Tre	atments		2007		2008		2009
Area	Storage ^x period	β	R^2	β^{y}	R ²	β	R ²
WBV		-0.402ns	0.947(<0.0001)	-0.466f	0.967(<0.0001)	-0.337ns	0.864(<0.0001)
	S2	-0.405	0.967(<0.0001)	-0.441e	0.967(<0.0001)	-0.466	0.976(<0.0001)
	S3	-0.381	0.869(<0.0001)	-0.393c	0.960(<0.0001)	-0.442	0.978(<0.0001)
Elgin	S1	-0.344	0.922(<0.0001)	-0.418d	0.963(<0.0001)	-0.294	0.867(<0.0001)
	S2	-0.393	0.974(<0.0001)	-0.383c	0.967(<0.0001)	-0.402	0.976(<0.0001)
	S3	-0.363	0.971(<0.0001)	-0.332a	0.968(<0.0001)	-0.368	0.985(<0.0001)
KBV	S 1	-0.380	0.939(<0.0001)	-0.435e	0.959(<0.0001)	-0.339	0.927(<0.0001)
	S2	-0.410	0.980(<0.0001)	-0.358b	0.971(<0.0001)	-0.410	0.980(<0.0001)
	S3	-0.387	0.975(<0.0001)	-0.329a	0.976(<0.0001)	-0.381	0.990(<0.0001)
Sig	nificance leve	<i>l: Pr>F</i>					
Area (A)		0.0105		<0.0001		0.1862	
Harvest i	time (H)	<0.0001		<0.0001		<0.0001	
Storage p	period (S)	0.0091		<0.0001		<0.0001	
H*A		0.2200		0.0004		0.6684	
A*S		0.5193		0.0043		0.3619	
H*S		<0.0001		0.0372		<0.0001	
A*H*S		0.03085		0.5200		0.4487	

^x 2007 season: $S_1 = 8$ weeks, $S_2 = 10$ weeks, $S_3 = 12$ weeks. 2008 and 2009 season: $S_1 = 8$ weeks, $S_2 = 12$ weeks, $S_3 = 16$ weeks. ^y Means followed by the same letter do not differ significantly at P = 0.05.

Table 5. Effect of harvest time and cold storage period (S) on rates of change.day⁻¹ (β) and R² (with significance levels at 5%) on ethylene production (μ L'kg⁻¹·h⁻¹), during ripening at 15°C of 'Forelle' pears. Fruit were harvested in three consecutive seasons from Warm Bokkeveld, Elgin and Koue Bokkeveld.

Trea	tments	200	7	2008			2009
Harvest time	Storage period	$d^x \overline{\beta^y}$	R^2	β^{y}	\mathbb{R}^2	β	R^2
Week 5 (H1)	\mathbf{S}_1	1.982b	0.775(<0.0001)	3.148a	0.974(<0.0001)	1.867ns	0.837(<0.0001)
	S_2	3.387a	0.885(<0.0001)	-2.665d	0.671(<0.0001)	3.169	0.833(<0.0001)
	S_3	2.067a	0.723(<0.0001)	-2.295d	0.598(<0.0001)	1.914	0.821(<0.0001)
Week 7 (H2)	\mathbf{S}_1	3.355a	0.947(<0.0001)	2.678a	0.852(<0.0001)	1.989	0.834(<0.0001)
	S_2	0.564c	0.616(<0.0001)	-0.893c	0.490(<0.0001)	1.994	0.757(<0.0001)
	S_3	0.067c	0.667(<0.0001)	0.176c	0.293(0.0044)	2.181	0.743(<0.0001)
Week 9 (H3)	\mathbf{S}_1	2.030b	0.891(<0.0001)	0.287c	0.690(<0.0001)	2.629	0.913(<0.0001)
	S_2	0.515c	0.725(<0.0001)	0.043c	0.505(<0.0001)	2.236	0.840(<0.0001)
	S_3	1.324bc	0.736(<0.0001)	0.043c	0.464(<0.0001)	1.821	0.644(<0.0001)
Week 11 (H4)	\mathbf{S}_1	0.828bc	0.770(<0.0001)	0.347c	0.679(<0.0001)	2.756	0.945(<0.0001)
	S_2	0.201c	0.605(<0.0001)	1.093b	0.564(<0.0001)	1.093	0.509(<0.0001)
	S_3	-0.105c	0.578(<0.0001)	2.321ab	0.767(<0.0001)	0.904	0.675(<0.0001)
Week 13 (H5)	\mathbf{S}_1	-0.262c	0.555(<0.0001)	-2.422d	0.731(<0.0001)	1.684	0.866(<0.0001)
	S_2	-0.364c	0.705(<0.0001)	-0.300c	0.464(<0.0001)	1.579	0.779(<0.0001)
	S_3	1.193bc	0.609(<0.0001)	1.826ab	0.833(<0.0001)	2.421	0.767(<0.0001)
Significance le	evel Pr>F		· · · ·		· · ·		
Area (A)		0.0019		0.0014		0.6713	
Harvest time (I	H)	<0.0001		<0.0001		0.5217	
Storage period	(S)	0.0315		<0.0001		0.6071	
H^*A		0.1530		0.0433		0.7646	
A*S		0.6214		0.2955		0.3153	
H*S		<0.0001		<0.0001		0.1949	
A*H*S		0.9100		0.8095		0.9821	

^x 2007 season: $S_1 = 8$ weeks, $S_2 = 10$ weeks, $S_3 = 12$ weeks. 2008 and 2009 season: $S_1 = 8$ weeks, $S_2 = 12$ weeks, $S_3 = 16$ weeks

^yMeans followed by the same letter do not differ significantly at P = 0.05.

Table 6. Influence of harvest time and growing area on the rates of change.day⁻¹ (β) and R² (with significance at 5%) on ethylene production (μ L'kg⁻¹·h⁻¹) during ripening at 15°C of 'Forelle' pears, harvested in 2007, 2008 and 2009 season from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV).

Treatme	ents		2007		2008		2009
Harvest time	Area	β	R^2	β^{x}	R^2	β	R^2
Week 5 (H1)	WBV	2.680ns	0.748(<0.0001)	-0.711c	0.781(<0.0001)	1.984ns	0.958(<0.0001)
	Elgin	1.961	0.801(<0.0001)	0.360bc	0.643(<0.0001)	1.907	0.798(<0.0001)
	KBV	2.800	0.836(<0.0001)	-1.747c	0.844(<0.0001)	3.059	0.823(<0.0001)
Week 7 (H2)	WBV	1.810	0.743(<0.0001)	0.991ab	0.595(<0.0001)	1.651	0.702(<0.0001)
	Elgin	0.969	0.795(<0.0001)	0.694b	0.516(<0.0001)	2.245	0.779(<0.0001)
	KBV	1.207	0.693(<0.0001)	0.149bc	0.519(<0.0001)	2.268	0.858(<0.0001)
Week 9 (H3)	WBV	2.124	0.856(<0.0001)	0.882ab	0.556(<0.0001)	2.151	0.740(<0.0001)
	Elgin	0.659	0.842(<0.0001)	0.086bc	0.701(<0.0001)	2.119	0.839(<0.0001)
	KBV	0.973	0.650(<0.0001)	-0.834c	0.351(0.0026)	2.417	0.819(<0.0001)
Week 11 (H4)	WBV	1.346	0.759(<0.0001)	1.994a	0.761(<0.0001)	1.978	0.759(<0.0001)
	Elgin	0.606	0.539(<0.0001)	0.700b	0.644(<0.0001)	1.523	0.682(<0.0001)
	KBV	-0.968	0.652(<0.0001)	1.005ab	0.584(<0.0001)	1.253	0.688(<0.0001)
Week 13 (H5)	WBV	0.570	0.767(<0.0001)	0.911ab	0.736(<0.0001)	2.385	0.877(<0.0001)
	Elgin	0.555	0.662(<0.0001)	-1.141c	0.695(<0.0001)	1.527	0.791(<0.0001)
	KBV	-0.463	0.476(<0.0001)	-0.385bc	0.591(<0.0001)	1.894	0.762(<0.0001)
Significance leve	l Pr>F		· · · ·		· · · ·		· · ·
Area (A)		0.0019		0.0014		0.6713	
Harvest time (H)		<0.0001		<0.0001		0.5217	
Storage period (S)	0.0315		<0.0001		0.6071	
H*A		0.1530		0.0433		0.7646	
A*S		0.6214		0.2955		0.3153	
H^*S		<0.0001		<0.0001		0.1949	
A*H*S		0.9100		0.8095		0.9821	

^xMeans in a column followed by the same letter do not differ significantly at P = 0.05.

Table 7. Effect of harvest time and cold storage period (- 0.5° C) on the rates of change.day⁻¹ (β) and R² (with significance at 5%) on ground colour index^x during ripening at 15°C of 'Forelle' pears, harvested in three consecutive seasons from Warm Bokkeveld , Elgin and Koue Bokkeveld.

Treatmer	nts	2007		2008		2009	
Harvest	Storage ^y	β ^z	\mathbb{R}^2	β ^z	R^2	β ^z	R^2
time	period						
Week 5 (H1)	\mathbf{S}_1	0.0893b	0.841(<0.0001)	0.110b	0.868(<0.0001)	0.024c	0.754(<0.0001)
	S_2	0.109ab	0.799(<0.0001)	0.125ab	0.925(<0.0001)	0.092ab	0.806(<0.0001)
	S_3	0.117ab	0.749(<0.0001)	0.120ab	0.924(<0.0001)	0.091ab	0.864(<0.0001)
Week 7 (H2)	\mathbf{S}_1	0.122a	0.915(<0.0001)	0.123ab	0.861(<0.0001)	0.055bc	0.816(<0.0001)
	S_2	0.108ab	0.822(<0.0001)	0.127ab	0.945(<0.0001)	0.058b	0.883(<0.0001)
	S_3	0.097ab	0.941(<0.0001)	0.070c	0.809(<0.0001)	0.078ab	0.869(<0.0001)
Week 9 (H3)	\mathbf{S}_1	0.094b	0.855(<0.0001)	0.111b	0.872(<0.0001)	0.093a	0.841(<0.0001)
	S_2	0.103ab	0.979(<0.0001)	0.096bc	0.798(<0.0001)	0.066ab	0.892(<0.0001)
	S_3	0.054c	0.859(<0.0001)	0.053c	0.864(<0.0001)	0.048bc	0.870(<0.0001)
Week 11 (H4)	\mathbf{S}_1	0.097ab	0.920(<0.0001)	0.149a	0.954(<0.0001)	0.065b	0.893(<0.0001)
	S_2	0.043cd	0.781(<0.0001)	0.092bc	0.949(<0.0001)	0.069ab	0.888(<0.0001)
	S_3	0.048cd	0.787(<0.0001)	0.088bc	0.827(<0.0001)	0.029c	0.785(<0.0001)
Week 13 (H5)	\mathbf{S}_1	0.034cd	0.760(<0.0001)	0.091bc	0.883(<0.0001)	0.076ab	0.912(<0.0001)
	S_2	0.028d	0.536(<0.0001)	0.077bc	0.789(<0.0001)	0.044bc	0.805(<0.0001)
	S_3	0.021d	0.655(<0.0001)	0.023d	0.697(<0.0001)	0.019c	0.847(<0.0001)
Significance level:	• Pr > F						
Area (A)		0.0069		0.0687		0.0095	
Harvest time (H)		<0.0001		<0.0001		0.0302	
Storage period (S)		0.003		<0.0001		0.0972	
$H^*\!A$		0.1206		0.2145		0.8505	
A*S		0.6080		0.6593		0.6326	
H*S		<0.0001		0.0182		<0.0001	
A*H*S		0.6672		0.9959		0.9982	

^x Colour index of 0.5 = dark green and 5 = deep yellow. ^y2007 season; $S_1 = 8$ weeks, $S_2 = 10$ weeks, $S_3 = 12$ weeks. 2008 and 2009 season; $S_1 = 8$ weeks, $S_2 = 12$ weeks, $S_3 = 16$ weeks. ^zMeans followed by the same letter do not differ significantly at P = 0.05.



Fig. 1. Firmness of 'Forelle' pears harvested four and two weeks before commercial harvest (H1 and H2), at commercial harvest (H3), two and four weeks after commercial harvest (H4 and H5) in 2007 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), then stored for weeks at -0.5°C. Star symbol (A) and broken line (C) indicate firmness at harvest. Vertical bars show LSD at 5%.



Fig. 2. Effect of harvest time and storage period on firmness of 'Forelle' pears harvested biweekly (H) in 2007 season from Warm Bokkeveld, Elgin and Koue Bokkeveld. Fruit were stored for weeks at -0.5°C then transferred to 15°C for 7 (A) and 11 (B) days. Dotted line at 3.5 kg represents optimum "edible firmness".



Fig. 3. Firmness of 'Forelle' pears, harvested biweekly (H) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in 2008 season and stored for 8, 12 and 16 weeks at -0.5°C. Broken line (A) and star symbol (B) indicate firmness at harvest. Vertical bars show LSD at 5%.



Treatment	Significance level: Pr > F				
	7 days (15°C)	11 days (15°C)			
Area (A)	< 0.0001	< 0.0001			
Harvest time (H)	< 0.0001	< 0.0001			
Storage period (S)	< 0.0001	< 0.0001			
A*H	0.7795	0.6782			
A*S	0.0077	0.0233			
H*S	< 0.0001	< 0.0001			
A*H*S	0.7722	0.9705			

Fig. 4. Effect of harvest time and storage period on firmness of 'Forelle' harvested biweekly (H) from Warm Bokkeveld, Elgin and Koue Bokkeveld in 2008, then stored at -0.5°C followed by 7 (A) and 11 (B) days at 15°C. Dotted line at 3.5 kg represents optimum "edible firmness". Vertical bars show LSD at 5%.



Fig. 5. Effect of growing area and storage period on firmness of 'Forelle' pears harvested biweekly (H) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in 2008, then stored at -0.5°C followed by 7 (A) and 11(B) days at 15°C. Dotted line at 3.5 kg represents optimum "edible firmness". Vertical bars show LSD at 5%.



Fig. 6A. Effect of area and harvest time (H) on firmness of 'Forelle' pears, harvested biweekly from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in 2009, then stored at -0.5°C. Vertical bars show LSD at 5%.



Fig. 6B. Average flesh firmness for 'Forelle at harvest in the 2009 season from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV).



Treatment	Significance level: Pr > F				
	7 days (15°C)	11 days (15°C)			
Area (A)	0.0002	0.6352			
Harvest time (H)	< 0.0001	< 0.0001			
Storage period (S)	< 0.0001	< 0.0001			
A*H	0.8124	0.9636			
A*S	0.1115	0.2651			
H*S	< 0.0001	< 0.0001			
A*H*S	0.5616	0.9318			

Fig. 7. Firmness of 'Forelle' pears harvested biweekly (H) in 2009 season from Warm Bokkeveld, Elgin and Koue Bokkeveld, stored for weeks at -0.5°C followed by 7 (A) and 11(B) days at 15°C. Dotted line at 3.5 kg represents optimum "edible firmness". Vertical bars show LSD at 5%.


Fig. 8. Ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly (H) in 2007 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), then stored for 8, 10 and 12 weeks at -0.5°C. Vertical bars show LSD at 5%.



Treatment	Significance level: Pr > F	
	7 days (15°C)	11 days (15°C)
Area (A)	0.0602	0.0055
Harvest time (H)	< 0.0001	< 0.0001
Storage period (S)	< 0.0001	< 0.0001
A*H	0.5033	0.4898
A*S	0.6624	0.2783
H*S	0.0007	< 0.0001
A*H*S	0.9669	0.8945

Fig. 9. Ethylene production (μ L'kg⁻¹h⁻¹) of 'Forelle' pears harvested biweekly in 2007 from Warm Bokkeveld, Elgin and Koue Bokkeveld, stored for weeks at -0.5°C then followed by 7 (A) and 11 (B) days at 15°C. Vertical bars show LSD at 5%.



Significance level			
Treatment	Pr > F		
Area (A)	0.0179		
Harvest time (H)	< 0.0001		
Storage period (S)	< 0.0001		
A*H	0.0264		
A*S	0.0567		
H*S	< 0.0001		
A*H*S	0.4295		

Fig. 10. Ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly (H) in 2008 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), after storage for 8, 12 and 16 weeks at -0.5°C. Vertical bars show LSD at 5%.



Fig. 11. Ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly (H) in 2008 season from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by 7 days at 15°C. Vertical bars show LSD at 5%.



Fig. 12. Ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly (H) in 2008 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by 11 days at 15°C. Vertical bars show LSD at 5%.



Fig. 13. Effect of harvest time (H) and storage duration on ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly in 2009 from Warm Bokkeveld, Elgin and Koue Bokkeveld, then stored for 8, 12 and 16 weeks at -0.5°C. Vertical bars show LSD at 5%.



Treatment	Significance level: Pr > F	
	7 days (15°C)	11 days (15°C)
Area (A)	0.0330	0.0311
Harvest time (H)	0.5278	0.5208
Storage period (S)	< 0.0001	< 0.0001
A*H	0.5580	0.4394
A*S	0.5545	0.5026
H*S	< 0.0001	0.0006
A*H*S	0.9962	0.9877

Fig. 14. Ethylene production (μ L'kg⁻¹·h⁻¹) of 'Forelle' pears harvested biweekly in 2009 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by 7 (A) and 11 (B) days at 15°C. Vertical bars show LSD at 5%.



Significance level				
Treatment	Pr > F			
Area (A)	< 0.0001			
Harvest time (H)	< 0.0001			
Storage period(S)	< 0.0001			
A*H	0.6305			
A*S	0.5354			
H*S	0.0010			
A*H*S	0.8263			

Fig. 15. Effect of storage duration and harvest time (H) on ground colour of 'Forelle' pears, harvested biweekly from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in 2007, and stored at -0.5° C. (Colour index of 0.5 = dark green and 5 = deep yellow). Broken line (A) and star symbol (B) indicate average ground colour at harvest. Vertical bars show LSD at 5%.



Fig. 16. Ground colour index of 'Forelle' pears harvested biweekly (H) in 2007 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by 7 days at 15°C. (Colour index of 0.5 = dark green and 5 = deep yellow). Vertical bars show LSD at 5%.



Fig. 17. Effect of storage period and harvest time (H) on ground colour of 'Forelle' pears, harvested biweekly from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) in 2007, stored for weeks at -0.5° C followed by 11 days at 15° C. (Colour index of 0.5 =dark green and 5 = deep yellow). Vertical bars show LSD at 5%.



Fig. 18. Ground colour of 'Forelle' pears harvested in 2008 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), then stored for 8, 12 and 16 weeks at -0.5° C (Colour index of 0.5 = dark green and 5 = deep yellow). Broken line (A) and star symbol indicate average ground colour at harvest. Vertical bars show LSD at 5%.



Fig. 19. Ground colour of 'Forelle' pears harvested in 2008 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for 8, 12 and 16 weeks at -0.5° C followed by 7 (A) and 11 (B) days at 15°C (Colour index of 0.5 = dark green and 5 = deep yellow). Vertical bars show LSD at 5%.

0.4404

< 0.0001

0.8877

0.2754

< 0.0001

0.8696

A*S

H*S

A*H*S



Fig. 20. Ground colour index of 'Forelle' pears harvested biweekly (H) in 2009 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), then stored for weeks at -0.5° C. (Colour index of 0.5 =dark green and 5 =deep yellow). Star symbol (A) and broken line (C) indicate average ground colour at harvest. Vertical bars show LSD at 5%.



Significance level: Pr > F		
7 days (15°C)	11 days (15°C)	
< 0.0001	< 0.0001	
< 0.0001	< 0.0001	
< 0.0001	< 0.0001	
0.7909	0.8779	
0.9382	0.3840	
< 0.0001	< 0.0001	
0.4681	0.7539	
	Significance 7 days (15°C) <0.0001	

Fig. 21. Ground colour of 'Forelle' pears harvested in 2009 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5° C, then transferred to 15° C for 7 (A) and 11 (B) days. (Colour index of 0.5 =dark green and 5 =deep yellow). Vertical bars show LSD at 5%.

Chapter 4: Paper 3

Influence of harvest maturity and cold storage periods on the incidence of mealiness and astringency in 'Forelle' pears

Abstract

Mealiness and astringency are the key eating quality disorders associated with South African 'Forelle' pears. These quality disorders, particularly mealiness, could be related to climatic differences in the growing regions. Hence, the objective of the trial was to determine the role of harvest maturity and cold storage duration on mealiness and astringency of 'Forelle' grown in three climatically diverse areas. Fruit were sourced from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Fruit were harvested biweekly for five harvest dates in three consecutive seasons (2007-2009). In 2007, fruit were stored for eight, 10 and 12 weeks, while in 2008 and 2009, fruit were stored for eight, 12 and 16 weeks, all in regular atmosphere at -0.5°C. Thereafter, fruit were ripened at 15°C for seven and 11 days. Significant incidence of astringency was observed in 'Forelle' harvested four weeks before commercial harvest time. In 2007, astringency was 36.6%, while in 2008 and 2009 only 12.0% of the fruit were astringent after eight weeks at -0.5°C. After eight weeks (-0.5°C) plus seven days at 15°C, mealiness was higher (>52.0%) in the 2007 season compared to 2008 (>40.0%) and 2009 (>40.0%). Extending cold storage from eight to 12 weeks at -0.5°C significantly reduced mealiness from 62.0% to 10.0% on fourth harvested fruit ripened for seven days in the 2007 season. Fruit from the WBV and Elgin, warmer areas compared to the KBV, were more prone to mealiness. In conclusion, the results of this trial do not support shortening the mandatory 12 weeks cold storage period, as eating quality is compromised.

Keywords: Flesh firmness; Fruit ripening; Heat units; Fruit Quality; Pyrus communis L.

1. Introduction

Generally, a good eating quality pear will ripen with a juicy, buttery and melting texture accompanied by good pear flavour (Eccher Zerbini, 2002). Fruit may fail to ripen to a good eating quality because of various factors; harvest maturity (Eccher Zerbini and Spada, 1993), storage condition and duration, and post-storage ripening condition (Martin, 2002; Mielke and Drake, 2005), and this impacts greatly on consumer acceptance. According to Harker et al. (1997), texture is one critical feature of pear quality influencing consumer acceptance, as it relates to changes in cell components (sugars, acids and volatile substances) during ripening (Eccher Zerbini, 2002).

Mealiness and astringency are key internal quality disorders associated with 'Forelle' eating quality in South Africa (Martin, 2002; Crouch et al., 2005; DFPT Technical Services, 2008; Crouch and Bergman, 2010). Attempts to understand mealiness and its related factors have not been very successful. Ben-Arie et al. (1989) links mealiness to the mechanism of juice released rather than the amount of juice present. Murayama et al. (2002) associate this inferior quality with lower polyuronide content, particularly the water-soluble polyuronides and alkali-soluble polyuronides. This is in agreement with Ben-Arie and Sonego (1980), who reported a similar observation in wooliness, a textural disorder linked to mealiness (Dawson et al., 1992). Contrary to 'Marguerite Marillat', 'La France' (Murayama et al., 2002) and 'd'Anjou' pears (Chen et al., 1983) that developed a dry floury texture following long storage periods, mealiness in 'Forelle' decreases with extended cold storage periods (> 12 weeks) at -0.5°C (Martin, 2002).

Microscopically, mealy flesh has its intercellular spaces filled with air rather than juice in apples (Harker and Hallet, 1992) and nectarines (Harker and Sutherland, 1993). Also, the cells appear separated due to dissolution of middle lamella, while non mealy tissue have well arranged non-detached cells (De Smedt et al., 1998). Moreover, the taste of such fruit gives a floury sensation in the mouth, with loss of crispiness and juiciness (Barreiro et al., 1998).

On the other hand, an astringent fruit gives a dry puckering mouth feel upon ingestion. This puckering mouth feel is due to plant phenolic compounds that bind with the oral mucopolysaccharides or proteins during mastication (Baxter et al., 1997). Astringency in pears and apples appears to be more of a maturity problem rather than that of storage (Eccher Zerbini and Spada, 1993; Young et al., 1999; Mielke and Drake, 2005), possibly due to high levels of tannins in less mature fruit (Ramin and Tabatabaie, 2003). However, in 'd' Anjou' pears harvested at optimum maturity, astringency was detected when fruit were stored for more than seven months at -1.1°C (Chen et al., 1983).

Seasonal and geographic differences also influence eating quality related disorders, particularly mealiness. An incidence of 53 to 70% mealiness was associated with growing seasons experiencing high total heat units (Hansen, 1961). This was further confirmed in 'd'Anjou' pears (Mellenthin and Wang, 1976) where fruit exposed to high daily temperatures six weeks before harvest ripened unevenly and were prone to mealiness. Cultural factors such as clay or heavy soils were also observed to favour astringency in pears (Downing, 2009 unpublished observation).

To ensure uniform ripening with 'acceptable' eating quality of 'Forelle', South African legislation enforces producers to store fruit for a minimum of 12 weeks at -0.5°C (De Vries and Hurndall, 1993; Du Toit et al., 2001). However, this duration does not seem to completely rectify the problem of inferior eating quality in 'Forelle'. Mealiness levels of more than 35% were reported in 'Forelle' stored for 12 weeks (Martin, 2002), hence, we suspect that other factors may play a role. Therefore, the objective of this study was to determine the role of harvest maturity together with cold storage duration, on mealiness and astringency of 'Forelle' pears grown in three climatically diverse areas, with the hypothesis that seasonal effects and growing location have an influence on 'Forelle' eating quality.

2. Materials and methods

2.1 Plant material and experimental lay-out

'Forelle' pears were harvested in three successive seasons (2007-2009) from three main growing areas of the Western Cape, South Africa: Warm Bokkeveld (WBV) (33°15'S; 19°15'E), Elgin (33° 54'S; 19°4'E) and Koue Bokkeveld (KBV) (33°8'S; 19°23'E). Four commercial farms were selected in each area and were considered as blocks for the trial. Harvesting was done at four and two weeks prior to commercial harvest (week 5 and 7, respectively), at commercial harvest (week 9), and two and four weeks after commercial harvest (week 11 and 13, respectively). Fruit harvested on these five dates were consequently referred to as H1, H2, H3, H4 and H5.

A uniform 240 fruit sample per block was harvested randomly at shoulder height from all sides of the trees at each harvesting date, to reduce the effect of fruit position. Only 20 of the fruit were used for maturity assessment at harvest. The remaining 220 fruit from each block were stored according to commercial packaging practice in regular atmosphere at -0.5°C. In the 2007 season, fruit were stored (-0.5°C) for eight, 10 and 12 weeks, while in 2008 and 2009, fruit were stored for eight, 12 and 16 weeks. Consequent to each storage period fruit were allowed to ripen at 15°C for seven and 11 days.

2.2 Quality measurements

Quality and ripening assessments were carried out after each storage period and again after ripening at 15°C for seven and 11 days. A sample of 20 fruit per block was used on every evaluation date. Fruit were individually evaluated for ground colour, mass, size, firmness, and starch breakdown (individual fruit identity was kept). A colour chart for apples and pears, developed by Unifruco Research Services (URS), South Africa (where 0.5 = dark green, 5 = deep yellow) was used to determine ground colour change from green to yellow. Fruit mass and size (diameter) were measured using an electronic balance and Cranston gauge (FTA 2007, Güss, Strand, South Africa). Firmness (kg) was determined using an electronic fruit texture analyser (FTA 2007, Güss, Strand, South Africa), fitted with an 8.0 mm diameter plunger. Two firmness readings were taken on peeled opposite sides of each fruit. The percentage starch breakdown was determined using the iodine test (50 g KI and 10 g of I₂ in 1 L distilled water) on the calyx-end half of the pear. A starch conversion chart for pears and apples developed by URS, South Africa (where 0% = totally stained surface and 100% = completely unstained) was then used to determine starch breakdown.

2.3 Ethylene production ($\mu L^{k}g^{-1}h^{-1}$)

Ethylene production was measured after storage (-0.5°C) and again after ripening at 15°C. Fruit were first allowed to warm up to room temperature before evaluation. Three replicates of five fruit (15 fruit) from each sample of the 20 fruit were placed in 5 L tight jars for 30 min. Samples were then collected using gas air tight syringes. These were injected into a gas chromatograph (Model N6980, Agilent technologies, Wilmington, U.S.A), assembled with PorapakQ and Molsieve packed column and flame ionization and thermal

conductivity detectors. Total fruit mass and free space volume of the jar were used to calculate ethylene production rates.

2.4 Evaluation for mealiness and astringency

The purpose of this assessment was to determine 'acceptable' eating quality of 'Forelle' pears after storage and ripening. 'Acceptable' eating quality was defined in terms of a fruit that was neither mealy nor astringent. Individual fruit assessment for mealiness and astringency was done subjectively, after each storage period at -0.5° C and again after every ripening period at 15° C. Longitudinal wedges ($\pm 1/6^{\text{th}}$ of fruit) were cut from each of the 20 fruit per evaluation date. Wedges were organoleptically assessed for astringency and mealiness as well as squeezed to assess free juice. Fruit that were dry with a coarse, floury texture were classified as mealy. Those that gave a dry puckering mouth feel were considered astringent. The same panel (evaluators) was used throughout the three trial seasons.

2.5 Data analysis

Logit transformed data on percentages of mealiness and astringency were analysed using the General Linear Means (GLM) procedure. Significant differences between means were separated using least significant difference at 5%. The Stepwise Discriminant Analysis (STEPDISC) procedure was used to determine the most important variables (ground colour index, fruit mass, size, firmness and starch breakdown) related to eating quality (astringent, non-astringent, mealy and non-mealy) of 'Forelle'. Thereafter, the variables selected from the STEPDISC procedure were used in a discriminant analysis (PROC DISCRIM) to determine whether the variables could be used to distinguish fruit in the different eating quality classes (astringent (1), non-astringent (2), mealy (3), non-mealy (4)). Finally, the data obtained from the STEPDISC analysis were further subjected to the Canonical Discriminant Analysis (CANDISC). All mentioned procedures were performed in Statistical Analysis System (SAS) program (SAS Institute Inc, Cary, North Carolina, 1990).

3. Results

3.1 Incidence of astringency in 'Forelle' pears over the seasons

3.1.1 2007 Season

Immediately after removal from -0.5°C (0 days at 15°C) 'Forelle' from the WBV had a significantly lower (5.9%) incidence of astringency than the Elgin (12.8%) and KBV (14.1%) areas (Fig. 1Ai). Harvest time and storage duration interacted significantly (P = 0.0196) on day zero at 15°C (Fig. 1Aii). Astringency was higher in fruit harvested four weeks before commercial harvest (H1) compared to other harvest dates (Fig. 1Aii). Occurrence of astringency was 36.6% on the first harvested fruit stored for eight weeks (-0.5°C). Extending the cold storage period to 12 weeks reduced the incidence to 12.5% (Fig. 1Aii). With week five (H1), nine (H3) and 11 (H5) harvested fruit, astringency appeared to decline with extended time at -0.5°C (Fig. 1Aii). Fruit harvested at commercial harvest and later (H3, H4, and H5) had significantly lower incidences of astringency (< 9.0%) after 10 and 12 week periods. By day seven at 15°C (Fig. 1B), no astringency was observed on commercial harvested fruit. On day 11 at 15°C, astringency was almost negligible (0 to 4%), except on H1 fruit stored for eight weeks (Fig. 1Ci and 1Cii). Directly after storage (0 days at 15° C), there was a significant interaction (P = 0.0143) between growing area and storage period (Fig. 2i). At any given storage period at -0.5°C, 'Forelle' from the WBV area experienced lower levels of astringency (< 5.0%) compared to the Elgin and KBV areas (Fig. 2i). However, these lower levels observed in the WBV area did not differ significantly to that of fruit from the Elgin (12 and 16 weeks (-0.5°C) and the KBV (8 and 16 weeks (-0.5°C)) (Fig. 2i). Astringency levels decreased significantly with harvest time. By the last harvest (H5), astringency was three times lower than in H1 (Fig. 2ii).

After seven and 11 days at 15°C, there was no significant interaction between factors, hence data were presented as main effects (Fig. 3A and 4B). By day seven (Fig. 3A) at 15°C, only 1.1% of astringency was observed in 'Forelle' from the WBV area. On day 11 at 15°C, less than 0.5% astringency was detected in the WBV fruit (Fig. 3B). Holding fruit for longer than seven days (11 days) at 15°C did not significantly reduce astringency in 'Forelle' from Elgin, but on KBV fruit, astringency levels were three times lower than at seven days (15°C) (Fig. 3A and 3B).

After seven days at 15°C, earlier harvested fruit (H1 and H2) had a higher (> 5.0%) incidence of astringency than fruit harvested at commercial harvest and later (H3, H4 and H5) (Fig. 3A). By day 11 at 15°C, astringency levels were less than 3.0%, and there was no significant difference (P = 0.5530) between the harvest dates (Fig. 3B).

Astringency levels observed after 12 weeks (4.8%) (day 7 at 15°C) did not differ significantly from fruit stored for eight (2.3%) and 16 (5.7%) weeks (Fig. 3A). By day 11, there was no significant difference in astringency levels between the storage periods (Fig. 3B).

3.1.3 2009 Season

There was a significant interaction between harvest time and storage period on astringency observed directly after storage (day 0 at 15° C). Astringency levels were higher (> 8.0%) in 'Forelle' harvested before commercial harvest (H1 and H2), particularly those that were stored for eight weeks (-0.5°C). On other harvest dates, astringency was less than 3.5% on any given period at -0.5°C (Fig. 4i). The astringency level observed in the KBV fruit (2.8%) directly after storage was not significantly different to that observed in the WBV (1.2%) and Elgin (3.9%) (Fig. 4ii).

On day seven of ripening $(15^{\circ}C)$, growing area and harvest time interacted significantly (P = 0.0135). In all treatment combinations (area by harvest time), except for the H1 fruit from the Elgin area, astringency did not differ significantly after seven days of ripening (Fig. 5i). Astringency appeared to decrease with prolonged storage at -0.5°C. Fruit stored for eight weeks (-0.5°C) had a higher incidence of astringency (3.5%), and differed significantly from fruit stored for 12 weeks (Fig. 5ii).

3.2 Incidence of mealiness in 'Forelle' pears over the seasons

3.2.1 2007 Season

There was almost no mealiness on day zero at 15° C (Fig. 6A). Only fruit harvested at week 11 and 13 (H4 and H5), stored for 10 and eight weeks showed an incidence of 8.9% (H4) and 3.6% (H5). Mealiness was unacceptably high (> 52.0 %) on fruit harvested after H1, and stored for eight weeks followed by seven days at 15° C (Fig. 6B). Extending cold storage from eight to 12 weeks significantly reduced mealiness to less than 25.0% on the H2,

H3 and H4 fruit (Fig. 6B). Mealiness levels for H3 fruit stored for 10 weeks (-0.5°C) plus seven days (15°C) did not differ significantly to those stored for eight weeks (Fig. 6B). The average fruit firmness (2.7 kg) at maximum mealiness (78.3%) after eight weeks (-0.5°C) was similar to that of fruit stored for 12 weeks on day seven of ripening (Fig. 6B).

Fruit from the Elgin area had higher mealiness levels (38.0%) than the WBV (23.0%) and KBV (30.0%), but this did not differ significantly from the KBV (Fig. 6Ci). The cold storage period had no significant effect in reducing mealiness for fruit harvested two weeks after commercial harvest (H4) (Fig. 6Cii). Mealiness reached more than 45.0% after all storage periods for the H4 fruit on day 11 at 15°C (Fig. 6Cii).

3.2.2 2008 Season

Immediately after cold storage, mealiness levels were very low (< 0.5%) (data not shown). Mealiness became evident after seven and 11 days at 15° C (Fig. 7A, 7Bi, and 7Bii). After storage at -0.5°C plus seven and 11 days at 15° C, harvest time and storage period interacted significantly (P < 0.05) on mealiness (Fig. 7A and 7Bi). Mealiness levels decreased with extended time at -0.5°C. In 'Forelle' harvested two and four weeks after commercial harvest time (H4 and H5), mealiness reached 66.0% (H4) and 43.0% (H5) after eight weeks (-0.5°C) plus seven days at 15°C (Fig. 7A). Prolonging the cold storage period to 12 weeks significantly reduced mealiness to 14.0% (H4) and 8.0% (H5) on the late harvested fruit (Fig. 7A). Storing fruit longer than 12 weeks did not have a significant effect on mealiness at day seven of ripening (15°C) (Fig. 7A), but after 11 days (15°C), a significant reduction to 4.5% was observed on the H5 fruit (Fig. 7Bi). Occurrence of mealiness differed significantly between the production areas after 11 days at 15°C. KBV fruit experienced approximately half the incidence (8.3%) observed in the WBV (16.1%) and Elgin (16.9%)

fruit (Fig. 7Bii). In fruit stored for eight weeks at -0.5°C, mealiness incidence increased with harvest maturity and peaked at H4 and thereafter it decreased again (Fig.7A and 7Bi).

3.2.3 2009 Season

No mealiness was detected directly after storage (-0.5°C) (data not shown). Mealiness was evident after seven and 11 days of ripening (15°C) (Fig. 8 and 9). The incidence of mealiness did not differ significantly for any storage period for 'Forelle' harvested at H3 or earlier (H2 and H1) (Fig. 8i). Later harvested fruit (H4 and H5) had 17.9% and 43.0% mealiness, respectively after eight weeks of storage (-0.5°C) followed by seven days at 15°C. For H4 and H5, extending storage period reduced mealiness. After 12 weeks at -0.5°C plus seven days of ripening, mealiness dropped to 11.7% (H4) and 5.4% (H5), respectively. After 16 weeks, the incidence of mealiness for the H4 and H5 fruit dropped further to 7.5% and 0.0%, respectively (Fig. 8i). The WBV fruit had a significantly higher (11.0%) mealiness incidence than Elgin (5.9%) and KBV (2.4%) (Fig. 8ii).

After storage at -0.5°C and 11 days at 15°C, there was a significant interaction of growing area by harvest time (P = 0.0059) and harvest time by storage duration (P = 0.0001) (Fig. 9i and 9ii). On day 11 (15°C), mealiness was significantly lower (< 5.0%) in all the areas for fruit harvested at commercial harvest and earlier (H3, H2 and H1) (Fig. 9i). Maximum mealiness of 37.9% was observed on the late harvested fruit (H5) of the Elgin area. However, this did not differ significantly to the WBV, but was significantly different to the KBV for H5 (Fig. 9i).

No mealiness was observed by day 11 at 15°C at any given storage period for 'Forelle' harvested before commercial harvest (H1 and H2) (Fig. 9ii). At commercial harvest, mealiness was less than 5.0% on all treatment combinations. Similar to the 2007 and

2008 seasons, prolonged storage time in 2009 significantly reduced mealiness on late harvested fruit (H4 and H5). Approximately half of the H5 fruit (43.9%) were mealy after eight weeks storage at -0.5°C plus 11 days at 15°C. Storing for the mandatory 12 weeks reduced mealiness to 19.1%. Extending the cold storage period further to 16 weeks, significantly reduced mealiness to 13.6% on the H5 fruit (Fig. 9ii).

3.3 Multivariate analysis for the incidence of mealiness and astringency in 'Forelle' pears

Tables 1, 2, 3 and 4 together with Figures 10 to 18, present a summary of the discriminant analysis of 'Forelle' pears that were astringent (1), non-astringent (2), mealy (3) or non-mealy (4).

3.3.1 2007 season

In all the areas, the discriminant analysis gave a stronger classification of 90.0 to 100.0% on fruit that were mealy in the 2007 season (Table 2 and Fig. 10 to 12). There was little distinction between 'Forelle' that were astringent and non astringent in the WBV and Elgin fruit (Fig. 10 and 11). Fruit from the KBV gave the weakest classification (< 50.0%) on fruit that were non-astringent and non-mealy (Fig. 12). Mealy fruit from the Elgin area were clearly discriminated from the rest of the groups (Fig. 11).

3.3.2 2008 season

In the WBV area, firmness, fruit size and ground colour were the variables selected to discriminate between the four classes (astringent, non-astringent, mealy and non-mealy) (Table 1). Astringent fruit were clearly separated from those that were non-astringent, but

only 65.8% were correctly classified (Fig. 13 and Table 3). Although 87.5% of the mealy fruit were correctly classified as mealy in the WBV area (Table 3), these fruit were not clearly distinguished from the rest of the group (Fig. 13). The WBV fruit also gave the weakest classification (37.5%) on non-mealy fruit (Table 3).

There was no clear differentiation between astringent and non-astringent fruit in the Elgin area. A proportion of 26.0% of the non-astringent fruit were misclassified within the astringent group (Table 3 and Fig. 14). A good classification of 98.0% on Elgin fruit were correctly identified as mealy, but this fruit could not be completely separated from the non-mealy and non-astringent groups (Fig. 14). Ground colour, fruit size, starch breakdown and firmness were the most important variables for explaining the differences in the four eating quality groups of 'Forelle' from the Elgin area.

In KBV fruit, there was no clear separation between the four eating quality classes (astringent, non-astringent, mealy and non-mealy) (Fig. 15). The points were scattered, with no distinct clusters. However, correct classifications for mealy and non-mealy were 84.0% and 69.4%, respectively (Table 3). Fruit size, firmness, ground colour and starch breakdown were parameters chosen in the stepwise discriminant analysis for 'Forelle' from the KBV area.

3.3.3 2009 season

In the WBV, astringent fruit were clearly separated from the non-astringent, mealy and non-mealy groups (Fig. 16). The discriminant analysis gave a 90.0% positive classification (Table 4) on astringent fruit. The rest of the groups were closely clustered. A proportion of 77.0% non-astringent and mealy fruit were identified correctly into their respective groups. Only 57.5% were correctly classified as non-mealy in the WBV (Table 4). In the Elgin area, mealy fruit were clearly separated from the other groups (Fig. 17), and 93.9% of the fruit were properly classified (Table 4). Points for the mealy group were clustered much closer compared to the other groups (Fig. 17). The weakest classification (45.0%) was observed on non-mealy fruit and the points were scattered (Fig. 17). Similar to the 2008 season, there was no clear distinction for astringent and non-astringent groups. A 28.0% proportion of the astringent fruit were misclassified as non-astringent (Table 4).

In the KBV area, astringent fruit were clearly discriminated from the other groups (Fig. 18), and 82.8% of the astringent fruit were correctly classified (Table 4). However, the points of this group were dispersed, while points in other groups were more clustered. There was no clear separation for non-astringent, mealy and non-mealy fruit (Fig. 18). More than 40.0% of the non-astringent fruit from the KBV were misclassified. A proportion of 70.0% and 72.5 % of the mealy and non-mealy fruit, respectively were correctly identified from the CANDISC procedure (Table 4).

4. Discussion

The incidence of 'Forelle' astringency was reduced with exposure to 15°C and advancing maturity. This indicated that the level of tannins in the fruit decreases as fruit matured and ripened. Astringency was mainly evident after fruit removal from cold storage (0 days at 15°C). The incidence was higher in the early season fruit, particularly the H1 fruit, than fruit harvested later. In the 2007 season, astringency reached the highest percentage measured during the trial, with a maximum of 37.0% in the H1 fruit, stored for eight weeks (-0.5°C). At this point, the average fruit firmness was 7.4 kg. However, in the 2008 and 2009 seasons, the incidence of astringency reported was less than 15.0% for the H1 fruit, and average fruit firmness was 7.8 kg (2008) and 6.6 kg (2009).

As fruit matured astringency decreased. Fruit harvested four weeks after the first harvest (H3) (2007) initially had an average firmness of 6.1 kg after eight weeks of cold storage, and astringency had reduced to 12.0% (Fig. 1Aii). After seven days of ripening (15°C), firmness dropped to an average of 2.3 kg and no astringency was observed. The reduction in the percentage of astringent fruit with delayed harvest suggested that, as fruit matures, it loses the ability to produce astringent compounds after storage (Mielke and Drake, 2005).

Fruit from the WBV in the 2008 season experienced the lowest incidence (< 4.5%) of astringency at any given storage period at -0.5°C, compared to the Elgin and KBV areas (Fig. 2i). This could possibly relate to the accelerated maturation (higher rate of change in firmness) earlier observed pre-harvest in the WBV area, as more mature fruit showed less astringency.

Contrary to 'Concorde' pears that experience a higher incidence of astringency following long-term storage (120 days at 1°C) (Mielke and Drake, 2005), with 'Forelle' (H1 fruit), the eight week storage period at -0.5°C resulted in higher astringency compared to 10, 12 and 16 weeks (Fig. 1Aii, 1B, 1Cii and Fig. 4i). Among other factors this could be ascribed to lack of ripening capacity in the H1 fruit stored for only eight weeks. This occurred in all the areas, despite of the variation in maturity between the different areas at H1.

There was a slight but significant incidence of astringency in fruit harvested after optimum maturity (H4 and H5), particularly in fruit stored for the longest duration in 2007 (12 weeks) and 2009 (16 weeks) seasons (Fig. 1B and Fig.4i). As fruit ripens the level of tannins decrease (Ramin and Tabatabaie, 2003). Hence, the higher incidence of astringency may be related to the lower softening rate (firmness rate of change) at 15°C in 'Forelle' that were stored for the longest duration at -0.5°C. Moreover, fruit size could be another

contributing factor, as we experienced inadequate fruit sampling after commercial harvest. This was typical of fruit from Kentucky (2007 season) and Platvlei (2009 season) (data not shown). It seems likely that some of the fruit were immature as low levels of TSS (< 14.0%) were observed at harvest, mainly on H5.

A lower incidence of mealiness was detected immediately after cold storage (0 days at 15° C). Fruit needed to soften, since firmness affects mealiness, and at higher firmness mealiness cannot be perceived. Only in the 2007 season, mealiness of 8.9% was observed upon removal from storage in the H4 fruit stored for 10 weeks (-0.5°C). In the 2008 and 2009 seasons, mealiness detected immediately after storage was less than 0.5%.

The incidence of mealiness after seven days at 15°C differed between the three seasons. The incidence was higher in the 2007 and 2008 seasons compared to 2009 (Fig. 6B, 7A, and 8i). In the 2007 season, mealiness reached a maximum of 78.0% on H2 fruit stored for eight weeks (-0.5°C) plus seven days at 15°C. The cold storage period had no significant effect in reducing mealiness in the H4 fruit in 2007 after 11 days at 15°C, because the firmness was already low and cold storage could not have an effect on cell wall integrity as the middle lamella had probably already disintegrated.

Hansen (1961) associated an incidence of 53 to 70% mealiness with seasons experiencing high total heat units (HU). In our study, the heat units between the seasons could not clearly explain the differences in mealiness, as the 2007 season where highest mealiness was reported experienced lower total heat units (45598 HU) compared to the 2008 (53742 HU) and 2009 (47357 HU) (Table 5).

In concurrence with Martin (2002), mealiness of 'Forelle' was significantly reduced with extended time in storage (-0.5° C). Extending cold storage from eight to 12 weeks (-0.5° C), reduced mealiness from 62.0% to 10.0% in 2007, H4 fruit ripened for seven days. In the 2008 season storing fruit for 16 weeks (-0.5° C) and seven days at 15°C reduced

mealiness to less than 6.0% in H2, H3 and H4 fruit (Fig. 7A). No mealiness was observed in the 2009 season for the same conditions in H3 and H5 (Fig. 8i). At this point of 0.0% mealiness, the average fruit firmness was at 3.5 kg for both harvest dates (H3 and H5), while ground colour index was 3.1 and 3.4, respectively. The length of cold storage could possibly affect the activity of cell wall degradation enzymes. It is most likely that storing 'Forelle' longer at -0.5°C (RA) causes cell wall degrading enzymes to be less active during fruit ripening, causing cell walls to break rather than slide during mastication, which in turn allows cell components (juiciness, sugars, acids and volatile substances) to be released.

Fruit from the WBV and Elgin appeared to be more prone to mealiness than those from the KBV (Fig. 7Bii and 9i). This could relate to the maturity at harvest, as fruit from the WBV and Elgin expressed higher rates of change both in ground colour index (0.028 and 0.030 colour index day⁻¹, respectively) and firmness (-0.044 and -0.037 kg day⁻¹, respectively) than the KBV (0.025 colour index day⁻¹ and -0.025 kg day⁻¹) (Chapter 2). Considering that the WBV and Elgin areas are warmer areas and generally accumulate more annual heat units than the KBV (Table 5). The behaviour of mealiness between the areas confirms previous work by Martin (2002) on WBV fruit, Hansen (1961) and Mellenthin and Wang (1976) on climate and postharvest quality relations.

Discrimination between fruit groups that were astringent, non-astringent, mealy and non-mealy was unsatisfactory. It is most likely that other factor (s) (eg. winter chilling, since it affects cell division during fruit growth) play a more important role on 'Forelle' eating quality than the used maturity indices. However, in most instances, the mealy fruit were clearly separated from the rest of the groups. This could be related to firmness, since most of the mealy fruit had an exceptionally low firmness (< 2.5 kg). The mealy group also gave a higher correct classification (> 70.0%) compared to the other groups. In the 2007 season, where a clearer discrimination was achieved in fruit from the Elgin area, among other

variables, firmness was the first selected variable with an R^2 value of 0.93 (Table 1 and Fig. 11). Both astringency and mealiness in 'Forelle' displayed a highly significant (p < 0.0001) correlation to firmness (Table 6). Mealiness was inversely correlated; the lower the firmness the higher the incidence of mealiness, while astringency showed a positive correlation; firmer fruit were associated with higher incidence of astringency (Table 6). This could suggest that firmness is one variable that still describes eating quality of 'Forelle' the best.

However, the extent to which the maturity indices (firmness, ground colour, fruit size, mass and starch breakdown) could precisely define the eating quality (astringency, non-astringency, mealy and non-mealy) appeared unpredictable, because at similar levels of the maturity indices, fruit expressed differences in eating quality. This might suggest that other quality attribute (s), in addition to the evaluated maturity indices, play a more important role with regard to mealiness and astringency in 'Forelle'.

5. Conclusion

Although eight weeks RA storage at -0.5° C instead of the mandatory 12 weeks was sufficient to induce ripening in 'Forelle' harvested at optimum and post-optimum harvest maturity, the eating quality was compromised. These fruit experienced maximum mealiness. Also, in the early season fruit (week 5 = H1) where astringency was more evident, the incidence was higher in fruit stored for eight weeks compared to the 10, 12 and 16 week periods. However, astringency is significantly reduced with delayed harvest (increased fruit maturity). Hence, the results of this trial do not support shortening the recommended mandatory 12 week cold storage period (-0.5°C) due to the higher mealiness incidence in the eight week storage period (-0.5°C). The three climatically diverse production areas had an influence on eating quality of 'Forelle'. Warmer areas were generally associated with higher incidence of mealiness, and therefore an advanced rate of maturation (ground colour and firmness rates of change). Fruit from the WBV and Elgin were more susceptible to mealiness disorder after ripening (15°C) compared to the KBV. Thus, producers within the WBV and Elgin areas may need to be extra cautious with regard to industry recommendations to reduce 'Forelle' mealiness.

The levels of mealiness differed between the seasons. This could not be clearly explained using the heat units accumulated early during the season. It would appear that factor (s), other than the heat units play a more important role with regard to 'Forelle' mealiness between seasons.

6. Recommendation

Further research on strategies to reduce 'Forelle' mealiness in shorter stored fruit is recommended. This will allow continuous market supply of premium quality for the South African bicolour pear cultivars since the mandatory 12 week for 'Forelle' causes a market gap after the supply of Rosemarie and Flamingo (Crouch and Bergman, 2010).

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Fig. 1A. Astringency (%) of 'Forelle' pears harvested in 2007 from Warm Bokkeveld (WBV) Elgin and Koue Bokkeveld (KBV), and stored for eight, 10 and 12 weeks at -0.5°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 1B. Effect of harvest time and storage period on astringency (%) of 'Forelle' pears harvested in the 2007 season from Warm Bokkeveld, Elgin and Koue Bokkeveld. Fruit were stored for eight, 10 and 12 weeks at -0.5°C followed by seven days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 1C. Astringency (%) of 'Forelle' pears harvested in 2007 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 10 and 12 weeks at -0.5°C followed by 11 days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



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Treatment	Pr > F					
Area (A)	0.0002					
Harvest time (H)	0.0005					
Storage period (S)	0.1180					
A*H	0.1866					
A*S	0.0143					
H*S	0.8275					
A*H*S	0.9566					

Fig. 2. Astringency (%) of 'Forelle' pears harvested in 2008 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 12 and 16 weeks at -0.5° C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 3. 2008 Incidence of astringency (%) in 'Forelle' after eight, 12 and 16 weeks at -0.5°C followed by seven (left) and 11 (right) days at 15°C. Fruit were harvested biweekly (H) from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 4. Effect of harvest time (H) and storage period on astringency (%) of 'Forelle' pears harvested in 2009 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 12 and 16 weeks at -0.5°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 5. Astringency (%) of 'Forelle' pears harvested in 2009 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 12 and 16 weeks at -0.5°C followed by seven days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 6A. Effect of harvest time (H) and storage period on mealiness (%) of 'Forelle' pears harvested in 2007 from the Warm Bokkeveld, Elgin and Koue Bokkeveld, stored for eight, 10 and 12 weeks at -0.5°C. (Logit transformed data).



Fig. 6B. Effect of harvest time (H) and storage period on mealiness (%) of 'Forelle' pears harvested in 2007 from the Warm Bokkeveld, Elgin and Koue Bokkeveld, stored for eight, 10 and 12 weeks at -0.5°C followed by seven days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 6C. Mealiness (%) of 'Forelle' pears harvested in 2007 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 10 and 12 weeks at -0.5°C followed by 11 days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 7A. Effect of harvest time (H) and storage period on mealiness (%) of 'Forelle' pears harvested in 2008 from the Warm Bokkeveld, Elgin and Koue Bokkeveld, stored for eight, 12 and 16 weeks at -0.5°C followed by seven days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 7B. Mealiness (%) of 'Forelle' pears harvested in 2008 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for eight, 12 and 16 weeks at -0.5°C followed by 11 days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 8. Mealiness (%) of 'Forelle' pears harvested in 2009 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by seven days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 9. Mealiness (%) of 'Forelle' pears harvested in 2009 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV), stored for weeks at -0.5°C followed by 11 days at 15°C (Logit transformed data). Letters indicate significant differences between treatment means, separated using LSD at 5%.



Fig. 10. Discrimination between 'Forelle' pears harvested in the 2007 season from the Warm Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Ground colour, fruit size firmness and starch breakdown were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 11. Discrimination between 'Forelle' pears harvested in the 2007 season from the Elgin area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, starch breakdown, fruit mass and ground colour were variables selected from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 12. Discrimination between 'Forelle' pears harvested in the 2007 season from the Koue Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, fruit mass, ground colour and fruit size were variables selected from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 13. Discrimination between 'Forelle' pears harvested in the 2008 season from the Warm Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, fruit size and ground colour were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 14. Discrimination between 'Forelle' pears harvested in the 2008 season from the Elgin area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Ground colour, fruit size, starch breakdown and firmness were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 15. Discrimination between 'Forelle' pears harvested in the 2008 season from the Koue Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Fruit size, firmness, ground colour and starch breakdown were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 16. Discrimination between 'Forelle' pears harvested in the 2009 season from Warm Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, ground colour, fruit size and starch breakdown were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 17. Discrimination between 'Forelle' pears harvested in the 2009 season from the Elgin area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, fruit size, ground colour, and starch breakdown were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.



Fig. 18. Discrimination between 'Forelle' pears harvested in the 2009 season from Koue Bokkeveld area that were astringent (1), non-astringent (2), mealy (3), and non-mealy (4). Firmness, starch breakdown, fruit size and ground colour were selected variables from a stepwise discriminant analysis and processed with a canonical discriminant analysis procedure.

Seasons	Areas	Selected variables	\mathbb{R}^2	F-value	Pr>F
2007					
	WBV	Ground colour	0.69	86.16	< 0.0001
		Fruit size	0.36	21.38	0.0001
		Firmness	0.26	13.04	< 0.0001
		Starch breakdown	0.11	4.44	0.0055
	Elgin	Firmness	0.93	495.35	< 0.0001
		Starch breakdown	0.46	32.41	< 0.0001
		Fruit mass	0.27	14.06	< 0.0001
		Ground colour	0.15	6.87	0.0003
		τ.	0.40	40.00	0.0001
	KBV	Firmness	0.43	49.02	< 0.0001
		Fruit mass	0.22	17.97	< 0.0001
		Ground colour	0.05	3.35	0.0201
		Fruit size	0.04	2.60	0.0532
2008			<u> </u>	10.51	0.0001
	WBV	Firmness	0.49	48.51	< 0.0001
		Fruit size	0.38	31.30	< 0.0001
		Ground colour	0.14	8.07	<0.0001
	Elgin	Ground colour	0.47	61.53	< 0.0001
	0	Fruit size	0.34	34.60	<00001
		Starch breakdown	0.21	18.13	< 0.0001
		Firmness	0.16	12.99	< 0.0001
	KBV	Fruit size	0.61	100.80	< 0.0001
		Firmness	0.28	24.75	< 0.0001
		Ground colour	0.06	4.20	0.0066
		Starch breakdown	0.06	3.79	0.0113
2009					
	WBV	Firmness	0.84	253.18	< 0.0001
		Ground colour	0.27	17.89	< 0.0001
		Fruit size	0.12	6.35	0.0005
		Starch breakdown	0.11	5.64	0.0011
	Elgin	Firmness	0.55	178.39	< 0.0001
		Fruit size	0.34	74.91	< 0.0001
		Ground colour	0.12	20.45	< 0.0001
		Starch breakdown	0.08	12.11	< 0.0001
	KRV	Firmness	0.71	119 52	<0.0001
	KD V	starch breekdown	0.17	0.76	<0.0001
		Fruit size	0.17	7 20	<0.0001 0.0002
		Ground colour	0.15	7.20 A 28	0.0002
		Ground colour	0.08	4.28	0.0063

Table 1. Stepwise selection summary on Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) (2007-2009) data for best classification results for astringent, non-astringent, mealy and non-mealy classes of 'Forelle' pears.

In class 2(non astringent) From class 1(astringent) 3 (mealy) 4 (non mealy) Total WBV 30 0 0 50 1 20 60.00 40.00 0.00 0.00 100.00 2 20 28 0 2 50 40.00 0.00 4.00 100.00 56.00 3 45 0 5 50 0 0.00 0.00 90.00 10.00 100.00 4 0 1 17 32 50 0.00 2.00 34.00 64.00 100.00 Total 50 49 62 39 200 25.00 24.50 19.50 31.00 100.00 Elgin 1 18 10 0 2 30 60.00 33.33 0.00 6.67 100.00 2 10 19 0 30 1 33.33 63.33 0.00 100.00 3.33 3 0 0 30 1 30 0.00 0.00 100.00 0.00 100.00 4 26 3 0 1 30 10.00 0.00 3.33 86.67 100.00 29 Total 31 29 31 120 25.85 25.83 24.17 100.00 24.17 KBV 31 9 3 48 1 5 64.58 18.75 6.25 10.42 100.00 2 13 4 50 23 10 26.00 46.00 20.00 8.00 100.00 3 1 1 45 3 50 2.00 2.00 90.00 6.00 100.00 4 7 5 17 21 50 14.00 10.00 34.00 42.00 100.00 Total 52 75 198 38 33 26.26 19.19 37.88 16.67 100.00

Table 2. Summary of the discriminant analysis for eating quality of 'Forelle' pears harvested in 2007 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Presented are number of observations and percent classified into astringent (1), non-astringent (2), mealy (3) and non-mealy (4).

		In class			
From class	1(astringent)	2(non astringent)	3 (mealy)	4 (non mealy)	Total
WBV					
1	25	5	5	3	38
	65.79	13.16	13.16	7.89	100.00
2	0	30	3	7	40
	0.00	75.00	7.50	17.50	100.00
3	0	3	35	2	40
	0.00	7.50	87.50	5.00	100.00
4	6	10	9	15	40
	15.00	25.00	22.50	37.50	100
Total	31	48	52	27	158
	19.62	30.38	32.91	17.09	100.00
Elgin					
1	35	13	0	2	50
	70.00	26.00	0.00	4.00	100.00
2	18	23	10	2	53
	33.96	43.40	18.87	3.77	100.00
3	0	1	54	0	55
	0.00	1.82	98.18	0.00	100.00
4	4	5	10	34	53
	7.55	9.43	18.87	64.15	100.00
Total	57	42	74	38	211
	27.01	19.91	35.07	18.01	100.00
KBV					
1	32	16	0	2	50
	64.00	32.00	0.00	4.00	100.00
2	20	25	0	5	50
	40.00	50.00	0.00	10.00	100.00
3	0	2	42	6	50
	0.00	4.00	84.00	12.00	100.00
4	0	4	11	34	49
	0.00	8.16	22.45	69.39	100.00
Total	52	47	53	47	199
	26.13	23.62	26.63	23.62	100.00

Table 3. Summary of the discriminant analysis for eating quality of 'Forelle' pears harvested in 2008 from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Presented are number of observations and percent classified into astringent (1), non-astringent (2), mealy (3) and non-mealy (4).

Table 4. Summary of the discriminant analysis for eating quality of 'Forelle' pears harvested in 2009 from Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Presented are number of observations and percent classified into astringent (1), non-astringent (2), mealy (3) and non-mealy (4).

		In class			
From class	1(astringent)	2(non astringent)	3 (mealy)	4 (non mealy)	Total
WBV	·	– ć	· • • ·	· • • • •	
1	30	3	0	0	33
	90.91	9.09	0.00	0.00	100.00
2	0	31	1	8	40
	0.00	77.50	2.50	20.00	100.00
3	0	0	27	8	35
	0.00 0.00 77.14		77.14	22.86	100.00
4	0	7	10	23	40
	0.00	17.50	25.00	57.50	100.00
Total	30	41	38	39	148
	20.27	27.70	25.68	26.35	100.00
Elgin					
1	70	30	1	6	107
	65.42	28.04	0.93	5.61	100.00
2	23	81	4	4	112
	20.54	72.32	3.57	3.57	100.00
3	0	2	107	5	114
	0.00	1.75	93.86	4.39	100.00
4	4	18	38	50	110
	3.64	16.36	34.55	45.45	100.00
Total	97	131	150	65	443
	21.9	29.57	33.86	14.67	100.00
KBV					
1	24	5	0	0	29
	82.76	17.24	0.00	0.00	100.00
2	6	23	0	11	40
	15.00	57.50	0.00	27.50	100.00
3	0	3	28	9	40
	0.00	7.50	70.00	22.50	100.00
4	0	3	8	29	40
	0.00	7.5	20.00	72.50	100.00
Total	30	34	36	49	149
	20.13	22.82	24.16	32.89	100.00

Table 5. Accumulated daily positive chill units with accumulated heat units (for 24 hours with base 10°C and upper limit 30°C) for each season (2007 - 2009) in the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV) areas (DFPT climate data base, 2007-2008).

Year	Area	Chill units ^x	Heat units ^x
2006			
	WBV	1093	10008
	Elgin	821	18523
	KBV	1480	17067
2007			
	WBV	990	19632
	Elgin	835	17995
	KBV	1385	16115
2008			
	WBV	939	20336
	Elgin	648	17117
	KBV	1567	9904

^x Accumulated Daily Positive Chill Units were recorded from May, 1 to August, 31 while heat units were recorded from September, 1 to December, 31 of each year, from an automatic industry weather station in the region.

Table 6. Relationship between firmness and mealiness and astringency of 'Forelle' pears harvested in three successive seasons (2007-2009).

Firmness vs mealiness or astringency of 'Forelle' pears								
			2007 2008		008	2009		
			r	P-value	r	P-value	r	P-value
Firmness	vs	Mealiness	-0.610	< 0.0001	-0.451	< 0.0001	-0.414	< 0.0001
Firmness	vs	Astringency	0.515	< 0.0001	0.318	< 0.0001	0.370	< 0.0001

General Discussion and Conclusions

The study was done over three successive seasons (2007 - 2009). 'Forelle' pears were harvested biweekly for five harvest dates from the Warm Bokkeveld (WBV), Elgin and Koue Bokkeveld (KBV). Various maturity indices and their rates of change were evaluated in order to identify maturity variables that behaved uniformly over the growing season and can be reliably used in a prediction model to determine optimum harvest maturity of 'Forelle' pears for good eating quality after storage. The effect of harvest maturity, storage time at -0.5°C and growing area were also investigated to determine the ripening potential and eating quality of 'Forelle', defined by an optimum "edible firmness" of 3.5 kg and absence or presence of astringency and mealiness.

Seasons had a significant influence on the rates of change of the evaluated maturity variables (ground colour, firmness, total soluble solids (TSS) and titratable acidity (TA)). This clearly indicated that seasonal differences have a direct effect on fruit quality, which confirms previous results (Frick, 1995; Wang et al., 1971; Mellenthin and Wang, 1976). The 2007 season expressed a significantly different behaviour in the rates of change for firmness, TSS and TA compared to the 2008 and 2009 seasons, while the latter two behaved similarly. A higher rate of change in TSS was associated with a decline in TA. This was linked to the relatively high heat units that prevailed prior to the 2007 harvest in the Elgin and KBV areas, since high heat units early in the season improve photosynthetic rates (Lötze and Bergh, 2005).

The firmness variable changed at a higher rate than the other parameters and was comparable to rates of change in ground colour. Firmness and ground colour further displayed a stronger linear relationship with days after full bloom (DAFB), showing R^2 values more than

90% and 70%, respectively. Contrary to that, TA changed at a slower rate in all the seasons. This confirmed that the different maturity variables do not behave in a similar pattern during maturation, with changes in fruit firmness and ground colour being more marked compared to changes of TA and TSS. Hence, the more noticeable changes such as the firmness and ground colour could be simpler and more accurate to use in predicting of harvest maturity for 'Forelle'.

Fruit from the Elgin area consistently reached optimum firmness earlier than the WBV and KBV in all the seasons. Furthermore, the Elgin area was earliest to attain optimum TSS and ground colour in the 2007 season. Optimum TSS was reached at 152 DAFB while optimum ground colour was achieved after 138 DAFB. This suggested that fruit maturation occurred at a much faster rate in the Elgin area. This was associated with the differences in climatic conditions between the three areas, with Elgin showing the highest spring temperatures, causing a more rapid drop in flesh firmness than other areas, confirming results from Lötze and Bergh (2005).

Flesh firmness and ground colour therefore proved to be the most reliable variables that could be fitted in a linear model of the equation $y = \alpha + \beta x$ to precisely predict harvest maturity of 'Forelle' pears, as these parameters also behaved consistently over three growing seasons. Henceforth, these two variables, together with ethylene production, were used to determine the ripening potential of 'Forelle' pears after exposure at -0.5°C followed by ripening at 15°C.

In agreement with Chen and Mellenthin (1981), earlier harvested fruit showed the lowest ripening potential when stored for a short period (8 weeks) at -0.5°C, compared to fruit harvested at commercial harvest time and later (optimum and post-optimum). In the 2007 season, fruit harvested four weeks before commercial harvest time and stored for eight weeks, did not reach an "edible firmness" (3.5 kg) by day 11 at 15°C. A similar case was observed in 2009, when

fruit was harvested both at four and two weeks before commercial harvest (pre-optimum), and stored for eight weeks (-0.5° C).

Furthermore, fruit from the 2007 and 2009 seasons had extremely low rates of ethylene production ($<0.5 \ \mu L^{\circ}kg^{-1}h^{-1}$) compared to fruit stored for more than eight weeks (10, 12 and 16) at -0.5°C. This indicated that, on earlier season fruit, an eight week period at -0.5°C is not sufficient to induce substantial amounts of ethylene to promote ripening changes. Our findings conformed to those of 'Bartlett' pears (Puig et al., 1996), where less mature fruit lacked the ability to start autocatalytic ethylene production at ripening. In South Africa, Frick (1995) found that an accelerated decline in 'Bon Chretien' flesh firmness was associated with a cool summer. This may be the case in 2008 for the early harvested fruit that ripened after eight weeks.

The rate of change in firmness at 15° C tended to increase with increased time in storage (-0.5°C) on 'Forelle' harvested before commercial harvest. In contrast, on fruit harvested at commercial harvest and later, shorter storage periods (<12 weeks) at -0.5°C resulted in rapid softening at 15° C, compared to longer periods. As a result, the incidence of mealiness was higher on fruit stored for eight weeks (-0.5°C) compared to 10, 12 and 16 weeks. Extending the cold period in optimum and post-optimum fruit seemed to influence cell wall degradation; the longer the storage duration, the less rapid the cell wall degradation and hence fruit were firmer. The late harvested fruit, at 15° C, further expressed a more pronounced yellow ground colour on shorter storage duration. This may possibly be associated with the rapid softening earlier observed on fruit stored for eight weeks, as firmness is highly correlated with ground colour (Eccher Zerbini, 2002).

The WBV fruit harvested in 2008 season, softened faster in response to a given storage period than fruit from Elgin and KBV. The differences between the growing areas were most

likely related to the rate of maturation (firmness rates of change), as influenced by DAFB. The WBV fruit matured at a faster rate (-0.044 kg day⁻¹) compared to Elgin and KBV. Also, the WBV area accumulated more heat units (HU) (19632 HU) before harvest compared to Elgin (17995 HU) and KBV (16115 HU). This may partly explain the differences in the ripening pattern between these areas, since high spring temperatures cause a rapid drop in firmness (Lötze and Bergh, 2005).

The last section of the study evaluated the eating quality of the fruit, which was defined in terms of presence or absence of mealiness or astringency. Fruit that were neither mealy nor astringent were regarded as having 'acceptable' eating quality. The harvest maturity, storage duration at -0.5°C and growing area played a significant role on the incidence of astringency and mealiness in 'Forelle'. Astringency was mainly evident after fruit removal from cold storage (0 day at 15°C) and it decreased over time at 15°C. Astringency was higher in the early season fruit, particularly the first harvested fruit (H1), than fruit harvested later (H2, H3, H4, and H5). In the 2007 season, astringency reached the highest percentage (37%) measured during the trial in H1 fruit (pre-optimum) stored for eight weeks (-0.5°C). At this point, the average fruit firmness was 7.4 kg. However, in the 2008 and 2009 seasons, the incidence of astringency reported was less than 15% for the H1 fruit, and average fruit firmness was 7.8 kg (2008) and 6.6 kg (2009). Astringency in 'Forelle' decreased with delayed harvest, which suggested that, as the fruit matures, it loses the ability to produce astringent compounds after storage (Mielke and Drake, 2005).

Generally, the WBV and Elgin had a lower percentage of astringent fruit compared to the KBV, but the incidence of mealiness was higher in the former two areas. For any given storage period at -0.5°C, less than 4.5% of fruit from the WBV were astringent in the 2008 season. This

was linked to the accelerated maturation (higher rate of change in firmness) earlier observed preharvest in the WBV, as more mature fruit showed less astringency.

In concurrence with Martin (2002), mealiness of 'Forelle' was significantly reduced with longer storage periods at -0.5°C. Extending the cold storage from eight to 12 weeks reduced mealiness from 62% to 10% in 2007 on 'Forelle' harvested two weeks after commercial harvest, and ripened for seven days at 15°C. In the 2008 season storing fruit for 16 weeks (-0.5°C) and seven days at 15°C reduced mealiness to less than 6.0% on H2, H3 and H4 fruit. The length of the cold storage period seemed to influence the activity of cell wall degradation enzymes. It is most likely that, by storing 'Forelle' longer at -0.5°C (RA), cell wall degrading enzymes become less active during fruit ripening, causing cell walls to break rather than slide during mastication, which in turn allows cell components (juiciness, sugars, acids and volatile substances) to be released.

Although an eight week period at -0.5° C (instead of the mandatory 12 weeks) was sufficient to induce ripening on 'Forelle' harvested at optimum and post-optimum harvest maturity, the eating quality of the fruit was compromised. Fruit stored for eight weeks experienced maximum mealiness. Moreover, in early season fruit (pre-optimum), where astringency was more evident, the incidence was higher in 'Forelle' stored for eight weeks. Therefore, the findings of this study do not recommend shortening the current mandatory 12 week period at -0.5° C due to the higher incidence of astringency and mealiness.

In this study we managed to quantify some of the physiological factors that influence harvest maturity and quality of South African 'Forelle' pears – incorporating three successive seasons, fruit produced in climatically diverse areas, harvested at various maturities and stored and ripened for different durations. This research project confirmed previous results on the

mandatory 12 weeks cold storage of 'Forelle' after harvest, and proposed that ground colour be used in conjunction with firmness as indicators of harvest maturity.

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