FACTORS AFFECTING SHRIVELLING AND FRICTION DISCOLOURATION OF PEARS (*Pyrus communis L.*)

BY

G.E. BURGER

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SUPERVISOR

Dr M. Huysamer – Department of Horticultural Science, University of Stellenbosch.

CO-SUPERVISOR

Prof G. Jacobs – Department of Horticultural Science, University of Stellenbosch.

DECLARATION

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

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Signature Date

SUMMARY

Shrivelling and friction discolouration (FD), as postharvest disorders, negatively influence the marketability and potential shelf life of pears. By investigating the contributing factors in each of the disorders, the potential involvement of handling and storage variables were determined. This allowed for a better understanding of the responsible factors that create susceptible environments for these disorders to occur.

From the moment that pears are harvested they lose weight by means of transpiration and, to a lesser extent, respiration. When excessive losses are experienced, the fruit will appear shrivelled and the marketability and shelf life are negatively influenced. By minimizing the rates of weight loss, the occurrence of shrivelling among pears during the postharvest handling can be lessened. The periods that proved to be most conducive to shrivelling (during a simulated postharvest handling duration) were where temperatures above 0 °C were experienced. These short periods proved more perilous for shrivelling than lengthy storage durations at low temperatures. This influenced the transpiration rate in such a way that the driving force accelerated the rate of weight loss in all the cultivars that were studied. Removing field heat from fresh produce and maintaining the cold chain reduces the driving force behind the transpiration of the pears.

In all the cultivars studied, 'Packham's Triumph', 'Beurrè Bosc' and 'Forelle', smaller and less mature fruit were more inclined to appear shrivelled. The surface area to volume ratio is fundamental in determining the rate of weight loss. This was most evident in 'Beurrè Bosc'. Although no reproducible results could be obtained from the morphological studies, literature has attributed this phenomenon to the composition and quantity of the cuticle layer.

Reduction of weight loss was obtained by sealing of the fruit stem. This obstructed water movement from the fruit through the xylem conducting tissue to the surrounding atmosphere. Not only did the stem appear greener and fresher, but less weight loss and subsequent shrivel was noticeable in the treated fruit. This effect was most evident in 'Packham's Triumph' and 'Beurrè Bosc', but not in 'Forelle'. 'Forelle' typically has a very short, thin stem in comparison to the other two cultivars.

All the cultivars showed visual shrivel symptoms after 11 days at 18 °C. Rate of weight loss was the lowest in 'Packham's Triumph', but due to its prominent dimensions, it appeared shrivelled before any of the other cultivars. 'Beurrè Bosc' lost weight at the highest rate ($0.42\%.day^{-1}$).

As fruit injury, in the presence of oxygen, is inevitable, the oxidative enzymatic browning of pears will always be troublesome. This defensive mechanism partially prevents the infection of the fruit where epidermal cells are injured. To minimize FD, impact and frictional forces need to be lower during both harvesting and handling practices.

A laboratory scale method was developed through which reproduceable treatments could be performed, thereby subjecting the fruit to industry related friction, rather than impact, injury. By assessing the discolouration in terms of both extent and intensity, the influence of variables could be determined on both 'Packham's Triumph' and 'Doyenne du Comice' pears. As also found in practice, 'Doyenne du Comice' proved to be far more susceptible to FD than 'Packham's Triumph', although the activity of the enzyme, polyphenol oxidase (PPO) was found to be higher in the latter.

Although no significant difference was found between the FD encountered at fruit temperature of 3 °C and 15 °C, discolouration was greater at the higher temperature. This might be attributed to a greater degree of water loss, lower cell turgidity or higher enzyme activity. Thus, fruit taken from storage and sorted directly thereafter will exhibit less FD. The contribution of condensation forming on the fruit, acting as lubrication, cannot be ignored. Such fruit, with high turgor pressure, might again be more susceptible to bruising which will only be revealed well after the injury. Since enzymes, which include PPO, catalyse biochemical reactions, the availability of sufficient substrate most probably regulates the extent of this biochemical discolouration.

Harvesting at optimum maturity and preventing any unnecessary friction will most definitely reduce the occurrence of FD. The ultimate challenge remains to optimize sorting and packing conditions without compromising on fruit quality.

OPSOMMING

Verrimpeling en friksie verbruining (FV), as na-oes defekte, het ’n negatiewe invloed op die bemarkbaarheid en potensiële raklewe van pere. Hierdie studie het die bydraende faktore vir elkeen van die defekte ondersoek. Daar is gepoog om die effek van verskillende hanterings- en opbergingsmetodes op bogenoemde defekte te bepaal. ‘n Beter begrip is verkry van die oorsaaklike faktore wat bydra tot die ontstaan van die betrokke defekte.

Direk na die oes van pere, begin die vrug gewig verloor as gevolg van veral transpirasie. Oormatige verliese sal lei tot ’n vrug wat verrimpeld voorkom, met ‘n negatiewe invloed op die bemarkbaarheid en raklewe daarvan. Die voorkoms van die verrimpeling van pere tydens die na-oes hantering van die vrugte, kan verminder word deur vermindering van die tempo van gewigsverlies. Die periodes waartydens die verrimpeling veral voorgekom het, (soos gevind in ’n gesimuleerde na-oes hanteringsmodel) was wanneer temperature bo 0 °C ondervind is. Sulke kort periodes was meer geneig om aanleiding te gee tot verrimpeling as die verlengde periodes van opberging by lae temperature. Sulke periodes van hoër temperature het gelei tot versnelde transpirasie en ’n versnelde tempo van gewigsverlies in al die kultivars wat ondersoek is. Die transpirasietempo van pere kan verlaag word deur die verwydering van veld-hitte en deur die streng behoud van die koue-ketting.

In al die kultivars wat ondersoek is, ‘Packham’s Triumph’, ‘Beurrè Bosc’ en ‘Forelle’, is gevind dat die kleiner en minder volwasse vrugte meer geneig was tot verrimpeling. Die oppervlak area tot volume verhouding is krities in die bepaling van die tempo van gewigsverlies. Hierdie bevinding was die prominentste in ‘Beurrè Bosc’. Alhoewel geen beduidende resultate verkry kon word van die morfologiese studies nie, is daar verskeie verwysings in die literatuur wat hierdie verskynsel toeskryf aan die samestelling en hoeveelheid van die kutikula laag.

Vermindering van gewigsverlies is verkry deur verseëling van die vrugtestingel. Hierdie tegniek het gelei tot ’n blokkering van die watervloei van die vrug na die omgewing deur die xileem weefsel. Verseëling van die stingel het dit groener en varser laat voorkom, en het ook ’n merkbare vermindering in gewigsverlies en die daaropvolgende verrimpeling tot gevolg gehad. Die effek van stingel-verseëling was die prominentste in ‘Packham’s Triumph’ en

‘Beurrè Bosc’. Dit was minder duidelik in ‘Forelle’ wat tipies gekenmerk word deur ‘n baie korter, dun stingel in vergelyking met die ander twee kultivars.

Al die kultivars het makroskopiese verrimpeling getoon na ‘n opbergingperiode van 11 dae by 18 °C. Die tempo van gewigsverlies was die laagste in ‘Packham’s Triumph’ alhoewel dit eerste verrimpeld voorgekom het. Dit kan toegeskryf word aan die spesifieke afmetings van hierdie betrokke kultivar. ‘Beurrè Bosc’ het vinnigste gewig verloor (0.42%.dag^{-1}).

Aangesien die besering van vrugte, in die aanwesigheid van suurstof, onvermydelik is, sal oksidatiewe, ensiematiese verbruining van pere altyd problematies wees. Hierdie verdedigingsmeganisme voorkom tot ’n mate die infeksie van die vrug wanneer epidermale selle beskadig word. FV kan beperk word deur die vrugte tydens oes en hanteringsprosedures so min as moontlik bloot te stel aan impak en friksie kragte.

’n Laboratorium-model is ontwikkel ter nabootsing van die omstandighede in die industrie. Die vrugte is aan friksie, eerder as impak, onderwerp, soos ondervind in die industrie. Die omvang asook die intensiteit van die verbruining is gemeet in beide ‘Packham’s Triumph’ en ‘Doyenne du Comice’ pere. Op hierdie wyse kon die invloed van die onderskeie veranderlikes in elke kultivar bepaal word. Alhoewel die ensiematiese aktiwiteit van die polifenol oksidase ensiem (PFO) die hoogste in ‘Packham’s Triumph’ was, is gevind dat ‘Doyenne du Comice’ veel meer geneig was tot FV as ‘Packham’s Triumph’. Hierdie bevinding bevestig die verskynsel soos in die praktyk gevind.

Alhoewel geen betekenisvolle verskil gevind is tussen FV by vrug temperatuur van 3 °C en 15 °C nie, was daar meer verbruining by die hoër temperatuur. Hierdie verskynsel kan toegeskryf word aan ’n groter mate van waterverlies, laer sel turgiditeit en hoër ensiem aktiwiteit. Dus sal vrugte wat direk na opberging gesorteer word, minder FV toon. Die bydrae van die kondensasie wat op die vrug vorm, en as ’n smeermiddel dien, kan nie geïgnoreer word nie. Sulke vrugte met hoër turgiditeit, mag egter meer vatbaar wees vir kneusing, wat egter eers ’n geruime tyd na die kneusing tevoorverskyn mag kom. Aangesien ensieme, wat PFO insluit, as katalis dien in biochemiese reaksies, sal die beskikbaarheid van voldoende substraat, na alle waarskynlikheid die omvang van die biochemiese verkleuring reguleer.

Die oes van pere tydens optimum volwassenheid, en die voorkoming van onnodige friksie sal definitief die voorkoms van FV verminder. Die uitdaging is steeds om sortering- en verpakkingstegnieke verder te verfyn sonder om ’n negatiewe invloed op vrugtekwaliteit te hê.

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1 LITERATURE REVIEW: SHRIVELLING SUSCEPTIBILITY

1.1 INTRODUCTION

Pears can be seen as highly developed living containers filled with a watery solution. During the postharvest handling of these "organisms", all precautions are taken to prolong quality and shelf life. In doing so, suppliers increase their chances of delivering a first class product to the market and in return receive first-rate rewards and eventually earn good profit on their product. When the water content of these containers is reduced to a certain extent, undesirable quality characteristics are known to develop. Losses of as little as 5 % of the total fruit weight have been shown to cause unwanted taste as well as a reduction in visible quality in apples (Hatfield and Knee, 1988). Not only does excessive weight loss decrease the total saleable weight of the product, and therefore the possible returns, but it also diminishes the palatability thereof.

The causes of an environment that promotes shrivelling in pears are mostly physical by nature. Everything possible, within financial reason, should be done to minimize water loss as an essentiality of quality maintenance, thereby prolonging the marketability live of the fruit and maintaining the quality thereof. By not managing these constituents correctly, from the producer to the consumer, huge financial losses can be experienced.

1.2 WEIGHT LOSS

During the postharvest handling of fresh produce, fruit inevitably loses weight. These losses are mainly due to two processes, namely transpiration and respiration. In the first instance water is lost to the atmosphere through the release of vapour. Secondly, carbon dioxide from organic sources like sugars or acids is exchanged for oxygen during the respiration process leading to a decrease in dry weight. The rate of both these processes differs during the postharvest period (Sastry, 1985). If these losses happen in excessive proportions, the fruit will develop a shrivelled appearance (Magness and Diehl, 1924; Allen and Pentzer, 1935; Claypool, 1940; Johnson, 1976; Hruschka, 1977; Hatfield and Knee, 1988; Hagenmaier and Baker, 1995). According to Hatfield and Knee (1988) and Hruschka (1977) a loss of as little

as 5 % in mass can cause apples to take on a shrivelled appearance. This will render a flaccid and unappealing appearance to the fruit (Hatfield and Knee, 1988).

1.2.1 Transpiration

Transpiration involves the diffusion and, later, evaporation of water from plant parts to the immediate environment. This process occurs spontaneously wherever there is a vapour concentration gradient present. In this case the moisture will migrate from the high concentration in the fruit to a lower concentration of the atmosphere (Nobel, 1991). Nobel (1991) further stated that diffusion in this system is regulated by the steady state solution of Fick's first law of diffusion.

1.2.1.1 Driving force behind water loss

As earlier stated, Fick's law of diffusion is fundamental in determining the diffusion rate from any environment.

$$r'_{H_2O} = AP'_{H_2O}\,\Delta p_{H_2O} \qquad [1]$$

Where:

r'_{H_2O} = Rate of water loss from product ($mol.s^{-1}$)

A = Surface area of fruit (m^2)

P'_{H_2O} = Effective permeance of fruit surface to the environment of water vapour under prevailing conditions ($mol.s^{-1}.m^{-2}.Pa^{-1}$)

Δp_{H_2O} = Difference in partial pressure of water vapour between the environment and inside the fruit (Pa)

As Maguire et al. (2001) stated, this fundamental and one-dimensional method can be applied to determine the causes responsible for water loss due to transpiration. From the equation it is clear to see that there are three main factors affecting this whole process. Firstly, the barrier properties of the fruit skin which retard vapour from leaving the fruit (P' H_2O). Secondly, the fruit surface that is exposed to the surrounding atmosphere (A), and thirdly the driving force behind vapour loss ($\Delta p\ H_2O$). When any of the above factors are affected, the diffusion rate will change and result in a different rate of fruit water loss.

According to Monteith and Unsworth (1990), dry air is comprised of 77 % nitrogen, 21 % oxygen, 0.034 % carbon dioxides, 0.934 % argon and a further 1 % of insignificant constituents. As we are dealing with the translocation of water, it is important to identify and determine the concentration gradient of precisely that. Thompson (1992) stated that if water were present in a container with dry air, the water would eventually become vapour. This vapour will spread itself evenly throughout the container until such a state that the previously dry air is saturated with the vapour. The amount of vapour that air can accommodate is, to a great extent, dependant on the temperature and pressure present in such a system. This amount of vapour present in air is referred to as the relative humidity (RH). RH is then used to describe the relative amount of water vapour in the air under specific conditions (Burton, 1982).

1.2.1.2 Barrier properties

All fruits are surrounded with a barrier called the fruit skin. This barrier needs to comply with two conflicting requirements. The fruit skin must provide adequate protection towards the prevention of water loss and, in contrast, needs to be permeable enough for the exchange of oxygen and carbon dioxide during normal aerobic respiration (Lendzian and Kerstiens, 1991). By rearranging equation [1], fruit skin permeance can be characterized using the following equation:

$$P'_{H_2O} = \frac{r'_{H_2O}}{A\Delta p_{H_2O}} \qquad [2]$$

Once again there are three main factors determining the rate of water loss. Of the three parameters required, determining the driving force is considered to pose the greatest difficulty in calculating accurately (Gaffney et al., 1985).

According to Bell (1937) the common fruit skin comprises four tissue layers. From the inside of the fruit there is the hypodermis followed by the epidermis, cuticle and, in some cases, an outside layer of epidermal hair. Unlike the two deeper cellular layers, it is these outside layers, cuticle and trichomes, which are most important in preventing the unnecessary water loss from the plant to its environment (Holloway, 1982). Especially the cuticle, which is the

outmost layer of mature pear fruit, has a significant role to play in reducing water loss. It is found on all above ground plant parts (Lendzian and Kerstiens, 1991).

Smith (1933), Pieniazek (1944) and Schonherr (1976) investigated the relationship between the cuticle thickness and its resistance to water permeance. Schonherr (1976) did work on pear and citrus leaves and found no correlation between the thickness of the studied cuticle and the permeance thereof. In 1933 and 1944 two researchers, Smith and Pieniazek, respectively, found no proof that a thicker cuticle results in less water loss due to transpiration. These studies were done on 'Cox's Orange Pippin' and various other apple cultivars. Thus, there had to be other factors, concerning the cuticle, which influenced the rate of water loss through this limiting barrier.

The first to suggest that the micro cracks in the cuticle may be responsible for the ineffective barrier properties of the cuticle was Meyer (1944). Micro cracks, as described by Opara et al. (1997), refer to multiple cracks on the fruit surface. There is evidence that these cracks can develop before harvest and while fruit is kept in storage (Verner, 1935; Meyer, 1944). Maguire et al. (1998) found that these cracks can result in up to 15 times the permeance of intact apple cuticles. Shutak and Schrader (1948) stated that the thicker the cuticle, the more likely it is to develop these cracks. Maguire et al. (1998) suggested that a thicker cuticle cracks more easily, which results in a greater transpiration rate and thereby negating any relationship between cuticle thickness and water loss.

The composition and the structure of the cuticle also seem to have a noteworthy responsibility towards that of weight loss from fruit. Holloway (1982) described two different lipid- like substances found in the cuticle layers of most plants or fruit. Lipid substances in the cuticle can either belong to the soluble polymeric cutins, or insoluble cuticular lipids. The skeleton of the cuticle is formed by the insoluble cutins, whilst the epicuticular wax is comprised of the soluble lipids that are embedded into the insoluble cutin skeleton. It is these soluble lipids that are a distinguishable feature of most above ground plant parts. It is found as a continuous- or partial covering of mostly higher plants. According to Lendzien and Kerstiens (1991) the polarity and the presence of charged groups in the cuticle is a factor of

the transport and sorption of substances like water vapour. This cuticle layer is also very common, and in much bigger quantities, on fruits like pears (Baker 1982).

Schonherr and Lendzian (1981) did research concerning the effect of the barrier properties of this soluble substance on tomatoes. Once the protective layer was removed, the permeability to water increased 20-fold. In the case of onions, the increase was as big as 1 500-fold (Schonherr and Merida, 1981). This illustrates the importance of the cuticle layer, and more specifically the soluble cuticle layer as a primary barrier to water movement.

Reynhardt and Riederer (1994) described the occurrence of three different fractions that are present in waxes found on plants. The three fractions are identified as the crystalline region, solid amorphous phase and the mobile amorphous region. The amorphous phase is present as the porous matrix into which the water-resistant flakes (crystalline phase) are implanted (Riederer and Schreiber, 1995). According to Riederer and Schreiber (1995), the reduction of water movement can be attributed to the presence of these water-resistant flakes in the cuticle. The explanation given is two fold. Firstly, the total area of the amorphous phase, from which water can be lost, is significantly reduced. Secondly, the path that water molecules have to travel to the atmosphere outside is made circumlocutory by the presence of the crystalline phase.

1.2.1.3 Means of gas exchange

Most of the stomata in mature fruit are replaced by lenticels, which also replace most of the stomatal gas exchange requirements needed for aerobic respiration (Burton, 1982). Pienazek (1944) studied the contribution that lenticels make to the total amount of transpiration from apple fruit. In the case of ‘Golden Delicious’ this amounted to 21 % of the total transpiration value, by far the highest percentage of any of the studied cultivars. This value indicates that there is a considerable percentage of transpiration taking place directly through the cuticle. There seems to be no correlation between size and number of lenticels and the rate of water loss due to transpiration. The cuticle’s barrier characteristics appear to be more important than those of lenticels in terms of water loss due to transpiration.

1.2.1.4 Changes in fruit during ripening

Pieniazek (1943) investigated the effect that apple fruit maturity has on permeance to water. It was found that the permeance of immature fruit was much higher than that of fruit from the commercial harvest date and that a steady decline in permeance occurs as the fruit reach maturity. Sastry (1985) found very much the same tendency. He added that immature, as well as over mature fruit have higher transpiration rates, i.e. optimally mature fruit show the lowest transpiration rates. This tendency can be attributed to the condition of the soluble cuticle lipids during fruit growth (Woods, 1990). The main barrier properties of the lipids can be ascribed to the amount and composition of these lipids present on the fruit. Young and immature plant organs seem be lack crystal structures associated with soluble cuticle lipids (Jenks and Ashworth, 1999). As the plant organs reach maturity, and most probably fruit as well, these crystals grow in both quantity and structure. This could well explain the higher permeance by immature fruit cuticle to water vapour (Maguire et al., 2001)

What is the effect then in fruit once commercial maturity is reached? Pieniazek (1943) reported that, in apples, permeance increased as the harvest date was delayed. Maguire et al. (1998) found similar results on different apple cultivars. The explanation given by Jenks and Ashworth (1999) is that tissues senesce over time. This degradation is a result of environmental weathering (rain, sun, and wind), mechanical damage as well as a decline in soluble cuticle lipids.

1.2.1.5 Surface area

Schouten et al. (2004) used two different ways of calculating the surface area of a pear, as this parameter is necessary to calculate the gas exchange of the fruit. In the first instance they assumed that the shape of a pear can be considered as a cone placed on top of a semi sphere. Equation 3 was then used to calculate the surface area thereof. In the second instance a quick and accurate computer imaging (CI) technique was developed and used to calculate the surface area.

$$A = \frac{O^2}{2\pi} + \frac{1}{2} O \sqrt{\frac{O}{4\pi^2} + \left(\frac{LO}{2\pi}\right)^2} \qquad [3]$$

Where:

A = Surface of a pear

L = Length of pear

O = Circumference of pear

Consider the relationship between fruit weight and surface area. As Maguire et al. (2001) stated, fruit surface area increases as the fruit weight increases. On the contrary, the ratio of fruit surface area to fruit weight decreases as the weight increases. Therefore, large fruit would have a smaller surface area to weight ratio than equivalently shaped smaller fruit. Thus, large fruit will lose more water in real terms, but less than small fruit terms of percentage of total weight. Jackson et al. (1971) also found that, in apples, smaller fruit tend to shrivel before larger fruit.

1.2.2 Respiration

Respiration is the second, and less critical, way in which fresh produce weight is reduced. This process converts complex carbon chains into simpler molecules. Oxygen is consumed during the process where substances like sugars, organic acid and starch are converted to carbon dioxide and water. The main purpose for these reactions is to generate energy that the cell can use for synthetic reactions. Energy generated from the respiration reactions is vital to sustain the biochemical functions of the cell (Burton, 1982; Wills et al., 1989).

As previously mentioned, the fruit need oxygen to undergo aerobic respiration. Maguire et al. (2001) used the following equation to highlight the chief controlling factors of respiration.

$$r_c' = M_a(C) r'_{CO_2} \qquad [4]$$

Where:

r'_c = Rate of carbon loss (kg. s^{-1})

r'_{CO_2} = Rate of respiration (mol. s^{-1})

$M_a(C)$ = Atomic mass for carbon (kg.mol^{-1})

In the case where little, or no oxygen at all is present, the fruit will start to ferment. Unlike the situation in aerobic respiration, fruit kept under such storage conditions produce more CO_2 relative to the O_2 consumption. Therefore, the rate of carbon loss will be greater and such fruit will lose more weight due to anaerobic respiration (Gran and Beaudry, 1993; Yearsley et al., 1996).

The percentage contribution that respiration makes to the total amount of weight loss depends to a large extent to the storage environment. If the RH is high, the water loss will be relatively low and carbon loss will play a more prominent role. In such a case the total weight loss will be small. The major fruit weight losses will occur under conditions that promote a high transpiration rate. If apples are kept at 0 °C and 90 % RH, the loss due to respiration will only account for approximately 7 % of the total weight loss (Maguire et al., 1998).

1.3 FACTORS AFFECTING WEIGHT LOSS AND THE REDUCTION THEREOF

There are a number of postharvest factors that can influence the extent of weight loss of fresh produce. To manage water loss in fruit, one needs to address each component of the equation mentioned in [1]. These are (1) the barrier properties of the fruit, (2) the driving force behind the water loss and (3) the fruit area exposed to the atmosphere. There is a twofold strategy to reduce these values, as summarised in Table 1 by Maguire et al. (2001). Firstly, the driving force causing the water loss can be reduced. Secondly, an enhanced barrier can be created around the fruit to prevent water loss from within, whereby the permeance towards water vapour loss through the fruit skin is lowered. The biggest contributor to weight loss is transpiration (Burton, 1982). The elements that have an effect on weight loss, either by increasing the rate of transpiration, respiration or both, as well as ways of limiting these losses will be discussed. The initial stage of storage proves to be the most important period in which to try and prevent these losses, as the degree of weight loss seems to decline as time continues (Burdon and Clark, 2001).

Table 1: Strategies for minimizing weight loss of apples (Maguire et al., 2001)

Strategy	Options	Actions
Minimize water vapour permeance	Reduce micro-cracking	Avoid low crop load
		Avoid sudden changes in tree water status
		Reduce irrigation immediately prior to harvest
		Harvest early
	Reduce mechanical damage	Reduce impacts throughout harvest, grading and packing
	Apply artificial barriers to water transfer	Apply surface coating after harvest
Reduce driving force	Reduce temperature	Pre-cool rapidly
		Maintain constant low temperature
	Elevate environmental RH	Insulate cold stores
		Maintain low temperature difference between evaporator and air
		Minimize door openings
		Eliminate moisture sinks
		Add water to system
		Use water transfer barriers in packaging
Segregate fruit	Match risk with packaging and handling chain	Assign high risk fruit to low driving force environment and short term storage

1.3.1 Temperature

Any object's temperature is a reflection of the amount of energy that is possesses. This energy level is the net result of all energy entering and leaving such an object (Nobel, 1991; Garby and Larsen, 1991). Another source of energy within all living organisms is the generation of heat due to respiration. While the fruit respire, complex carbon chains are converted into carbon dioxide and water along with the release of heat (Burton, 1982; Wills et al., 1989). As the fruit temperature increases, and the energy levels thereof rise, water molecules at the fruit surface are being excited. This causes water molecules to share kinetic energy in terms of thermal energy. With this rise in kinetic energy the Brownian motion of the water molecules also increases (Garby and Larsen, 1991, Hayne, 2001). This, in turn, results in an increase in partial pressure of the water vapour within the fruit as well as at the fruit surface. The amount of water vapour in the surrounding environment is generally much less that the fruit's. Therefore, according to the diffusion laws, water vapour will diffuse to the area of lowest concentration i.e. the air (Hayne, 2001). This results in the partial pressure difference that is one of the main driving forces behind fruit weight loss (Sastry, 1985; Woods, 1990).

According to Hoang et al. (2004) the swift and well-timed reduction of fruit temperature will lead to significant results concerning water loss levels. Ideally, the fruit surface temperature needs to be lowered and stabilized at the required level. This is paramount to achieve the objectives set out to obtain export quality fruit. Standard procedures to attain this, include the swift lowering of fruit temperature directly after harvest, without any unnecessary delay, and maintaining fruit at such temperature regimes (Mitchell, 1992).

According to Schonherr and Schmidt (1979) the ability of water to move through the cuticle is improved by an increase in temperature. Once temperatures of 45 °C or higher are encountered, the cuticle undergoes permanent structural change. The permeability of these fruit cuticles will be higher and therefore the fruit is likely to lose water more easily. The argument in such cases, according to Eckl and Gruler (1980), is that the soluble cuticle lipids undergo reorientation once the cuticle recools after high temperature was experienced. Once this phase transition occurs, the orientation of the lipids is altered and so-called hydrophilic holes are formed at the fruit surface. According to Schreiber and Schonherr (1990) the cuticle

changes that occur between the physiological temperatures of 0 °C to 30 °C prove to be significant, as they found a 300 % increase in permeance of citrus leaves between these temperatures.

1.3.2 Relative humidity

Atmospheric air is a mixture of water vapour and dry air. The dry air is comprised of 78 % argon, 21 % oxygen, 0,03 % carbon dioxide as well as other minor constituents (Monteith and Unsworth, 1990, Wills et al., 1989). Humidity is the general term that describes the amount of water vapour in the air. RH is the term used to describe the humidity of moist air and is temperature dependant. Therefore, RH is expressed as the percentage of actual water vapour in relation to the maximum amount of water vapour that the air can hold at that specific temperature (Wills et al., 1989). As the intercellular spaces will be very close to saturation, in relation to water vapour, one can assume that the RH will be 100 % at normal fruit temperature (Burton, 1982). Thus, to prevent a large driving force from withdrawing most of the moisture from fruit, the RH of the surrounding atmosphere needs to be close to 100 %. By keeping the RH high, one will reduce the amount of water vapour that is required to be added to the air before the saturation point is reached. In such cases the possible amount of water vapour that can be withdrawn from the fruit will be reduced (Wills et al., 1989).

Thus, at any given temperature, the partial pressure difference will be lowered if the RH is increased. To create such environments it is important to have good insulated cold stores. Another critical feature of well-kept cold stores is that the temperature difference between the air in the cold store and the evaporator should be kept as small as possible. Small differences in temperature between the evaporator and the air ensure that only small amounts of vapour condense onto the evaporator. To achieve this, the capacity of the cold store should be established and a sufficient evaporator needs to be installed. The larger the evaporator, the higher is the delivery air temperature at which it can still achieve similar refrigeration capacities as a smaller, colder evaporator. In such cases the larger evaporator will extract less water vapour from the cold store because the temperature difference between the evaporator and the air is less. This feature needs to be addressed while planning to build a new cold store, as well as in the daily running of the facility (Thompson, 1992).

Hoang et al. (2004) stated that the ideal conditions under which fruit should initially be kept are: low temperature, high air velocity and a low RH. Such an environment will allow product to be cooled down faster due to the coupled effect of heat and mass transfer. Although a low storage temperature will be reached more quickly, storing fruit for long periods of time at a low relative low humidity will increase weight loss. Storing apples (Tu et al., 2000) and pears (Dijkink et al., 2004) at a humidity of at least 95 %, will ensure less undesirable fruit weight losses (Grierson and Wardowski, 1978).

Another important constituent of this equation is the sink for moisture within the storage environment. All possible sinks would ideally be eliminated to acquire an enhanced storage atmosphere for the fruit. By adding extra moisture to the cold store, like dampening the floor beforehand or pre-soaking wooden bins, will create less of a sink to absorb moisture from the fruit. Another way in which the barrier can be increased is by packing the fruit in plastic bags, thereby creating a modified atmosphere around the fruit. Such liners can significantly lessen the partial pressure difference between the fruit and the environment, and thus reduce water loss from the apples (Wills et al., 1989).

Barrie (1968) states that the fruit cuticle has similar properties to that of a continuous hydrophobic polymer membrane. He found that the gas permeability of such a continuous hydrophobic polymer membrane increases with an increase in RH at any given temperature. Therefore one would suspect the same reaction from a hydrophobic fruit cuticle. Sastry et al., (1978) reported that the rate of water permeance through a fruit skin is independent of environmental alterations. This statement, concerning fruit cuticles, can be questioned due to the fact that various researchers found the contrary (Smith, 1933; Lentz and Rooke, 1964; Fockers and Meffert, 197; Shirazi and Cameron, 1993). All these researchers found that an increase in RH caused an increase in water vapour permeability.

Schonherr (1976) investigated the relationship between water vapour permeability and water content of isolated apple cuticles. A positive correlation was found to exist between these two factors. Chamel et al. (1991) also studied the relationship between increased water vapour pressure and absorption. These studies on apples, tomato and pepper fruit showed the same

tendency. Schonherr and Schmidt (1979) studied the relationship between atmospheric vapour pressure and water vapour permeability. Results illustrated that these two factors coincide in trials done on citrus leaves and eggplant fruit.

A possible explanation for increasing water permeance associated with increasing water content is given by Lendzian and Kerstiens (1991). It appears that water-coated pathways are formed through which water vapour can move more easily. These pathways are formed throughout the cuticle when water molecules attach themselves to polar groups within the cuticle. Such water-coated channels provide paths of little resistance through which water vapour can diffuse easily. Therefore it is believed that water vapour permeance within the cuticle is increased by an increase in air water vapour content. Chau et al. (1998), Gaffney et al. (1985) and Sastry (1985) had drawn a different conclusion regarding this matter. They alleged that the permeance of the exposed cuticle is unaltered by the atmospheric water vapour pressure.

As Paull (1999) correctly state, RH is dependant on the cooling coil's surface area as well as the temperature difference between the coil and surrounding air. Aspects that influence RH include: fresh produce, packing material, air exchange rate and the temperature distribution within the cold store.

1.3.3 Barrier properties

It is evident that, under similar conditions, fruit with a high permeance will lose water more rapidly than fruit with a low permeance. The primary goal regarding this aspect must be to prevent micro cracking of the cuticle (Maguire et al., 2001). Opara et al. (1997) mentioned that an excessive change in tree water status is unfavourable in this regard. It is believed that such sudden changes result in micro cracks of the cuticle. Volz et al. (1993) recommend that trees should not be irrigated immediately prior to harvest. The incidence of cuticle cracking will apparently be less in such cases. However, the consequence of such treatments must be thoroughly assessed prior to commercial application. Volz et al. (1993) also comment that fruit from trees with a low crop yield are especially in danger of micro cracking due to the large growth potential of the fruit.

Another approach to avoid micro cracking is to try and harvest more immature fruit by harvesting earlier in the season. The theory behind it is to deny enlarging fruit the opportunity to develop faster than what the cuticle can expand. An example of this is that of 'Braeburn' apples that tend to develop a large number of cuticle cracks as the harvest date is delayed (Maguire et al., 1998).

Postharvest applications of edible fruit coatings have proven to be an effective way to increase the barrier properties of the fruit. Such coatings will eliminate areas with a high permeance to water such as cracks in the cuticle (Banks et al., 1997). The application of considerably diluted wax coatings to fruit supplies the most prospective requirements needed from a fruit coating. Such applications will give reduced permeance, without suffocating the fruit. The latter is easily achieved when undiluted wax coatings are used.

1.3.3.1 Fruit packaging

Both skin as well as boundary layer permeance has an influence on the rate of water loss from fresh produce (Gaffney et al., 1985). The way in which produce is packed interferes with the way in which free air flows around the product. This area, the so-called 'boundary layer', is described by Nobel (1975) as: "the zone of gas around the fruit within which there is limited turbulent mixing". As the air velocity is increased the effective thickness of the boundary layer decreases, as can be seen in the following equation.

$$\Delta x^{bl} = \frac{2.8\sqrt{\frac{d^f}{v}} + \frac{0.25}{v}}{1000} \qquad [5]$$

Where:

Δx^{bl} = Effective boundary layer thickness (m)

d^f = Diameter of fruit (m)

v = Velocity of air (m)

As the air velocity increases, the boundary layer thickness will decrease and thus expose the fruit to these higher air velocities. Gaffney et al. (1985) and Nobel (1975) both stated that fruit under such conditions are expected to lose more weight due to transpiration.

Not only does fruit packaging guard the protective boundary layer, but packaging can also increase the RH in the immediate air surrounding the fruit. By reducing the air velocity that passes the fruit, this environment around the fruit is more saturated with water vapour and thus increases the water vapour partial pressure. This will result in less weight loss due to water loss. This then is the main purpose for plastic liners found in fruit cartons (Lentz and Rooke., 1964, Wills et al., 1989).

1.3.3.2 Waxing of the fruit

Fruit and other fresh produce are waxed for two reasons. Firstly, to enhance the cosmetic quality thereof (sheen, perceived depth of colour) and secondly, to create a modified atmosphere by improving the barrier properties of the fruit (Banks et al., 1997). The application of edible wax to fruit has been practised from as early as the 1920's on apples to try and reduce weight loss (Magness and Diehl, 1924). Lately there is a considerable increase in research done on this topic (Hagenmaier and Shaw, 1992; Hagenmaier and Baker, 1995; Nussinovich and Lurie, 1995; Gontard et al., 1996; Mannheim and Soffer, 1996; Amarante and Banks, 2000). This reflects the industries interest in using such applications more frequently, either as an application to enhance fruit appearance or a preventative measure against shrivelling.

As previously mentioned, such an added layer would modify the protective barrier around the fruit. Therefore, such an application poses a certain risk associated with it in terms of gas exchange. Possible postharvest disorders that could arise due to poor gas exchange are fermentation due to anaerobic conditions and the occurrence of off fruit flavours (Banks et al., 1997).

1.3.4 Air movement

To ensure adequate lowering of fruit temperature, there has to be an effective energy gradient whereby heat can be removed as thermal energy from the fruit to the surrounding air. Good product ventilation is needed as cold air from the cooling coil flows past the hotter produce. Swift removal of such heat can only be achieved if there is adequate air circulation around the fruit itself. As the air velocity increase, the boundary layer will be altered and the amount of water vapour that can be lost from the fruit will increase (Wills et al., 1989). Thus, as Grierson and Wardowski (1978) stated, a higher air velocity will result in quicker lowering of produce temperature and therefore less weight loss. This principle is true for most fruit like pears, which have a barrier protection in the presence of the cuticle, but another set of rules applies to those with a wet surface, e.g. mushrooms, that have no cuticle.

There is, however, a compromise to be made in this aspect. Adequate air movement is necessary to create a big enough temperature gradient for sufficient temperature exchange, at a rate that limits the loss of weight due to boundary layer disturbance.

1.3.5 Mechanical damage

Any way in which the cuticle is broken or disrupted will have a negative effect on the barrier properties thereof. Literature clearly states that any cuts (Sastry, 1985), cuts and stem punctures (Burton, 1982) or bruises (Wills et al., 1989) that rupture the protective cuticle layer around the fruit will inevitably alter the permeance thereof, and therefore the rate of diffusion and, subsequently, water loss. This is a result of the unprotected underlying cell layers being exposed to the outside atmosphere.

Such cuticle cracks can either occur prior to harvest (Verner 1935; Meyer 1944) or thereafter (Goode et al. 1975). Opara et al. (1997) describe these cuticle cracks as the presence of several minuscule surface faults on the cuticle. Such cracks can result in an increased permeance of up to 15% higher than that of an intact apple cuticle (Maguire et al., 1998). Maguire et al. (1998) further stated that most of the variation in different cuticle permeance found in her trials could be attributed to the degree of cuticle cracks that were present.

Meyer (1944) studied the relationship between fruit size and the occurrence of cuticle cracks on 'Golden Delicious' apples. It was found that the rate of epidermal cell division influenced the integrity of the fruit cuticle. As the rate of fruit growth increased, the occurrence of cuticle cracks also grew. Such cracks resulted in epidermal and even hypodermal tissue of mature fruit being exposed without any protection from either the cuticle or cork formation.

Conflicting results remain in the literature regarding the side (sun or shade) of a fruit that is likely to show a tendency towards cuticle cracking first (Tetley 1930, Verner 1935, Shutak and Schrader 1948). However, Opara (1997) stated that cuticle cracking occurs most often where the fruit cuticle is thicker and less elastic.

A common disorder associated with shrivel in apples, is that of russetting (Pieniazek, 1944). Apparently the side of the apple which had the most severe condition of russet, showed shrivel first. In such cases, the cork layers will penetrate deeper into the fruit skin than normal. Not only will these circumstances compromise the barrier properties of the cuticle, but micro cracks will also develop micro cracks in these areas. Such circumstances will increase water loss from the fruit considerably.

1.4 CONCLUSION

The onset of shrivel in pears is more an adverse result of transpiration than respiration. The degree to which transpiration takes place is mostly a result of the physical conditions, of which the most important are temperature and the RH at which the fruit is kept. Therefore, one can argue that, to prevent shrivel from setting in on pears, one must apply good postharvest handling practices. Therefore, the most favourable environment needs to be created for the fruit during the postharvest handling thereof. Whether addressing the harvesting procedure, transport, storage or fruit retailing outlets, optimising these physical factors will minimize water loss from pears significantly. Because the rate of moisture loss decreases with time, the initial stages within the postharvest chain might prove to be the most critical in minimising the extent to which shrivel could set in. Dealing with these areas might prove to be the most effective with the least amount of effort.

The occurrence of cultivar difference cannot be ignored. The fact that the physical appearance of cultivars differs, gives rise to different volume to surface area ratios, especially in the neck area of pears. Due to this small ratio in the neck of some pear cultivars, only a small degree of moisture loss might prove to show visible reduction in the quality thereof. It is, therefore, no wonder that shrivel proves to be most troublesome in this area. The influence of the fruit stalk might also prove to play a significant role towards the development of shrivelling thereof.

Maguire et al. (2001) stressed the contributions that micro cracking of the fruit skin adds to the degree of shrivel of fruit. This problem can be addressed by doing further research in this field and linking this phenomenon to that of shrivelling.

1.5 LITERATURE CITED

Allen, F.W., Pentzer, W.T., 1935. Studies on the effect of humidity in the cold storage of fruits. Proc. Am. Soc. Hort. Sci. 33, 215 223.

Amarante, C., Banks, N.H., 2000. Postharvest physiology and quality of coated fruits and vegetables. Hort. Rev. 26, 161-232.

Baker, E.A., 1982. Chemistry and morphology of plant epicutucular waxes. In: D.F. Cutler, K.L. Alvin and C.E. Price, The plant cuticle. Academic press, London. p.139.

Banks, N.H., Cutting, J.G.M., Nicholson, S.E., 1997. Approaches to optimising surface coatings for fruits and vegetables. New Zeal. J. Crop Hort. Sci. 25, 261-272.

Barrie, J.A., 1968. Water in polymers. In: T. Crank and G.S. Park, (eds.), Diffusion in polymers. Academic Press, London. pp. 259-313.

Bell, H.P., 1937. The protective layers of the apple. Canadian J. Res. 15, 391-402.

Burdon, J., Clark, C., 2001. Effect of postharvest water loss on 'Hayward' kiwifruit water status. Postharv. Biol. and Technol. 22, 215-225.

Burton, W.G., 1982. Postharvest physiology of food crops. Longman, London, New York.

Chamel, A., Pineri, M., Escoubes, M., 1991. Quantitive determination of water sorption by plant cuticles. Plant Cell Environ. 14, 87-95.

Chau, K.V., Romero, R.A., Baird, C.D., Gaffney, J.J., 1998. Transpiration coefficients for certain fruits and vegetables. Am. Soc. Heat. Refrig. Aircond. Eng. Trans. 94, 1553-1562.

Claypool, L.L., 1940. The waxing of deciduous fruits. Proc. Am. Soc. Hort. Sci. 115, 775-778.

Dijkink, B.H., Tomassen, M.M., Willemsen, H.A., Van Doorn, W.G., 2004. Humidity control during bell pepper storage, using a hollow fiber membrane contractor system. Postharv. Biol. and Technol. 32, 311-320.

Eckl, K., Gruler, H., 1980. Phase transitions in plant cuticles. Planta. 150, 102-113.

Fockers, F.H., Meffert, H.F.T., 1972. Biophysical properties of horticultural products related to loss of moisture during cooling down. J. Sci. Food Agr. 23, 285-298.

Gaffney, J.J., Baird, C.D., Chau, K.V., 1985. Influence of airflow rate, respiration, evaporative cooling, and other factors affecting weight loss calculations for fruits and vegetables. Am. Soc. Heat Refrig. Aircond. Eng. Trans. 91, 690-707.

Garby, L., Larsen, P.S., 1991. Bioenergetics: Its thermodynamic foundations. Cambridge University Press. pp.

Gontard, N., Thibault, R., Cup, B., Guilbert, S., 1996. Influence of the relative humidity and film composition on oxygen and carbon dioxide permeabilities of edible films. J. Agr. Food Chem. 44, 1064-1069.

Goode, J.E., Fuller, M.M., Hydrycz, K.J., 1975. Skin-cracking of 'Cox's Orange Pippin' apples in relation to water stress. J. Hort. Sci. 50, 265-269.

Gran, C.D., Beaudry, R.M., 1993. Determination of the low oxygen limit for several commercial apple cultivars by respiration quotient breakpoint. Postharv. Biol. and Technol. 3, 259-267.

Grierson, W., Wardowski, W.F., 1978. Relative humidity effects on the postharvest life of fruits and vegetables. HortScience. 13, 570-574.

Hagenmaier, R.D., Baker, R.A., 1995. Layered coatings to control weight loss and preserve gloss of citrus fruit. HortScience. 30, 296-298.

Hagenmaier, R.D., Shaw, P.E., 1992. Gas permeability of fruit coating waxes. J. Am. Sco. Hort. Sci. 117, 105-109.

Holloway, P.J., 1982. Structure and histochemistry of plant cuticular membranes: An overview. In: D.F. Cutler, K.L. Alvin and C.E. Prince (eds.), The plant cuticle. Academic Press, London. pp. 1-32.

Hatfield, S.G.S., Knee, M., 1988. Effects of water loss on apple in storage. Int. J. Food Sci. Technol. 23, 575-583.

Hayne, D.T., 2001. Biological thermodynamics. Cambridge University Press. pp. 1-57.

Hoang, M.L., Verboven, P., Baelmans, M., Nicolaï, M., 2004. Sensitivity of temperature and weight loss in bulk of chicory roots with respect to process and product parameters. J. of Food Engin. 62(3), 233-243.

Hruschka, H.W., 1977. Postharvest weight loss and shrivel in five fruits and five vegetables. Agricultural Marketing Service, U.S. Dept. of Agr., Marketing Res. Rep. 1059. In: Factors affecting weight loss of apples. Maguire, K.M., Banks, N.H., Opara, L.U., 2001. Hort. Rev. 25, 197-234.

Jackson, J.E., Sharples, R.O., Palmer, J.W., 1971. The influence of shades and within-tree position on apple fruit size, colour and storage quality. J. Hort. Sci. 46, 277-287.

Jenks, M.A., Ashworth, E.N., 1999. Plant epicuticular waxes: Function, production, and genetics. Hort. Rev. 23, 1-67.

Johnson, D.S., 1976. Influence of water loss on the storage quality of apples. Chem. Indust. 18, 1044-1046.

Lendzian, K.J., Kerstiens, G., 1991. Sorption and transport of gases and vapours in plant cuticles. Rev. Environ. Contam. Toxicol. 121, 65-128.

Lentz, C.P., Rooke, E.A., 1964. Rates of moisture loss of apples under refrigerated storage conditions. Food Technol. 18, 119-121.

Magness, J.R., Diehl, H.C., 1924. Physiologocal studies on apples in storage. J. Agr. Res. 27, 4-35.

Maguire, K.M., Banks, N., Lang, A., 1998. Harvest and cultivar effects on water vapour permeance in apples. p. 246-251. In: E. J. Mitcham (ed.), Proc. 7th Int. Controlled Atmosph. Res. Conf. Davis, CA, USA. pp. 246-251.

Maguire, K.M., Banks, N.H., Opara, L.U., 2001. Factors affecting weight loss of apples. Hort. Rev. 25, 197-234.

Mannheim, C.H., Soffer, T., 1996. Permeability of different wax coatings and their effect on citrus fruit quality. J. Agr. Food Chem. 44, 919-923.

Meyer, A., 1944. A study of the skin structure of Golden Delicious apples. Proc. Am. Soc. Hort. Sci. 45, 105-110.

Mitchell, F.G., 1992. Postharvest handling systems: Temperate fruits. In: A.A. Kader, Postharvest technology of horticultural crops, Special publication. 3311. Agricultural and natural resources publications, Univ. California, Oakland.

Monteith, J.L., Unsworth, M.H., 1990. Heat transfer. In: Principles of environmental physics, 2nd ed. Edward Arnold, London, New York. pp. 121-145.

Nobel, P.S., 1975. Effective thickness and resistance of the air boundary layer adjacent to spherical plant parts. J. Expt. Bot. 26, 120-130.

Nobel, P.S., 1991. Cells and diffusion. In: Physicochemical and enviromental plant physiology. Academic Press, San Diego. pp. 1-46.

Nussinovich, A., Lurie, D., 1995. Edible coatings for fruits and vegetables. Postharv. News and Inform. 6, 53-57.

Opara, L.U., Studman, D.J., Banks, N.H., 1997. Fruit skin splitting and cracking. Hort. Rev. 19, 21-262.

Paull, R.E., 1999. Effect of temperature and relative humidity on fresh commodity quality. Postharv. Biol. and Technol. 15, 263-277.

Pieniazek, S.A., 1943. Maturity of apple fruits in relation to the rate of transpiration. Proc. Am. Soc. Hort. Sci. 42, 231-237.

Pieniazek, S.A., 1944. Physical characters of the skin in relation to apple fruit transpiration. Plant Physiol. 19, 529-536.

Reynhardt, E.C., Riederer, M., 1994. Structure and molecular dynamics of plant waxes. Cuticular waxes from leaves of *Fagus sylvatica* L. and *Hordeum vulgare* L. Europ. Biophys. J. 23, 59-70.

Riederer, M., Schreiber, L., 1995. Waxes: The transport barriers of plant cuticles. In: R. J. Hamilton (ed.), Waxes: Chemistry, Molecular biology and functions. The Oily Press, West Ferry, Scotland. pp. 131-159.

Sastry, S.K., 1985. Factors affecting shrinkage of fruits in refrigerated storage. Am. Soc. Heat Refrig. Aircond Eng. Trans. 91, 683-689.

Sastry, S.K., Baird, C.D., Buffington, D.E., 1978. Transpiration rates of certain fruits and vegetables. Am. Soc. Heat Refrig. Aircond. Eng. Trans. 84, 237-155.

Schonherr, J., 1976. Water permeability of isolated cuticular membranes: The effect of cuticular waxes on diffusion of water. Planta. 131, 159-164.

Schonherr, J., Lendzian, K.J., 1981. A simple and inexpensive method of measuring water permeability of isolated plant cuticle membranes. Z. Pflanzenphysiol. 102, 321-327, abstract.

Schonherr, J., Merida, T., 1981. Water permeabilities of plant cuticular membranes: The effects of humidity and temperature on the permeability of non-isolated cuticles of union bulb scales. Plant Cell Environ. 4, 459-466.

Schonherr, J., Schmidt, H.W., 1979. Water permeability of plant cuticles: Dependance of permeability coefficients of cuticular transpiration on vapour pressure saturation deficit. Planta. 144, 391-400.

Schouten, R.E., Vedltman, R.H., De Wild, H.P.J., Koopen, T.J., Staal, M.G., Tijskens, L.M.M., 2004. Determination of O_2 and CO_2 permeance, internal respiration and fermentation for a batch of pears (cv. Conference). Postharv. Biol. and Technol. 32, 289-298.

Schreiber, L., Schonherr, J., 1990. Phase transition and thermal expansion coefficients of plant cuticles: The effect of temperature on structure and function. Planta. 182, 186-193.

Shirazi, A., Cameron, A.C., 1993. Measuring transpiration rates of tomato and other detached fruit. HortScience. 28, 1035-1038.

Shutak, V., Schrader, A.L., 1948. Factors associated with skin-cracking of 'York Imperial' apples. Proc. Am. Soc. Hort. Sci. 51, 245-257.

Smith, W.H., 1933. Evaporation from apples in relation to temperature and atmospheric humidity. Ann. Applied Biol. 20, 220-235.

Tetley, U., 1930. A study of anatomical development of the apple and some observations in the 'pectic constituents' of the cell-walls. J. Pomol. Hort. Sci. 8, 153-171.

Thompson, J.F., 1992. Psychometrics and perishable commodities. p. 79-84. In: A.A. Kader (ed.)., Postharvest technology of horticultural crops, Special publ. 3311. Agricultural and Natural Resources Publ., Univ. California, Oakland.

Tu, K, Nicolaï, B., De Baerdemaeker, J., 2000. Effects of relative humidity on apple quality under simulated shelf temperature storage. Scientia Hort. 85, 217-229.

Verner, L., 1935. A physiologival study of cracking in Stayman Winesap apples. J. Agr. Res. 51, 191-222.

Voltz, R.K., Ferguson, I.B. Bowen, J.H., Watkins, C.B., 1993. Crop load effects on fruit mineral nutrition, maturity, fruiting and tree growth of 'Cox Orange Pippin' apple. J. Hort. Sci. 68, 127-137.

Wills, R.B.H., McGlasson, W.B., Graham, D., Tee, T.H., Hall, E.G., 1989. Effects of water loss and humidity. p. 53-60. In: Postharvest: An introduction to the physiology of handling of fruit and vegetables. 3rd ed. New South Wales Univ. Press, Kensington.

Woods, J.L., 1990. Moisture loss from fruits and vegetables. Postharv. News and Inform. 1, 195-199.

Yearsley, C.W., Banks, N.H., Ganesh, S., Cleland, D.J., 1996. Determination of lower oxygen limits for apple fruit. Postharv. Biol. and Technol. 8, 95-109.

2 LITERATURE REVIEW: FRICTION DISCOLOURATION SUSCEPTIBILITY

2.1 INTRODUCTION

Friction discolouration (FD) is one of the few troublesome disorders remaining in pear postharvest handling (Chen and Varga, 1996). Fruit skin discolourations are produced by either friction or bruising of the fruit (Mitcham et al., 2001). As early as 1974, Mellenthin and Wang commented on FD by saying: "it is one of the most serious postharvest problems in the pear industry". Meheriuk et al. (1982) describe FD as: "diffuse, brown skin discolouration, especially at high points of irregular fruit surfaces". Raese (1989) stated that such unappealing skin appearances result in purchaser dissatisfaction. This problem poses huge customer discontent that will inevitably lead to financial complications. Fruit that is typically more in danger from this includes: fruit stored for long periods, the handling of immature fruit and fruit of inferior size. The two most important aspects that should be addressed are fruit maturity and the speed at which the pack line operates. Slowing down the pack line speed could have significant advantages.

2.2 ENZYMATIC BROWNING

Enzymes are large, specialised proteins, made up of 20 or more amino acids, and are very reaction specific. These enzymes are fundamental in catalysing some of the essential reactions taking place within cells. By lowering the activation energy required for certain reactions, they accelerate the reaction speed, and consequently, reduce the time necessary for the reaction to take place (Salisbury and Ross, 1992). Polyphenol oxidase (PPO) is primarily located in the plastids, whereas the related phenols are predominantly located in the vacuole or cytoplasm (Barrett et al., 1991). This enzyme (PPO) is a hydrophilic protein that can attach itself to the thylakoid membrane by means of its short, hydrophobic tail (Marques et al., 1994).

Therefore, when plant cells are injured, decompartmentalisation occurs and the three constituents needed for enzymatic browning (substrate, enzyme and oxygen) are brought together. Under such conditions enzymatic browning will inevitably take place. This phenomenon will occur rapidly in most fruits (apple, pear, apricot, peach and cherries), but

not in all fruit (e.g. citrus, pineapple, tomato). The reason for this is that, in the cases of citrus, pineapple and tomato, the presence of the enzyme, PPO, is lacking (Meheriuk et al., 1982). Therefore, if this copper-containing enzyme is present, as is the case with pear fruit, this browning will result due to a series of biochemical reactions whereby phenols, such as cholorogenic acid and catechol, are ultimately oxidised. Firstly, PPO catalyses the hydroxylation of monophenols to form *o*-diphenols (monophenolase activity), and secondly, it catalyses the oxidation reaction of *o*-diphenols to *o*-quinones (diphenolase activity). It is these unstable *o*-quinones that undergo polymerisation to form dark coloured pigments (Fenoll et al., 2004). Espin et al. (1997) were the first to report on the monophenolase ability of PPO after isolating it from 'Blanquilla' pear.

Mayer and Harel (1990) and Sgarbi et al. (2003) suggested that this whole process acts as a defensive mechanism against tissue damage and subsequent infection. Once these reactions have taken place, unstable orthoquinones are formed, which in turn undergo polymerisation to form severely coloured pigments (Goupy et al., 1995, Saspers, 1989, Nicolaï et al., 1994). These quinones are believed to possess effective antifungal properties. Therefore, in cases where fruit injury is experienced, possible fungal infection will be localized to the area of injury (Salisbury and Ross, 1992). Thus, any action that might lead to the rupture of pear cells, be it pre- or postharvest, will eventually lead to enzymatic browning (Meheriuk et al., 1982).

Proteins, and therefore also enzymes, are very sensitive to temperature changes. Not only will low temperatures negatively affect the activity of the enzyme, but high temperatures can also cause protein denaturation and subsequent loss of catalytic ability. This thermal characteristic of the PPO enzyme has been exploited in many a way to try and inactivate the enzymes by means of different heat treatments. Weemaes et al. (1998) stated that heat inactivation of pear PPO becomes progressive at 60-65 °C. Exposing PPO to brief periods of high temperatures will successfully destroy the oxidative ability of the enzyme (Weemaes et al., 1998). Tate et al. (1964) stressed that the duration of exposure has to be brief and adequate, because a slow blanching technique will only result in the activation of the enzyme. The application of such heat inactivation applications on fresh fruit could pose a problem where aspects like texture

and taste might be negatively influenced. The effectiveness thereof proves to be most valuable on processed products like fruit puree and -juice.

Yue-Ming et al. (1997) and Jiang et al. (2004) studied the thermo stability and activity of litchi PPO. It is reported that at 25 °C, the enzyme operates at only 28 % of its optimum ability, which is achieved at 70 °C. Only when the enzyme was kept for 10 minutes at 90 °C did it lose 50 % of it activity due to inactivation. Dogan and Dogan (2004), Abreu et al. (2003) and Fujita et al. (1995) found similar results in terms of heat inactivation for '*Thymus*', pear and cabbage respectively. The time necessary for this reaction to take place is very short. Cheng and Crisosto (1995) experienced that such browning in peach and nectarine took place within the first hour of incubation. As previously mentioned, this reaction is temperature sensitive and it will be more rapid in warmer environments than at environments with a lower temperature, as Feng et al. (2004) found with pears.

Enzymatic activity is also pH dependant. Weemaes et al. (1998) found that the optimum activity of PPO in pear occurs at pH 7. This correlates well with what was found in litchi; pH = 7,0 (Yue-Ming et al., 1997), avocado; pH = 7.5 (Gomez-Lopez, 2002) and cabbage; pH = 7.4 (Fujita et al., 1995). A noticeable decrease in activity was observed once the pH was increased to above 8. When the pH was lowered to below 4 a similar decrease in enzyme activity was observed (Weemaes et al., 1998). This correlates well with the results found by Halim and Montgomery (1978) and Yue-Ming et al. (1997). On the contrary, Espin et al. (1997) found that the optimum pH for PPO activity from 'Blanquilla' pear fruit was achieved at 4.3.

Aquino-Bolanos and Mercado-Silva (2004) challenged the belief that PPO is primarily responsible for catalysis of discolouration reactions in injured tissue. In the jicama roots (*Pachyrizus erosus* L. Urban) that were studied, the presence and catalytic activity of peroxidase (POD) proved to be instrumental in the discolouration of the samples. This discolouration is attributed to lignification of the cells in an attempt to protect them from further decay. Phenols that act as precursors for lignification, such as coumaric, caffeic- and ferulic acid proved to be good substrates for the POD.

Saltveit (2000) found that the enzyme phenylalanine ammonia-lyase (PAL) is induced during wound-induced stress of lettuce. This increase in enzymatic activity catalyses the production, and accumulation, of phenolic compounds like chlorogenic acid that can eventually undergo oxidation and cause subsequent browning. Fig. 1 illustrates the supposition of Saltveit (2000) on the alteration in phenolic metabolism due to a wound induced stress signal.

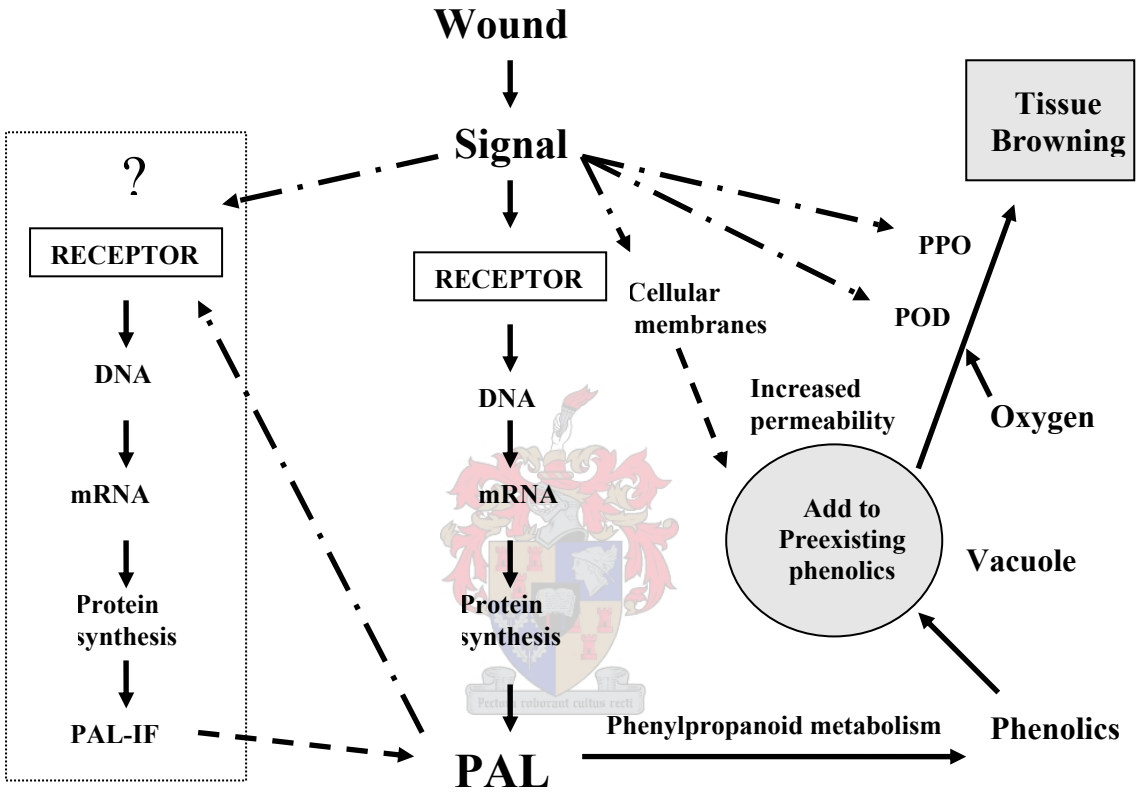

Fig. 1. The interrelationship between wounding lettuce leaf tissue and the subsequent changes in phenolic metabolism that leads to tissue browning. Many control points for postharvest modification of the wound-induced browning processes are evident in this diagram (adopted from Saltveit, 2000). PAL - Phenylalanine ammonia-lyase; PAL-IF - PAL-inactivating factor; PPO - Polyphenol oxidase; POD – Peroxidase.

2.3 DESCRIPTION OF BROWNING SYMPTOMS

This unsightly discolouration has been called many a name during the years. Most of the names are related to the action or conditions that give rise to the disorder. Names that exist

include: Finger prints, belt- or brush burn and scald (Smith, 1946), friction marking (Meheriuk et al., 1982), scuffing disorder (Chen and Varga, 1996), skin browning (Mitcham et al., 2001), peel browning (Feng et al., 2004) and friction discolouration (Mellenthin and Wang, 1974, Amarante et al., 2001). As this literature search will investigate the discolouration of pears caused by frictional forces, this disorder will be referred to as friction discolouration (FD).

2.4 FACTORS AFFECTING FRICTION DISCOLOURATION

Kvåle (1988) made the comment that different pear cultivars reacted differently to similar grading practices used during postharvest handling. Harvested pear fruit also respond differently from season to season even though similar grading procedures were used. From the literature it is evident that there are a number of different fruit and environmental factors that should be taken into consideration if the occurrence and extent of FD must be limited.

2.4.1 Phenolic composition

Not only does the severity of discolouration depend on the degree of the injury, but it also depends on the amount and activity of polyphenol oxidase and the quantity of the substrate, polyphenols. Fruit size and fruit maturity are two of the many factors affecting the amount of phenolic compounds, whether in the peel or in the flesh of pear fruit. As previously mentioned, it is the presence of the PPO that rapidly oxidises natural phenolic compounds like chlorogenic acid and catechol into unstable quinones. These highly unstable quinones will consequently polymerise along with other quinones, proteins, phenolic substances or amino acids to form dark coloured pigments. Sometimes these unsightly black, brown and red polymerised pigments are referred to as melanins (Goupy et al., 1995, Nicolaï et al., 1994, Weemaes et al., 1998).

Burges (1963) suggested that most plant phenolics are derived as secondary metabolites from at least three of the following different metabolic pathways: Prephenic- and shikimic acids, mevalonic acid and via an acetate condensation. Plant phenols accommodate a wide variety of secondary metabolites that are produced from carbohydrates during the shikimac pathway and phenylpropanoid metabolism (Ryan et al., 2002). This pathway, and therefore the

production of these aromatic amino acids, is only found in plants and micro organisms (Robards, 2003). Phenolic substances are particularly important in fruit and vegetables for their contribution to colour and flavour. All phenolic compounds possess an aromatic ring structure onto which various substitute groups, like hydroxyl, carboxyl and methoxyl, are attached. Due to their structure, phenols are more soluble in polar solvents, like water, than in nonpolar solvents (Salisbury and Ross, 1992). Wang and Mellenthin (1973) found, during their study on 'd'Anjou' pears, that the phenol, chlorogenic acid, correlated best with the incidence of enzymatic browning. This conclusion is supported by Gauillard and Richard-Forget (1997) who found that chlorogenic acid is a better substrate for 'Williams' pear PPO than catechin. Kvåle (1979) also stated that skin discolouration can be positively correlated to the chlorogenic acid content, but not to total phenol content in both 'Moltke' and 'Herrepaere' pear cultivars.

Such a situation could be explained on the basis of coupled oxidation (Goupy et al., 1995). As expected, they found that the degree of apple browning is a function of the amount of degraded phenols. Because the PPO activity does not stay constant, the initial amount of phenols and the rate of phenol synthesis are unknown; it is difficult to predict the amount of degraded phenols present in any apple sample. The effect of coupled oxidation causes variation within the different phenol classes, even though the total phenol content might stay the same. This variation in phenol categories, quantitatively and qualitatively, is the cause for conflicting results found in terms of chlorogenic acid content, total phenol content and enzymatic browning in apples.

As this effect of enzymatic browning is a costly affair, many researchers have studied the phenolic composition and enzyme activation properties in pear fruit (Amiot et al., 1995, Espin et al., 1997, Gauillard and Richard-Forget, 1997, Weemaes et al., 1998, Carbonaro and Mattera, 2001), puree (Dimick et al., 1951, Sioud and Luh, 1966, Amiot et al., 1995), juice (Cornwell and Wrolstad, 1981, Spanos and Wrolstad, 1990, Amiot et al., 1995) and fresh-cut pear slices (Gorny et al., 2000). As Goupy et al. (1995) stated, many a researcher attempted to correlate the browning intensity in apples to the main contributing factors, i.e. enzymatic activity and the phenolic content. Cosetang and Lee (1987) found that both these constituents played a role in the browning intensity in apples.

Saltveit (2000) reported on the phenolic metabolism following injury to fresh lettuce leaves. Wounding lettuce causes the production of enzymes that catalyse phenolic production and subsequent browning (Peiser et al., 1998). One of these enzymes, the first, and also the limiting factor in the pathway, is PAL. This enzyme is the first committed step towards the synthesis of the phenolpropanoid compounds by means of the primary phenolic pathway. Saltveit (2000) concluded that the eventual increased production of phenols due to injury does not act through the ethylene signal, but through another unknown signal. The highest level of newly synthesised phenols found in the injured lettuce was chlorogenic acid. Such tissue browning in lettuce, is a result of newly produced oxidised phenols, by means of the PAL synthesis, rather that the passive oxidation of pre-existing phenols.

Amiot et al. (1995) found that the browning susceptibility of pears at harvest was closely correlated to the initial amount of hydroxycinnamic esters and flavonols. Further results stated that chlorogenic acid (5'-caffeolquinic) resulted for between 70 and 85 % of the hydroxycinnamoyl derivatives and (-)-epicatechin between 27 and 60 % of the total flavanol content. Both these phenolic substances have been correlated to browning susceptibility of pear peel. These phenolic compounds were highest in the peel of the pear, and subsequently the browning susceptibility due to injury was also the highest in the pear peel. Supporting these results, Gauillard and Richard-Forget (1997) found, in 'Williams' pears, that chlorogenic acid is the most substrate specific phenol for PPO reactions.

Cheng and Crisosto (1995) studied the relationship between phenolic acid, chlorogenic acids in particular, PPO and browning potential in buffer extracts from peach- and nectarine skin tissue. They stated that the browning potential of such tissues is strongly correlated to the content of chlorogenic acid in these tissues. Thus, it seems that the activity and amount of the enzyme, PPO, is not the limiting factor, but rather the availability of the chlorogenic acid as the main contributor for the enzyme-mediated reaction. Mitcham et al. (2001) correctly stated that a higher PPO activity and phenolic content does not necessary imply that a higher incidence of skin browning, but that these constituent only contribute towards the discolouring intensity of the injured area.

2.4.2 Cultivar susceptibility

In relation to other fruit, pears are classified as very susceptible to FD. Even among pears, there are some cultivars that produce fruit of even higher vulnerability than other cultivars (Meheriuk et al., 1982).

The occurrence of FD has been studied in a wide variety of pear cultivars in the past, including ‘d’Anjou’ (Smith, 1946, Wang and Mellenthin, 1973, Mellenthin and Wang, 1974), ‘Bartlett’ (Smith, 1946, Mitcham et al., 2001) and ‘Doyenne du Comice’ (Amarante et al., 2001). Kvåle (1979, 1988) also studied this phenomenon in an array of Norwegian pears (‘Moltke’, ‘Herrepaere’, ‘Amanlis’, ‘Moltke’, ‘Philip’ and ‘Herzogin Elsa’). Amiot et al. (1995) found, within the studied cultivars, that ‘Abate Fetel’, ‘Comice’, ‘P2198’ and ‘Guyot’ to be the most vulnerable in terms of FD. In all these cultivars, except ‘Guyot’ a high phenolic content was correlated to an increased susceptibility to browning. The higher phenolic content of the cultivars is then given as an explanation to the degree of browning that was encountered in the trials. Cultivars that were less susceptible to enzymatic browning in both peel and flesh included ‘Conference’, ‘Williams’ and ‘Harrow Sweet’. Berardinelli et al. (2005) also found ‘Conference’, followed by ‘Doyenne du Comice’ and ‘Abate Fetel’, to be the most susceptible to FD within the studied cultivars.

Not only do the phenolic, and more specifically chlorogenic acid (Carbonaro and Mattera, 2001), content have a significant role to play in the extent of skin discolouration, but other cultivar characteristics might also play a role. The way in which the epidermal cells react on impact will definitely affect the decompartmentalization that is needed for extensive browning to occur. Epidermal cells, and therefore fruit, that are weak and fragile will be much more prone to such decompartmentalisation. The protective cuticle layer that is produced to protect the fruit will also differ from cultivar to cultivar. This trait will determine to what extent oxygen, which is needed for oxidative purposes, is permitted within close proximity of the injured cells. Because these discolorations occur predominantly on elevated areas of an uneven fruit surface, cultivars that produce fruit with these characteristics will be more susceptible than pears with dissimilar features (Meheriuk et al., 1982).

2.4.3 Fruit size

There is sound evidence that smaller fruit are more vulnerable to FD than larger fruit (Chen and Varga, 1996). Mellenthin and Wang (1974) simulated packing conditions that promote FD and evaluated the fruit samples 72 hours after the simulation. 'd'Anjou' pears from 3 different maturities were sampled and the smaller fruit had a higher susceptibility to FD throughout the different maturities. Gorny et al. (2000) also reported that freshly cut fruit slices from small pears showed more rapid cut surface discolouration than bigger fruit from similar maturity. No explanation was given for this phenomenon. If one speculated that smaller fruit is most often less mature than larger fruit at a specific harvesting date, it is easy to explain by means of the phenolic presence within the fruit, where more mature fruit are less susceptible to FD than immature fruit (Mellenthin and Wang, 1974, Kvåle, 1988). Then, such discolouration is a result of fruit maturity and only indirectly related to fruit size.

At commercial harvest all the fruit has undergone complete cell division. Thus, from a purely physical point of view, the major difference between small and large fruit is the size of the cells, which is a result of different cell enlargement rates. On a micro scale, one can argue that the 'concentration' of cells is higher in small fruit than in larger fruit. Therefore, frictional injury will rupture more cells in small than in larger fruit. In smaller fruit more cells will be injured; liberating more phenols and enzymes which contribute to a higher incidence of FD.

2.4.4 Fruit maturity

Throughout the literature researchers agree that fruit maturity plays a significant role in determining the susceptibility of pear to FD. Mellenthin and Wang (1974) found a decrease in phenolic concentration with an increase in fruit maturity of pears. Amiot et al. (1995) also found that the phenolic concentration, and more specifically chlorogenic acid and (-)-epicatechin decreased with a delayed harvesting date. Mellenthin and Wang (1974) associated a decrease in FD with an increased maturity, and attributed it to the decrease in phenolic substances available for the oxidation reactions after injury. This is confirmed by the findings of Kvåle (1979, 1988) and Meheriuk et al. (1982).

Fruit harvested before the climateric rise in respiration were more susceptible to FD. This is attributed to the higher levels of chlorogenic acid found in these less mature fruit. Higher substrate availability will also result in a higher incidence of FD in less mature fruit (Kvåle, 1979). Kvåle (1988) concluded that skin browning occurred more readily in fruit of inferior firmness. Norwegian pears with firmness values of 5 kg or lower showed more visible discolouration marks. This effect of fruit firmness has been related to fruit ripening and cell turgor pressure, and has been supported by numerous researchers (Smith, 1946, Wang and Mellenthin, 1974, Amiot et al., 1995, Mitcham et al., 2001). The literature is also unanimous in stating that phenolic content decreases with maturity (Wang and Mellenthin, 1973, Mellenthin and Wang, 1974, Amiot et al., 1995). Wang and Mellenthin (1973) found that PPO showed an opposing trend in relation to PPO activity with an increase in maturity. This fact could possibly explain the decrease of pre-existing phenols in the fresh, uninjured fruit.

Another possibility is that, as the fruit mature, an improved barrier is formed by means of a thicker and more adequate cuticle layer. This will not only physically protect the fruit against water loss and subsequent loss of cell turgidity, but it will also minimize oxygen access to the ruptured epidermal cells in the case of frictional injury. In all these papers the mutual advice is that fruit must be harvested at optimum maturity to ensure the least possible amount of FD damage.

2.4.5 Fruit temperature

Various researchers have contemplated the effect that fruit temperature has on FD susceptibility of pears, with some contradictory findings. One of the first papers published on this subject was by Smith in 1946. Smith (1946) stated that there was a common belief among exporters that the handling of cold pear fruit, straight out of storage, resulted in skin discolouration during handling. The reaction of both ‘d’Anjou’ and ‘Bartlett’ pears was studied in relation to temperature, specifically after certain storage durations. No correlation could be found between the low fruit core temperature and the degree of discolouration of these two cultivars (Smith, 1946). In retrospect, this belief of exporters might probably have been due to the storage effect rather than one of maturity.

Sommer et al. (1960) found conflicting results in relation to fruit temperature and subsequent vibration damage. Apparently warm fruit exhibited a far greater degree of injury than cold fruit. Kvåle (1979), however, found that less FD damage occurred on fruit that was graded right after storage removal (0 °C) than similar fruit that was warmed for two days (10 °C - 15 °C). The question arises whether the injury that was observed was in fact, due to friction or whether it was due to impact (Amarante et al., 2001). It is a well-known fact that warm and cold fruit react differently towards bruising- and friction discolourations. In the case of bruising, warmer fruit, which are more plastic, tend to show less injury as a result of the impact because they can absorb the impact better than colder fruit. Cold fruit are believed to be more elastic. Even though the warmer, more plastic fruit showed less impact damage, more epidermal damage were found in warm fruit (Sommer et al., 1960). This effect of temperature on bruising is supported by Schoorl and Holt (1978) but opposed by Saltveit (1984). The turgidity in warm fruit will be less than in cold fruit and the former will therefore be less vulnerable to impact damage (Banks and Joseph, 1991).

Amarante et al. (2001) highlighted the fact that higher temperatures can lead to conditions that are more conducive for the shrinkage of the fruit skin due to an increased rate of water loss. Such fruit skin will be rougher in texture and will therefore be more susceptible to friction damage. Not only does the higher temperature dehydrate the fruit skin, thereby making it a more uneven surface, but it also causes disorder in the cellular membrane and thereby increases the level of soluble, active PPO (Mayer and Harel, 1979).

Kvåle (1988) stated that skin discolouration due to grading practises could be linked to climacteric and crop differences. Seasons that had a particularly high incidence of discolouration were seasons that yielded relatively low crops and where abnormally high summer temperatures were experienced. These findings can, however, not be relied on too heavily as Kvåle (1979) himself stated that dissimilar results were found in relation to an abnormally cool season.

Saltveit (2000) studied the efficacy of heat shock treatments on the manifestation of tissue browning after wounding of lettuce. After wounding of the tissue, the enzyme PAL is produced as a wounding signal whereby an increased rate of phenolic metabolism is

observed. This is in reaction to the higher activity of PAL present in the cells. Subjecting the wounded lettuce to heat treatments (45 °C for 90 s) reduced the production of PAL in these cells. Heat treatments of this magnitude are not adequate for complete denaturising of enzymes (e.g. PPO, PAL, and POD) (Loaiza-Velarde et al., 1997). It does, however, interfere with the wounding signal by producing a heat shock signal. In reaction to such a signal, the cell allocates most if its synthetic capacity to the production of heat shock proteins (hsps) to try and prevent any further damage due to this increase in temperature. Under such simultaneous inductions of heat- and wounding stress, the cell will give highest priority to the production of hsps. This advantageous effect will only be present in the cells that experience both stress signals simultaneously. Heat shock of this magnitude in the wounded cell prevents the synthesis of PAL, but not to the same degree in the neighbouring intact cells. This kind of treatment will therefore be most advantageous in commodities that possess relatively small amounts of phenolic compounds prior to the injury. If the commodity has large amounts of phenolics available to be oxidised in the case of injury, this will not be as useful.

2.4.6 Storage

Several researchers have investigated the effect of fruit storage on FD. All evidence conclusively declares that FD susceptibility increases with an increase in storage duration. This finding compares well with the fact that the phenolic, and hence chlorogenic acid (Gil-Izquierdo et al., 2001) concentration also increases as storage time is extended (Smith, 1946, Wang and Mellenthin, 1973, Mellenthin and Wang, 1974, Meheriuk et al., 1982, Kvåle, 1988). Amiot et al. (1995) found only a slight increase in phenolic accumulation of 'Williams' pears after 1 month of storage. After the second month of storage a considerable increase in phenolic content was observed. The most notable increase was that of flavanol. Storing the fruit in a controlled atmosphere (CO_2: 1 %, O_2: 1 %) effectively reduced the fruits ability to synthesize phenols.

Mellenthin and Wang (1974) suggested that the accumulation in phenols after a prolonged storage could be attributed to the decreased activity of PPO that was found in 'd'Anjou' pears. Because PPO uses phenolic compounds as its substrate, a lower activity will result in the accumulation of these compounds. The contribution to the accumulation due to this lack

in phenolic conversion by an inactive PPO is uncertain. This might only contribute to a small amount of the increased phenolic content during prolonged storage durations. Even if the PPO activity were high, the conversion of phenolic compounds can in any case only occur in the presence of oxygen. Thus, in uninjured fruit, the accumulation in phenolic compounds is most probably due to an increased rate of phenolic synthesis within the cell itself.

Amarante et al. (2001) also found that an increase in storage showed an increased susceptibility towards FD. One possible reason given is that the fruit loses water during prolonged storage periods and that such fruit have the tendency to discolour more easily. This is attributed to the loss in cell turgidity and subsequent loss of skin integrity. Another reason given is that the fruit ripen during these storage periods and more ripe fruit are more vulnerable to FD (Kvåle, 1988, Amiot et al., 1995, Amarante et al., 2001).

2.5 MEANS OF PREVENTION

Enzymatic browning, and more specifically FD, leads to large financial losses because of consumer discontent and therefore a lot of research has been done on the prevention thereof (Feng et al., 2004). Not only has the application of certain waxes and coatings enjoyed recent attention, but the application of antioxidants has also been widely studied (Mellenthin et al., 1982, Amarante et al., 2001, Mitcham et al., 2001, Feng et al., 2004). In general, most coatings are applied to evade, or rather minimize, the abrasion damage that fruit will inevitably undergo during handling. The mode of action in the case of the antioxidants is to keep the PPO and the phenols separate, even though cellular damage has occurred, thereby preventing enzymatic browning from setting in (Tomas-Barberan and Espin, 2001).

With the new understanding of the mechanism responsible for wound induced phenolic metabolism, Saltveit (2000) proposed a number of points where the process can be interrupted. These possibilities range from stopping propagation of the wound signal to strengthening the membranes with calcium salt applications. Although most of the postharvest handling procedures target some of the ideas mentioned, new preventative treatments might arise from this research.

Lately an interesting preventative measure has been proposed by Kim et al. (2005). This totally organic method, that uses an onion extract, was successfully used to avoid enzymatic browning from setting in on pear juice. A possible explanation given for this is that such an onion extract contains certain PPO inhibitors like volatile sulphur compounds in which thiols are commonly found. The effectiveness of this application was increased if the onion extract was heat treated (100 °C for 10 minutes) before adding it to the pear juice. Even though this whole mechanism is not yet fully understood, enzymatic browning due to pear PPO could be significantly reduced by this method. Similar results were also found by Lee et al. (2002) with potato PPO.

2.5.1 Coatings and antioxidants

It has been reported that the application of antioxidants and fruit coatings can reduce the effect of enzymatic browning in pears (Meheriuk et al., 1982, Amarante et al., 2001, Thomas-Barberan and Espin, 2001). An array of products has been studied through the years. Inconclusive results were more often than not found in the literature. Such results were found for treatments that comprised of a coating, antioxidant or a combination thereof. The effect of genetic, agronomic and postharvest differences that inevitably occur might be responsible for such erratic results (Mellenthin et al., 1982, Mitcham et al., 2001, Feng et al., 2004). Some of the most popular and effective treatments will be discussed.

2.5.1.1 Coatings

Epidermal cells of the fruit skin only discolour when they undergo injury that leads to the inevitable damage to these cells. When fruit are covered in a coating it is expected that friction that gives rise to these injuries will be reduced (Amarante et al., 2001). Not only does such a coating reduce these frictional forces, but it also blocks the lenticells through which gas exchange takes place, and therefore reduces the permeance of water vapour through this barrier (Amarante and Banks, 2000). Depending on the chemical nature (Hagenmaier and Shaw, 1992), character (Banks et al., 1993) and the thickness thereof (Hagenmaier and Baker, 1993) such coatings create a modified internal atmosphere inside the fruit. Consequently, as the CO_2 concentration increases and that of O_2 decreases, the lesser availability of O_2 as an

essential co-substrate for the oxidation of the phenols, will result in less enzymatic browning (Amarante et al., 2001). Amarante and Banks (2000) stated that fruit coatings also reduce the following factors that can lead to the occurrence of FD: Water loss from fruit (Mayer and Harel, 1979) and fruit ripening (Mellenthin and Wang, 1974, Kvåle, 1988, Amiot, et al., 1995).

Mellenthin et al., (1982) reported that the application of 'Fresh-Cote' as a fruit coating significantly reduced the occurrence of FD on 'Bartlett' and 'd'Anjou' pears. This coating protected the epidermal tissue from experimental belt- and brush burns that lead to discolouration. Although the application of 'Semperfresh' delays ripening in pears (Van der Merwe et al., 2002), and would therefore be expected to reduce the occurrence of FD (Amiot et al., 1995), Feng et al. (2004) found that it, as well as 'NatureSeal', gave no significant protection against FD on 'Bartlett' pears.

Amarante et al. (2001) found that the degree of FD decreased with an increase in coating concentration. 'Doyenne du Comice' pears were treated with a carnauba-based wax emulsion (Capsicum/ Zucchini Wax® Castle Chemicals, Australia) just prior to cold storage. After a storage period of two months, a coating concentration of 40 % proved to be most beneficial. Increasing concentrations to above 40 % only led to very little, insignificant reductions in FD. Berardinelli et al. (2005) also found that one could significantly reduce the incidence of FD by treating pears with a wax emulsion coating.

2.5.1.2 Antioxidants

Antioxidants that showed the most promise in preventing or reducing FD, include: 2-mercaptobenzothiazole (Mellenthin et al., 1982), 4-hexylresorcinol (Dong et al., 2000), ascorbic acid and sulphur dioxide (Meheriuk et al., 1982). However, Feng et al. (2004) reported that none of these applications proved to be effective on 'Bartlett' pears, even though lower and higher concentrations were used during the trials. In the case of 4-hexylresorcinol, serious phytotoxicities were encountered, even though a concentration as low as 0.01 % was used.

Feng et al. (2004) studied a wide variety of coatings and antioxidants on 'Bartlett' pears. The most effective proved to be the application of ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) and diphenylamine (DPA). These treatments are also considered to be the most successful against scald, which is a similar oxidative pear disorder (Meheriuk et al., 1982, Curry and Sugar, 1994). Results showed that treatments of ethoxyquin (0.3 %) or DPA (0.2 %) constantly reduced the occurrence of FD, either by vibration, rolling or scuffing, on 'Bartlett' pears. However, Feng et al. (2004) reported that these applications only have an effect on FD for 5 days after the application. After this period, newly induced fruit injury may lead to discolouration as a result of newly synthesised PPO. With warmer fruit (20 °C) it was found that the efficacy of the treatment lasted for even shorter periods of time. This could be attributed to the fact that the effect of DPA and ethoxyquin wore off more rapidly at high temperatures. This phenomenon could also be attributed to the increased activity of PPO at similar temperatures, or the higher rate of and phenolic synthesis that occur with an increase in temperature.

Mitcham et al. (2001) studied the efficacy of a variety of antioxidants on 'Bartlett' pears. They stated that even though some methods were shown to be effective in the past, similar results could not be duplicated. Of all the possibilities evaluated, the most effective proved to be a combination of 2 % ascorbic acid, 1 % calcium lactate and 0.5 % cysteine (pH 7), a 1 % cysteine or a 1-methylcyclopropene (1-MCP) treatment of the fruit. 1-MCP was applied at 0.3 $\mu L.L^{-1}$ for 12 hours at 0 °C and was found to be most effective on fruit samples that were removed from 0 °C storage after 1, 2 and 3 months. Not only did this ethylene inhibiting agent reduce the browning potential of the fruit, but less PPO and total phenolics were found to be present in the skin of the fruit. Similar results were found on warmer fruit (20 ^{o}C) that received the same treatment. Saltveit (2004) studied the effect of 1-MCP on the phenolic compounds, metabolism and concentration thereof in terms of subsequent browning in 'Iceberg' lettuce. Treatments were performed on both injured and uninjured samples. It was found that treatments of 1-MCP (0.5 $\mu L.L^{-1}$ for 3 hours at 5 °C) significantly reduced the occurrence of browning in experiments that included whole heads of uninjured lettuce. When excised tissue was treated in the same way, no difference was found. Saltveit (2004) further commented that 1-MCP prevented the accumulation of phenols due to the ethylene inhibiting nature of the treatment, but that newly produced phenols due to injury could not be prevented

by the 1-MCP treatment. Because the phenolic concentration increased in injured tissue even though it was treated with 1-MCP, it proves that either wounding or ethylene can act independently upon the induction of phenolic production. Therefore, unlike Mitcham et al. (2001) reported, beneficial performance of 1-MCP treatment would only be achieved in uninjured product where it will decrease tissue browning by inhibiting ethylene production and subsequently phenolic metabolism. Another way in which 1-MCP can lessen pear susceptibility towards FD, is by inhibiting ethylene production and thereby delaying fruit ripening. Amarante et al. (2001) found that the incidence of FD was less if the ripening of pears could be delayed.

2.5.2 Harvesting precautions

Chen and Varga (1996) stated that harvesting fruit at the optimum maturity is the first vital step to ensure the least amount of damage caused by FD. Other than ensuring sound harvesting procedures, there is not a lot that can be done to prevent the incidence of FD on pears. Without considering factors like temperature, fruit size and fruit maturity, there are only a few other harvesting procedures that one can implement to try and diminish FD. Ensure that the bulk bins used in the orchard have a firm base and that the bins are securely placed during transport within the orchard. If a very susceptible cultivar is harvested, soft, smooth liners can be placed inside the bins, as well as between the fruit layers to minimize the contact and the impact that fruit have on each other. To prevent excessive amount of water loss, which will negatively affect the turgidity of the cells, Amarante et al. (2001) advised that fruit be cooled as soon as possible. The best way in which to try and reduce pears susceptibility to FD is to implement good general harvesting practises (Meheriuk et al., 1982).

2.5.3 Handling precautions

Meheriuk et al. (1982) commented that the modification of ones handling and packing procedures will be the most effective approach in minimizing the occurrence of FD. This includes handling throughout the postharvest chain, from the harvesting and transportation in the orchard throughout the pack line as well as the packaging and transit to markets. Since

FD susceptibility increases as storage time increases, late packing of fruit is not advised (Meheriuk et al., 1982). Harvested fruit should be packed as soon as possible (Smith, 1946, Meheriuk et al., 1982). Another strategy is to decrease the speed of the pack line (Meheriuk et al., 1982, Chen and Varga, 1996), to reduce, and if possible do without, the use of brushes and to make sure that the packaging material is rigid so as to prevent any unnecessary movement of the fruit during transit (Sommer et al., 1960, Meheriuk et al., 1982).

Slaughter et al. (1996) reported that vibration injury during simulated transport was reduced when 'Bartlett' pears were packed in polyethylene film bags. Amarante et al. (2001) also found that pears packed in perforated plastic bags showed significantly less FD. This phenomenon was attributed to the reduction of water loss that can play a role in the occurrence of FD. Covering bulk bins with plastic film to prevent water loss from the fruit might also prevent excessive water loss during prolonged storage. Fruit should, where possible, be kept under CA storage. This has been shown to significantly reduce the incidence of FD on pears (Amarante et al., 2001).

2.6 CONCLUSION

Recent studies have shed some light onto the mechanisms and understanding of enzymatic browning in an array of horticultural products. Most notable is the research done by Saltveit (2000, 2004) on the mechanics of wound and heat induced phenolic metabolism. By means of his research a number of new possibilities by which phenolic metabolism, and subsequent browning, can be limited or even prevented. Aquino-Bolanos and Mercado-Silva (2004) stated that it is not just enzymatic browning that causes tissue discolouration, but that lignification also contributes to the discolouration of cells under certain situations.

Advances in preventative measures include harvesting, storage, packing precautions and postharvest treatment by which the extent of the disorder can be limited. However, when harvesting susceptible cultivars, fruit should be picked at optimum maturity in an attempt to reduce the occurrence of FD (Meheriuk et al., 1982, Chen and Varga, 1996).

2.7 LITERATURE CITED

Abreu, M., Beirao-da-Costa, S., Goncalves, E.M., Beirao-da-Costa, M.L., Moldao-Martins, M, 2003. Use of mild heat pre-treatments for quality retention of fresh-cut 'Rocha' pear. Postharv. Biol. and Technol. 30, 153-160.

Amarante, C., Banks, N.H., 2000. Postharvest physiology and quality of coated fruits and vegetables. Hort. Rev. 26, 161-238.

Amarante, C., Banks, N.H., Ganesh, S., 2001. Effects of coating concentration, ripening stage, water status and fruit temperature on pear susceptibility to friction discolouration. Postharv. Biol. and Technol. 21, 283-290.

Amiot, M.J., Tacchini, M., Aubert, S.Y., Oleszek, W., 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. J. Agric. Food Chem. 43, 1132-1137.

Aquino-Bolanos, E.N., Mercado-Silva, E., 2004. Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. Postharv. Biol. and Technol. 33, 275-283.

Banks, N.H., Dadzie, B.K., Cleland, D.J., 1993. Reducing gas exchange of fruits with surface coatings. Postharv. Biol. and Technol. 3, 269-284.

Banks, N.H., Joseph, M., 1991. Factors affecting resistance of banana fruit to compression and impact bruising. J. Sci. Food Agric. 56, 315-323.

Barrett, D.M., Lee, C.Y., Lui, F.W., 1991. Changes in the activity and subcellular of PPO in 'Delicious' apples during controlled atmosphere storage. J. Food Biochem. 15, 185-199.

Berardinelli, A., Donati, V., Giunchi, A., Guarnieri, A., Ragni, L., 2005. Damage to pears caused by transport. J. Food Engin. 66 (2), 219-226.

Burges, N.A., 1963. Introductory address, Enzymes associated with phenols. In: Enzymes chemistry of phenolic compounds. Proceedings of the plant phenolic group symposium. Pridham, J.B., (ed.) Liverpool. pp. 1-67.

Carbonaro, M., Mattera, M., 2001. Polyphenol oxidase activity and polyphenol levels in organically grown peach (*Prunus persica* L., cv. 'Regina bianca') and pear (*Pyres communis* L., cv. 'Williams'). Food Chem. 72, 419-424.

Chen, P. M., Varga, D.M., 1996. Storage challenges with winter pears. WSU- TFREC Postharvest information network: Washington Tree Fruit Postharvest Conference, 1996 Proceedings, Storage challenges with winter pears. http://postharvest.tfrec.wsu.edu/PC96A.pdf.

Cheng, W.G., Crisosto, C.H., 1995. Browning potential, phenolic composition, and polyphenol oxidase activity of buffer extracts of peach and nectarine skin tissue. J. Am. Soc. Hort. Sci. 120(5), 835-838.

Cornwell, C.J., Wrolstad, R.E., 1981. Causes of browning in pear juice concentration during storage. J. Food. Sci. 46, 515-518.

Cosetang, M.Y., Lee, C.Y., 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. J. Food. Sci. 52, 985.

Curry, E., Sugar, D., 1994. Reducing scald in 'Red d'Anjou' pears with hot water and reduced rates of ethoxyquin. Acta Hort.. 367, 426-431.

Dimick, K.P., Ponting, J.D., Makower, B., 1951. Heat inactivation of polyphenol oxidase: Purification and fractionation on sephadex thin layers. J. Food Sci. 35, 27-35.

Dong, X., Wrolstad, R.E., Sugar, D., 2000. Extending shelf life of fresh-cut pears. J. Food Sci. 65, 181-168.

Dogan, S., Dogan, M., 2004. Determination of kinetic properties of polyphenol oxidase from *Thymus* (*Thymus longicaulis* subsp. *Chaubardii* var.*chaubardii*). Food Chem. 88, 69-77.

Espin, J.C., Morales, M., Varon, R., Tudela, J., Garcia-Canovas, F., 1997. Monophenolase activity of polyphenol oxidase from 'Blanquilla' pear. Phythochem. 44(1), 17-22.

Feng, X., Biasi, B., Mitcham, E.J., 2004. Effects of various coatings and antioxidants on peel browning of 'Bartlett' pears. J. Sci. Food Agric. 84, 595-600.

Fenoll, L.G., Penalver, M.J., Rodriquez-Lopez, J.N., Varon, R., Garcia-Canovas, F., Tudela, J., 2004. Tyrosinase kinetics: Discrimination between two models to explain the

oxidation mechanism of monophenol and diphenol substrates. Internat J. Biochem Cell Biol. 36: 235-246.

Fujita, S., Bin Saari, N., Maegawa, M., Tetsuka, T., Hayashi, N., Tono, T., 1995. Purification and properties of polyphenol oxidase from cabbage (*Brassica oleracea* L.). J. Agric. Food Chem. 43, 1138-1142.

Gauillard, F., Richard-Forget, F., 1997. Polyphenol oxidases from 'Williams' Pear (*Pyres communis* L, dv. 'Williams'): Activation, purification and some properties. J. Sci. Food Agric. 74, 49-56.

Gil-Izquierdo, A., Gil, M.I., Conesa, M.A., Ferreres, F., 2001. The effect of storage temperature on vitamin C and phenolics content of artichoke (*Cynara scolymus* L.,) heads. Inn. Food Sci. Em. Technol. 2, 199-202.

Gomez-Lopez, V.M., 2002. Some properties of polyphenol oxidase from two varieties of avocado. Food Chem. 77, 163-169.

Gorny, J.R., Cifuentes, R.A., Hess-Pierce, B., Kader, A.A., 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. J. Food Sci. 65(3), 541-544.

Goupy, P., Amiot, M.J., Richard-Forget, F.C., Duprat, F., Aubert, S., Nicolas, J.J., 1995. Enzymatic browning of model solutions and apple phenolic extracts by apple polyphenol oxidase. J. Food Sci. 60, 497-501.

Halim, D.H., Montgomery, M.W., 1978. Polyphenol oxidase of d'Anjou pears (*Pyrus communis* L.). J. Food Sci. 48, 1479-1483.

Hagenmaier, R.D., Baker, R.A., 1993. Reduction in gas exchange of citrus fruit by wax coatings. J. Agric. Food Chem. 41, 283-287.

Hagenmaier, R.D., Shaw, P.E., 1992. Gas permeability of fruit coating waxes. J. Am. Soc. Hort. Sci. 117, 105-109.

Jiang, Y., Duan, X., Joyce, D., Zhang, Z., Li, J., 2004. Advances in understanding of enzymatic browning in harvested litchi fruit. Food Chem. 88(3), 443-446.

Kim, M., Kim, C.Y., Park, I., 2005. Prevention of enzymatic browning of pear by onion extract. Food Chem. 89, 181-184.

Kvåle, A., 1979. FD of two pear cultivars in relation to date of harvest and phenolic compounds in the fruit. Acta Agric. Scand. 29, 29-32.

Kvåle, A., 1988. Skin discolouration of four pear cultivars in relation in to maturity, degree of ripening and duration of storage. Norw. J. Agric. Res. 2, 139-142.

Lee, N., Kim, Y., Kim, N., Kim, G., Kim, S., Bang, K., Park, I., 2002. Prevention of browning in potato with a heat-treated onion extract. Biosci. Biotechnol. Biochem. 66(4), 856-858.

Loaiza-Verlande, J.G, Tomas-Barbera, F.A., Saltveit., M.E., 1997. Effect of intensity and duration of heat shock treatments on wound-induced phenolic metabolism in 'Iceberg' lettuce. J. Am. Soc. Hort. Sci. 122, 873-877.

Marques, L., Fleuriet, A., Cleyet-Marel, J.C., Macheix, J.J., 1994. Purification of an apple polyphenol oxidase isoform resistant to sds-proteinase K digestion. Phytochem. 36, 1117-1121.

Mayer, A.M., Harel, E., 1979. Polyphenol oxidases in plants. Phytochem. 18, 193-215.

Mayer, A.M., Harel, E., 1990. Phenol oxidase and their significance in fruit and vegetables. In: P.F. Fox, Food enzymology. London Elsevier, Applied Science. pp. 378-398.

Meheriuk, M., Prange, R. K., Lidster, P. D. Porritt, S.W., 1982. Postharvest disorders of apples and pears. Agriculture Canada, Publication 1737/E.

Mellenthin, W.M., Chen, P.M., Borgic, D.M., 1982. In-line application of porous wax coating materials to reduce FD of 'Bartlett' and 'd'Anjou' pears. HortScience. 17(2), 215-217.

Mellenthin, W. H., Wang, C. Y., 1974. Friction discolouration of 'd' Anjou' pears in relation to fruit size, maturity storage and polyphenol oxidase activities. HortScience. 9, 592-593.

Mitcham, E.J., Feng, X., Biasi, B., 2001. Susceptibility to and the control of skin browning in 'Bartlett' pears. Report to Californian pear advisory board.

Nicolaï, J.J., Richard-Forget, F.C., Goupy, P.M., Amiot, M.J., Aubert, S., 1994. Enzymatic browning in apple and apple products. Crit. Rev. Food Sci. Nutrit. 34, 109-157.

Peiser, G., Lopez-Galvez, G., Cantwell, M., Saltveit, M.E., 1998. Phenylalanine ammonia lyase inhibitors control browning of cut lettuce. Postharv. Biol. and Technol. 14, 171-177.

Raese, J.T., 1989. Physiological disorders and maladies of pear fruit. Hort. Rev. 11, 357-411.

Robards, K., 2003. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. J. Chromatography. 1000, 657-691.

Ryan, D., Antolovich, M., Prenzler, P., Robards, K., Lavee, S., 2002. Biotransformations of phenolic compounds in *Olea eyropaea* L. Scientia Hort.. 92, 147-176.

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, fourth ed. Wadsworth Publishing Company, Belmont Calif. p. 191-192, 318-321.

Saltveit, M.E., 1984. Effects of temperature on firmness and bruising of 'Starkrimson Delicious' and 'Golden Delicious' apples. Hort. Sci. 19(4), 550-551.

Saltveit, M.E., 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. Postharv. Biol. and Technol. 21, 61-69.

Saltveit, M.E., 2004. Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut 'Iceberg' lettuce. Postharv. Biol. and Technol. 34, 75-80.

Saspers, G.M., 1989. Browning of foods: Control by sulphites, antioxidants and other means. Food Technol. 47(10), 75-84.

Schoorl, D., Holt, J.S., 1978. The effects of storage time and temperature on the bruising of 'Jonathan', 'Delicious' and 'Granny Smith' apples. J. Text. Stud. 8, 409-416.

Sgarbi, E., Fornasiero, R.B., Lins, A.P., Bonatti, P.M., 2003. Phenol metabolism is differentially affected by ozone in two cell lines from grape (*Vitis vinifera* L.) leaf. Plant Sci. 165, 951-957.

Sioud, F.B., Luh, B.S., 1966. Polyphenolic compounds in pear pureè. Food Technol. 20, 535-538.

Slaughter, D.C., Hinsch, R.T, Thomson, J.F., 1996. Vibration induced injury during transportation of pears. Tree Fruit Postharv. J. 7(1), 8-11.

Smith, E., 1946. Handling injuries on pears following cold storage. Proc. Am. Soc. Hortic. Sci. 47, 79-83.

Sommer, N.F., Mitchell, F.G., Guillou, R., Luvisi, D.A., 1960. Fresh fruit temperature and transit injury. Am. Soc. Hort. Sci. 76, 156-162.

Spanos, G.A., Wrolstad, R.E., 1990. Influence of variety, maturity, processing, and storage on the phenolic composition of pear juice. J. Agric. Food Chem. 38, 817-824.

Tate, J.N., Luh, B.S., York, G.K., 1964. Polyphenol oxidase in 'Bartlett' pears. J. Food Sci. 29, 829-836.

Thomas-Barberan, F.A., Espin, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric. 28, 853-876.

Van der Merwe, J.A., Nielsen, M., Lamont, M., 2002. Effect of Semperfresh on the post-storage quality of 'Packham's Triumph' pears and 'Golden Delicious' apples. SA Fruit J. 47-52.

Wang, C.Y., Mellenthin, W.M., 1973. Relationship of friction discoloration to phenolic compounds in 'd'Anjou' pears. HortScience. 8(4), 321-323.

Wang. C.Y., Mellenthin, W.M., 1974. Inhibition of discolouration on 'd'Anjou' pears by 2-mercaptobenzothiazole. HortScience. 9, 196.

Weemaes, C.A., Ludikhuyze, L.R., Van den Broeck, I., Hendrickx, M.E., Tobback. P.P., 1998. Activity, Electrophoretic characteristics and heat inactivation of polyphenol oxidase from apples, avocados, grapes, pears and plums. Lebensm-Wiss. U. Technol. 31, 44-49.

Yue-Ming, J., Zauberman, G., Fuchs, Y., 1997. Partial purification and some properties of polyphenol oxidase extracted from litchi pericarp. Postharv. Biol. and Technol. 10, 221-228.

ARTICLE I EFFECT OF HARVESTING DATE, FRUIT SIZE AND STORAGE DURATION ON SHRIVELLING SUSCEPTIBILITY OF PEARS.

ABSTRACT

The influence of variables (harvesting date, fruit size and storage duration) has been studied on the occurrence and susceptibility of shrivelling in 'Packham's Triumph', 'Beurré Bosc' and 'Forelle' pears. The periods during simulated postharvest handling that proved the most conducive were those of high temperatures (i.e. after harvest, packing, recooling and shelf life). These short periods of relative high temperatures increased the rate of transpiration to such a level that high rates of weight loss were recorded. The contribution of transpiration to the total degree of weight loss far outweighed that of respiration. Early harvested (i.e. fruit of inferior maturity) and small fruit were more susceptible to shrivel and showed visual signs of shrivel before larger, more mature fruit did. The surface area to volume ratio determined the rate at which transpiration could take place. The smaller the fruit, the bigger the ratio and the higher the risk of experiencing shrivel. This renders the pear neck very susceptible to weight loss and subsequent shrivel. The highest rate of weight loss, at 18 °C, was recorded for 'Beurré Bosc' (0.43 %.day^{-1}) followed by 'Forelle' (0.35 %.day^{-1}). However, 'Packham's Triumph' required the least amount of time to display signs of shrivel (10 days). This can be attributed to the prominent shape of this particular pear's neck. Average percentage moisture loss required before shrivel was visible ranged from 2.5 %, 3.9 % and 4.4 % for 'Packham's Triumph', 'Beurré Bosc' and 'Forelle', respectively. Sealing of the fruit stem reduced the amount of moisture that was lost through it and extended the possible shelf life of the pears. This was most notable in 'Beurré Bosc' and 'Packham's Triumph'.

KEYWORDS: Shrivelling, weight loss, 'Packham's Triumph', 'Beurré Bosc', 'Forelle', stem waxing, fruit dimension, surface area: volume.

INTRODUCTION

Fresh fruit is bound to lose weight during postharvest handling (Sastry, 1985). A weight loss of as little 5% can cause an apple to take on a shrivelled appearance (Hatfield and Knee, 1988). These losses are mainly due to the processes of respiration and transpiration, of which the latter is by far the greater contributor (Sastry, 1985). With such obvious differences in the

apple and pear dimensions, volume to surface area, one would suspect that even a smaller degree of weight loss is required to cause such an unwanted appearance in the so-called "neck" of a pear. It is also this ratio, along with the possible maturity effect (Sastry, 1985), that renders smaller fruit more susceptible to shrivelling, due to the loss in cell turgidity, than larger fruit (Jackson et al., 1971). Reducing these losses during storage or fruit ripening assists in maintaining fruit quality (Burdon and Clark, 2001).

The degree of transpiration, or weight loss due to water loss, is predominantly a spontaneous physical reaction of the fruit towards it surroundings (Nobel, 1991). This then is the reason behind the modern postharvest handling procedures of today, to create atmospheres between harvest and fruit consumption in which fruit quality and quantity are maintained (Kader, 1985). Some of these storage precautions include high relative humidity (RH, 95 %; Grierson and Wardowski, 1978), low storage temperatures (<5°C; Kader, 1985) and low oxygen concentrations (<5%; Kader, 1985). When highly susceptible cultivars are handled and stored for long periods, these and other precautions become very important in maintaining cuticle integrity (Baker, 1982) and fruit quality (Maguire et al., 2001).

Although transpiration can be classified as a physical response (Nobel, 1991), the fruit is not completely passive towards these losses (Lendzian and Kerstiens, 1991). Fruit has made numerous adaptations, in terms of the fruit skin, to prevent loss of excessive amounts of moisture through transpiration. Some of these adaptations are being artificially emulated to further increase the barrier properties of fruit. The likes of fruit waxing (Amarante and Banks, 2000) and packaging (Wills et al., 1989) imitate some of the fruit qualities responsible for the prevention of water loss from fresh commodities.

Harvesting date, and indirectly fruit maturity, plays a significant part in water loss potential of fruit. Transpiration rates are believed to be the lowest in fruit harvested at optimum maturity (Sastry, 1985). This can be attributed to the condition of the protective cuticle layer surrounding the fruit (Woods, 1990). During fruit growth this layer is still being deposited (Maguire et al., 1998), and during advanced maturity it has undergone severe damage that renders it ruptured and cracked (Jenks and Ashworth, 1999). Such conditions will increase

the permeability of the cuticle and affect the transpiration rate negatively (Jenks and Ashworth, 1999).

This study was conducted to evaluate the different cultivars' susceptibility towards shrivelling in terms of harvesting date, storage duration and fruit size. By simulating a temperature related postharvest handling procedure, the critical periods of fruit weight loss could be identified. Respiration rate of the fruit could be determined under controlled conditions and the contribution of transpiration to the total weight loss could be established. This study also allowed for the calculating of the degree of weight loss necessary in pears before signs of shrivelling became noticeable.

MATERIALS AND METHODS

Season 1: 2003

Postharvest simulation

'Packham's Triumph', 'Beurré Bosc' and 'Forelle' pears were randomly selected from bins in the orchard during commercial picking. The fruit were sampled on the different harvesting dates at the farm Jagerskraal in the Warm Bokkeveld, Ceres (Lat: 33,17 °S and Long: 19,19 °E). Twenty fruit per replicate were selected at each maturity and placed into polyethylene mesh citrus bags. Bags were placed inside bins and underwent the commercial handling procedures during transport and storage. The harvested fruit, along with the sampled bags, were taken to commercial cold stores at CFG (Ceres Fruit Growers) in Ceres. Fruit mass was determined at various intervals during storage with an electronic scale (UWE, NBK-30, 30 kg x 0.005 kg).

Due to unforeseen injuries encountered by the stored fruit, a separate set of fruit was used from the shipping period onwards. This period included simulated 'shipping' (0 °C for 14 days) and 'shelf life' (20 °C for 12 days) periods. Although the commercially packed fruit units (±12 kg) were of similar maturity, small differences would inevitably be present between the different fruit batches, thereby influencing the reliability of the results. The weight of each replicate was determined after each simulation period with an electronic scale.

Fruit maturity

Each of the cultivars was harvested on two different dates. 'Packham's Triumph': 11 and 22 February 2003, 'Beurré Bosc': 11 and 22 February 2003 and Forelle': 22 February and 11 March 2003. Fruit underwent firmness evaluation on arrival at Stellenbosch. Readings were taken on opposite, peeled sides of the fruit with a penetrometer (Southtrade, FT 327, Italy) fitted with an 8 mm tip.

Fruit stem waxing.

A laboratory warmer (Soiltest 0-144-8, Evanston Illinois, USA) was used to apply melted candle wax to the stem of the fruit and thereby obstruct water movement through it. The treatments consisted of wax applied to 0 % (control), 15 %, 50 % and 100 % of the uppermost part of the stem length. Twenty single fruit replicates were used. Fruit was placed at 23 °C for 3 weeks to simulate shelf life conditions and to encourage weight loss. Fruit were weighed weekly with an electronic scale (UWE, Everyweigher, EEW-5000, 5500g x 0.5 g) to determine weight loss.

Anatomical study

Anatomical studies were conducted on epidermal tissue using a light microscope. Epidermal tissue segments were prepared by fixing in FAA (90 parts (50%) ethanol, 5 parts acetic acid and 5 parts formalin) for 48 hours. Thereafter the tissue underwent dehydration by means of an ethanol series as stipulated by Johansen (1940). The tissue was embedded in paraffin wax (Naidoo et al., 1990) and transections were made with a rotary microtome. De-waxing of the transections was done whereafter they were mounted on glass slides using DPX adhesive. Samples were then dyed with the Alcian-green/ Safranine method of staining (Brooks et al., 1950). Glass cover slips were placed over the samples. A Leica light microscope was used to study the samples at a 150-fold magnification. Images were captured using the Leica Qwin LIDA software program.

Season 2: 2004

Postharvest simulation

‘Packham’s Triumph’, ‘Beurré Bosc’ and ‘Forelle’ pears were selected from bins in the orchard during commercial picking at the farm Jagerskraal in the Warm Bokkeveld, Ceres (Lat: 33,17 °S and Long: 19,19 °E). Fruit, 3 kg per replicate, were selected by fruit size (described below) at each maturity and placed into polyethylene mesh citrus bags. The sampling bags were taken to commercial sized experimental cold stores in Stellenbosch. Fruit were treated with a 2 % iprodione (dicarboximide, Aventis CropScience) solution to prevent decay. Fruit mass was determined at various intervals during the postharvest simulation period with an electronic scale (UWE, NBK-30, 30 kg x 0.005 kg). After the postharvest simulation the number of fruit in each replicate that showed visual signs of shrivelling were determined.

Fruit core- and air temperature were determined with copper-constantan Type-T thermocouples. During the postharvest simulation, fruit was subjected to the following temperature regimes: ‘cooling down’ (± 30 °C to 0 °C within 3 days), ‘storage’ (0 °C for 4 weeks), ‘packing’ (0 °C to 15 °C in 1 day), ‘recooling’ (15 °C to 0 °C in 1 day), ‘shipping’ (0 °C for 2 weeks) and ‘shelf life’ (15 °C for 7 days). In the case of ‘Forelle’ pears, the storage period was increased to 8 weeks, which is also done commercially to prevent mealiness.

Fruit maturity

Each of the cultivars was harvested on three different dates. ‘Packham’s Triumph’: 24 February, 2 and 9 March 2004, ‘Beurré Bosc’: 24 February, 2 and 9 March 2004 and ‘Forelle’: 9, 16 and 23 March 2004. Fruit underwent firmness evaluation on arrival at Stellenbosch. Readings were taken on opposite, peeled sides of the fruit with a penetrometer (Southtrade, FT 327, Italy) fitted with an 8 mm tip.

Fruit size

Fruit, used in ‘postharvest simulation’, were sampled into three different fruit size classes (large, medium and small) by equatorial diameter. Each of the six replicates comprised of 3 kg fruit, and thus had different numbers of fruit per size class. The size classes were as follows: ‘Packham’s Triumph’ and ‘Beurré Bosc’: 84 to 75, 74 to 65 and 64 to 55 mm, and ‘Forelle’: 80 to 73, 72 to 65 and 64 to 57 mm.

Respiration measurements

The respiration rate of each cultivar was determined at 0 °C and 15 °C. Five fruit per replicate were placed inside buckets (5 L). Humidified air was supplied to the buckets via flowboards. The flow rate, governed by glass capillaries, was 667 ml/min. A CO_2 analyzer (S151, Qubit systems, Canada) was used to calculate the CO_2 emission rate over a period of seven days.

Fruit dimensions

The average dimensions (volume, surface area and weight) of each fruit size class were calculated. Three fruit per cultivar, per size class were measured. Fruit were peeled and the surface area was determined by photocopying the whole fruit's peeled skin. Then, by cutting out the "peel" and weighing it, the surface area could be calculated proportionally since the weight per unit area of the paper is known. Volume was calculated by means of water displacement. Fruit weight was determined with an electronic scale (UWE, Everyweigher, EEW-5000, 5500g x 0.5 g).

Fruit stem waxing

Fruit stems were waxed as previously described. The influence of fruit size was investigated by sorting fruit samples into either a 'large' or 'small' category. The cut off diameter between the classes was as follows: 'Packham's Triumph' and 'Beurré Bosc': 69 mm and 'Forelle': 64 mm. After waxing, fruit were kept at 18 °C for 12 days to simulate shelf life conditions and to encourage weight loss. Twenty single fruit replicates were used and were weighed every second day with an electronic scale (UWE, Everyweigher, EEW-5000, 5500g x 0.5 g).

Visual shrivelling detection

By monitoring the 'control' fruit of the 'fruit stem waxing' trial we were able to determine the time and amount of weight loss needed before visual shrivelling of the pear neck sets in. The topmost (± 3 cm) part of the pear neck was examined for any signs of shrivel. Shrivelled fruit acquire a spongy feel when lightly pressed between thumb and forefinger and the fruit skin becomes wrinkled in this area. Twenty single fruit replicates were used.

Statistical analysis

Statistical Analysis Systems (SAS), Enterprise Guide VI version 1.3 was used to determine the analysis of variance (ANOVA) and LSD values with a 5 % significance level. A complete randomised design was used for the data set. In the cases where interactions were present, contrasts were examined.

RESULTS

Postharvest simulation

During the 2003 season fruit samples were stored under controlled atmosphere at commercial facilities. This limited the opportunities at which samples could be weighed, and this particular aspect was addressed during 2004. Statistical analysis of weight loss was done on the final values obtained. During 2003 these were obtained 86 days after harvest when fruit was retrieved from storage and data are presented in Fig. 1a as the average of both harvesting dates. Fruit samples used in Fig. 1b consisted of separate, commercially packed, comparable fruit. Both 'Packham's Triumph' and 'Beurrè Bosc' were collected on the same day and a very sharp increase in weight loss can be seen in both these cultivars during the 'shipping' section that is continued into the 'shelf life' section. Eventually this resulted in a combined loss of 7.19 % and 6.61 %, respectively. In the case of 'Forelle' a less steep gradient is present that only led to an eventual loss of 3.32 %.

A single set of fruit was used throughout the 2004 simulation and values used for analysis were obtained after the respective 'shelf life' periods. The representation of the results in both Fig. 2 and 3 is the average of both variables (harvesting date and fruit size) for each of the cultivars. Noticeable changes in the rate of weight loss can be observed between storage temperature of 0 °C and 15 °C for all the cultivars (Fig. 2 and 3). A similar temperature dependant trend is evident in the response of stored fruit during 2003 (Fig. 1a and 1b).

Weight loss during 2003 was significantly higher in the fruit sampled at harvesting date 1 than from harvesting date 2 (Table 1). Insignificant results were obtained in respect to harvesting date and the percentage of weight loss for 'Beurrè Bosc' and 'Forelle' during 2004

(Table 2). Even though harvesting date influenced weight loss of 'Packham's Triumph' during 2004, significant interaction between harvesting date and fruit size prevent the discussion thereof (Table 2).

Harvesting date

Fruit used in both seasons were harvested within the commercial picking window. However, during 2004, the three harvesting dates were more representative of the whole picking period than what was the case during 2003. Fruit firmness significantly decreased as fruit maturity increased for all cultivars in both seasons (Table 3). During both seasons 'Packham's Triumph' and 'Beurré Bosc' was picked on the same date, within a season, while 'Forelle' was typically harvested 2 weeks later.

Fruit size

During the postharvest simulation of 2004, both 'Beurré Bosc' and 'Forelle' showed similar trends in terms of fruit size and degree of weight loss (Table 4). 'Small' 'Forelle' showed the greatest percentage of weight loss (6.47 %). Fruit from the 'large' category showed the least amount of weight loss in both 'Beurrè Bosc' (5.46 %) and 'Forelle' (5.40 %).

This fruit size dependant response was also observed during the 2004 'fruit stem waxing' trial done on 'Packham's Triumph' (Table 5) where a loss of 3.29 % and 2.74 % was recorded for small and large fruit, respectively. The percentage weight loss experienced by 'Beurrè Bosc' and 'Forelle' during the 'postharvest simulation' trial of 2004, were much worse than 'Packham's Triumph' encountered during the 'fruit stem waxing' trial. During the postharvest simulation trial fruit was stored for a much longer period and only evaluated after 86 days of storage, whereas during the 'fruit stem waxing' trial fruit were only evaluated for 12 days. Due to significant interaction in the 'fruit stem waxing' trial, data obtained from 'Beurrè Bosc' and 'Forelle' are not shown (Table 6). In all of these cases, the smaller fruit lost significantly more weight, on percentage basis, than the larger fruit.

Respiration measurements

Significant respiration differences were found in terms of harvesting date and storage temperature for both 'Packham's Triumph' and 'Beurré Bosc' (Table 7). An increase in

respiration rate was found for both cultivars as fruit maturity increased. A marked increase was also evident between storage temperatures of 0 °C and 15 °C for both cultivars (Table 8). Due to significant interaction between harvesting date and storage temperature of 'Forelle' (Table 7), the main effects are not shown in Table 8.

By transforming the data in Table 8, the rate of dry mass loss can be calculated for each cultivar (Table 9). The contribution of transpiration and respiration to the total percentage of weight loss can therefore be calculated.

Fruit stem waxing

Significant interactions between harvesting date and treatment during 2003 prevent the discussion of stem waxing treatment on all of the cultivars concerned. 'Packham's Triumph' showed no significant interactions during 2004, while 'Beurrè Bosc' (harvesting date and treatment) and 'Forelle' (harvesting date and treatment as well as fruit size and treatment) did have significant interactions (Table 6).

The differences within 'Packham's Triumph' for each of the main effects (harvesting date, fruit size and treatment) are presented in Table 5. As previously mentioned, weight loss decreased with an increase in fruit size and fruit maturity. The stem waxing treatment also significantly reduced the degree of weight loss from the fruit. Similar trends were observed with the other two cultivars.

Visual shrivelling

The average time (days) and amount of weight loss (%) that is needed before visual signs of shrivel become evident, is shown in Fig. 4. The results presented in Fig. 4 are the averages across size classes and maturities. These values were obtained from the control fruit of the 'fruit stem waxing' trial. The time needed, at 18 °C, before this disorder became apparent ranged from 10.3 ('Packham's Triumph') and 10.4 ('Beurrè Bosc') to 11.2 ('Forelle') days. The percentage of weight lost during this time ranged from 2.5 ('Packham's Triumph') to 3.9 ('Forelle') and 4.4 ('Beurrè Bosc'). It is evident that the rate of loss was the fastest in 'Beurrè

Bosc' (0.42 %.day^{-1}) followed by 'Forelle' (0.35 %.day^{-1}) and 'Packham's Triumph' (0.25 %.day^{-1}).

Fruit dimensions

The dimensions (mass, volume and surface area) for small and large fruit are presented in Fig. 5 and 6 respectively. As suspected, in all the cultivars, average fruit volume decreased as average fruit mass decreased. In both fruit size classes there are, however, marked increases in the surface area of 'Beurrè Bosc'.

Anatomical studies

No consistent anatomical differences were found between 'Packham's Triumph', 'Beurrè Bosc' and 'Forelle'. In all three cultivars there was a clearly defined cuticular layer protecting the epidermis (Fig. 7, 8 and 9). The cortex cells underlying the epidermis were significantly larger than the epidermal cells. In some cells, the cuticle appeared to be damaged, thereby exposing the dermal tissue which would hasten moisture loss (Jenks and Ashworth, 1999).

DISCUSSION

Postharvest simulation

Most of the work done here was at higher temperature and lower relative humidities than found in commercial practice and would therefore illustrate the "worst case scenario" in terms of storage conditions and the rate, as well as the extent, of water loss. Fruit was deliberately placed in well-ventilated bags to ensure good air movement around the product. The use of regular atmosphere (RA) storage during 2004 contributed to these conducive conditions.

The period following harvest (day 0) proved to be crucial in influencing the degree of weight loss that was experienced. This is evident in both years and in all cultivars that were studied (Fig. 1a, 2 and 3). During this period the rate of weight loss is relatively high and can be attributed to the high temperatures and lack of high RH that promoted the loss of moisture (Hoang et al. 2004). Removing field heat from the commodity as quickly and effectively as possible has been reported to extend the shelf life of fresh commodities (Maharaj and Sankat,

1990). Managing these abiotic factors, temperature and RH, will be most important in governing the transpiration rate, and to a lesser extent the rate of respiration, that have been reported as the main driving forces behind moisture loss from fruit (Burton, 1982, Sastry, 1985).

The other areas that proved to be critical are those of 'packing' and 'recooling'. This can clearly been seen in the case of 'Forelle' (Fig. 3). The two-day break in storage temperature (from 0 °C to 15 °C and back to 0 °C) greatly increased the amount of weight that was lost by the fruit. This short period proved to be just as important, if not more important, than the lengthy 'storage' and 'shipping' periods. The rate of weight loss during 'packing' and 'recooling' is far greater than that experienced during 'storage' and 'shipping' in 2004. During industry related conditions this will prove to be even more so when fruit is kept under a controlled atmosphere (CA). The rate of weight loss during 'storage' proved to be far less under CA conditions used during 2003 (Fig. 1a) than under the RA conditions of 2004 (Fig. 2 and 3). By storing fruit under CA, rather than RA conditions, the RH is elevated and the driving force behind water loss is reduced (Tu et al., 2000). This will then undoubtedly reduce the rate and lessen the eventual percentage of weight loss.

The sudden increase in weight loss experienced during 2003 with 'Packham's Triumph' and Beurrè Bosc' (Fig. 1b) after 'shipping' might be attributable to high temperatures encountered during transportation. The packed fruit units were collected from Ceres and transported to Stellenbosch (130km, 1½ h) for further examination. Although the fruit was subsequently subjected to a shelf life temperature of 20 °C, far greater temperatures (±32 °C) were experienced on this particular day. This sharp increase in temperature must surely have influenced the fruits core temperature, and subsequently the transpiration and respiration rate, negatively. The coupled effect of increase in temperature and decrease in RH might be responsible for this sudden increase in weight loss rate. This also illustrates the importance of maintaining the cold chain throughout the postharvest handling of fruit, thereby ensuring longevity of the commodity (Brosnan and Sun, 2001).

Most notable is the large increases in weight loss when the fruit is not kept at 0 °C, i.e. 'cooling down', 'packing', 'recooling' and 'shelf life'. Although these periods are relatively

brief, substantial weight is being lost during these stages. Because the fruit is bound to lose weight from the moment that it is harvested, every step should be taken to minimize weight loss and prolong the marketability of the product. The importance of initiating and maintaining the cold chain cannot be ignored. The eventual amount of weight loss can be minimized considerably when harvested fruit is cooled swiftly (Paull, 1999). This will reduce respiration and transpiration and will result in a smaller amount of weight being lost (Maguire et al., 1999). Therefore, when excessive weight is lost during these first few days, whether due to poor handling or cooling practices, the fruit begin the postharvest handling chain with a disadvantage that cannot be reverted even if exceptional procedures are followed. The increased rate of weight loss during 'shelf life' was expected. Increasing the temperature and lowering the RH, as was done during these periods, will influence the rate of weight loss adversely.

Although 'Beurrè Bosc' exhibited a higher respiration rate than 'Packham's Triumph' (Table 8), the possible difference between the eventual degrees of weight loss during 2004 (Fig. 2), might be attributed to the difference in fruit dimension. As the loss of dry mass, due to respiration, is very low (Fig. 9, Refer to 'Respiration') in comparison to transpirational losses, one can assume that factors affecting the transpiration rate of fruit will be most influential in affecting the degree of weight loss. Therefore, intercultivar differences like surface area to volume (SA:V) ratio become an important factor to consider (Fig. 5 and 6; Refer to 'Fruit dimension').

Harvesting date

Fruit firmness significantly decreased with an increase in harvesting date (Table 3). Although a random 20 fruit sample was used to determine fruit firmness, no discrimination was made in fruit size. On each occasion one can assume that fruit classed into the 'small' category might have been firmer, and therefore most probably less mature, than those of the 'large' category.

Weight loss is generally more severe in immature fruit than in mature fruit. This was evident in results obtained from 'Packham's Triumph' (Table 1 and 5), 'Beurrè Bosc' and 'Forelle' (Table 1). Sastry (1985) related this phenomenon to the increased transpiration rate found in

immature fruit. Woods (1990) experienced the same tendency and associated it with the decrease in cuticle permeance as the fruit mature. This decline in cuticle permeance is associated with an increase in cuticle quantity and quality found on maturing fruit. This however, was only observed up to a certain maturity, whereafter the cuticle permeance increased. Jenks and Ashworth (1999) found that in over mature fruit the cuticle deteriorates due to a number of abiotic factors (wind, rain and heat) and the fact that wax production is reduced.

Fruit size

Small fruit lost more weight (on percentage basis) than larger fruit. During the 2004 'postharvest simulation' trial, both 'Beurré Bosc' and 'Forelle' showed a similar trend in terms of fruit size and the degree of weight loss (Table 4). This was also found in the 2004 'fruit stem waxing' trial done on 'Packham's Triumph' (Table 5). A number of reasons could be given for this phenomenon. Smaller fruit is most often less mature than larger fruit and a number of qualities come into play when fruit maturity is considered along with fruit size. (Refer to 'Harvesting date'). This is reflected in the number of significant interactions found between fruit size and harvesting date (Table 2 and 6).

Fruit of different sizes possess different volumes. The most obvious difference between these size classes would then evidently be the dissimilarity in the SA:V ratio. A high ratio means that there is a greater diffusional area, from which water can diffuse, per water-saturated fraction of fruit volume. This will relate into a higher incidence of water loss from the fruit.

Although larger fruit have the ability to lose more weight in actual mass, the higher degree of potential loss, as percentage, from smaller fruit will render them more susceptible to shrivel (Woods, 1990). This can be attributed to the higher SA:V found in small fruit. Smaller apple fruit tend to shrivel before larger fruit do (Jackson et al. 1971), and the rate of weight loss (%) of eggplant tends to be slower in larger fruit (Diaz-Perez, 1998).

Respiration measurements

An increase in temperature resulted in a higher respiration rate (Table 8). This was evident in both 'Packham's Triumph' and 'Beurrè Bosc'. Significant interaction between harvesting

date and storage temperature was found in the case of 'Forelle' (Table 7). As fruit temperature increases, so do the metabolic processes within the fruit. This is illustrated by the temperature quotient (Q10) equation that demonstrates the logarithmic association between metabolic processes and temperature (Reid, 1991). For each 10 °C rise in temperature, the metabolic deterioration rate doubles. Therefore, as Senti and Rizek (1975) found, the rate of deterioration at 25 °C is 2.5 times greater than at 0 °C. This accentuates the importance of lowering fruit temperature directly after fruit harvest and maintaining the cold chain throughout postharvest handling. Such practices encourage the deceleration of the deterioration rate found during maturation of flowers (Farnham et al., 1975) and fruit (Senti and Rizek, 1975).

The rate of dry mass loss due to respirations is presented in Table 9. It is interesting to note that the rate at 15 °C is 3.78 and 2.48 times greater than at 0 °C for 'Packham's Triumph' and 'Beurré Bosc', respectively. Thus, for an average 'Packham's Triumph' weighing 180 g, stored for 4 weeks at 0 °C, the contribution of respiration to weight loss will be only 0.15 % and 0.56 % at 0 °C and 15 °C, respectively. A similar contribution is observed with 'Beurré Bosc', with 0.18 % and 0.44 % at 0 °C and 15 °C, respectively. Therefore, from the results obtained during the 'storage' period of the 'postharvest simulation' trial of 2004, it appear as if transpiration is responsible for 82.2 % and 87.1 % of the total weight loss from 'Packham's Triumph' and 'Beurré Bosc', respectively during these 4 weeks. During the simulated 7 days of shelf life, the contribution will be 78.4 % and 89.8 % from 'Packham's Triumph' and 'Beurré Bosc', respectively.

This demonstrates that respiration is a less critical constituent in the greater weight loss equation. The effect of transpiration rate is far greater and should also be regarded in this light when shrivelling of horticultural commodities is evaluated.

Fruit stem waxing

The pedicel of the Rosaceae has a dual purpose during its existence. During anthesis the pedicel serves as a flower stalk, whereafter it eventually develops into a fruit stalk. Throughout this period the pedicel undergoes fundamental changes in terms of its function and structure (Roth, 1977).

Thus, during flowering, the pedicel is responsible for positioning the flower in the most favourable position to intercept light. Secondly, the pedicel must provide the flower with adequate water supplies during this time. After fertilization the pedicel must supply much needed structural support and a far greater amount of water and nutritive substances. Therefore, it acts as a mechanical aid in addition to the 'conductor' role that it plays towards the ever-increasing weight of the growing fruit (Roth, 1977).

The roles that the pear stem, or rather the pedicel, plays in the extent of shrivel experienced in the 'neck' of the fruit was studied. By covering the stem to different degrees with melted candle wax, this 'water conduit' could be blocked off. Significant differences were found in all three cultivars, but significant interactions prevent the discussing of main effects in the case of 'Beurré Bosc' and 'Forelle' (Table 6).

Although the predominant area for diffusion is the cuticle (Salisbury and Ross, 1985), it has been reported that diffusion does occur through stem scar tissue. Cameron and Yang (1982) studied the attached, unsealed stem of tomato fruit and found that diffusion does occur though it. Diaz-Perez (1998) found that the calyx of the eggplant fruit accounted for at least 60 % of the transpirational weight loss.

Although a number of significant interactions were found (Table 5), similar trends to that of 'Packham's Triumph' were found in 'Beurrè Bosc' and 'Forelle' (data not shown). From the results obtained from 'Packham's Triumph', one can conclude that by sealing off the pear stem, the loss of weight through it is reduced. The fact that the only significant difference is between the control and the rest of the treatments, suggests that moisture is lost predominantly through the tip and not the sides of the pedicel.

Visual shrivelling detection

When all of these aspects are taken into consideration, the following question arises. What percentage weight loss is needed before signs of shrivel are detected in pears? From Fig. 4 it is clear to see that it took, for all practical purposes, 11 days at 18 °C before the fruit showed any signs of shrivel. The percentage loss required was the lowest in 'Packham's Triumph'

although it also proved to be reached at the slowest rate (0.25%.day^{-1}). 'Beurrè Bosc' revealed the fastest rate of weight loss (0.42%.day^{-1}), but also required the largest loss before shrivel became visible. 'Forelle' needed the biggest percentage weight loss, and this was reached at a rate of 0.35%.day^{-1}.

Although the rate of weight loss was the slowest in 'Packham's Triumph', it also acquired a shrivelled appearance before the other cultivars did. The specific shape of the 'Packham's Triumph' might be responsible for this response. The average 'Packham's Triumph' normally has a very distinct 'neck'. This area lends itself perfectly towards shrivel due to its relatively large SA:V. It is for this reason that 'Packham's Triumph', coupled with the higher respiration rate, is more prone to shrivel.

The physical difference between the average pear and apple is quite obvious. This can possibly also be the explanation for the differences in the weight loss rate and degree required for shrivelling. All these values are less than what is required by apples (5 %) to obtain a shrivelled appearance (Hatfield and Knee, 1988). Differences in pear shape and SA:V for each of the cultivars are discussed in 'Fruit dimensions'.

Fruit dimensions

The dimensions (mass, volume and surface area) for both size categories (small and large) for all cultivars concerned are presented in Fig. 5 and 6. As suspected, average fruit volume decreases as average fruit mass decreases. Thus, one can assume that the density of the different pear cultivars is practically equal. In the case of surface area, 'Beurré Bosc' appears to have a greater surface area than the other cultivars of the same size class. Because a perfect sphere has the smallest surface area in relation to a specific volume, one can assume that the shape of the 'Beurré Bosc' pear is further from a sphere than either 'Packham's Triumph' or 'Forelle'. The shape and size of the so-called neck can be responsible for this prominent proportion of surface area value in 'Beurré Bosc'. Therefore, among the three cultivars studied, a 'Beurré Bosc' pear has the largest surface area that is exposed to the surrounding environment per unit of pear volume. As previously stated, a large SA:V ratio will encourage diffusion from taking place at a faster rate than from fruit with a smaller ratio. This explains the high rate of fruit weight loss that was observed in 'Beurrè Bosc'.

Anatomical studies

The variation and development in cuticular thickness and quality were to be studied in the cultivars concerned. It has been reported that storage (Veraverbeke et al., 2001) and an array of abiotic stresses such as temperature (Roy et al., 1994), wind (Gardingen et al., 1991), rain (Rinallo and Mori, 1996) and UV-B (Gordon et al., 1998) cause variation in the composition and quantity of the cuticular layer in apples. It is also thought that, as the fruit mature, this protective barrier is deposited in greater amounts (Maguire et al., 1999). No conclusive cultivar differences could be found from the microscopy studies (Fig 7, 8 and 9). Very large variations were found within the studied material. This might be because the eventual dissection is only representative to a small part (1 x 1cm) of the whole fruit.

CONCLUSION

The highest rates of weight loss were experienced during the short, warmer (> 0 °C) periods of the postharvest simulation. These also proved to contribute the most towards the total mount of weight loss that was experienced by all the cultivars. Reducing the driving force behind the transpiration rate will lower the rate, and thereby the amount of weight that is lost from pears. Although the respiration rate, and eventual dry mass loss, was higher at 15 °C than at 0 °C, the contribution thereof, in comparison to transpiration, is almost negligibly small.

Smaller and less mature fruit are more susceptible to shrivelling. The surface area to volume ratio is fundamental in determining the rate and susceptibility of pears to visual shrivelling symptoms. The protective cuticle layer, acting as a barrier, prevents excessive rates of weight loss from more mature fruit. Similar properties were revealed by sealing of the fruit stem which prevented moisture loss through it. This phenomenon was most evident in the cultivars with shorter, thicker stems.

LITERATURE CITED

Amarante, C., Banks, N.H., 2000. Postharvest physiology and quality of coated fruits and vegetables. Hort. Rev. 26, 161-232.

Baker, E.A., 1982. Chemistry and morphology of plant epicutucular waxes. In: D.F. Cutler, K.L. Alvin and C.E. Price, The plant cuticle. Academic press, London. p. 139.

Brooks, R.M., Bradley, M.V., Anderson, T.I., 1950. Plant microtechnique manual. Department of Pomology, Univ. Calif.

Brosnan, T., Sun, D., 2001. Precooling techniques and applications for horticultural products – A review. Inter. J.of Refrig. 24, 154-170.

Burdon, J., Clark, C., 2001. Effect of postharvest water loss on 'Hayward' kiwifruit water status. Postharv. Biol. and Technol. 22, 215-225.

Burton, W.G., 1982. Postharvest physiology of food crops. Longman, London, New York.

Cameron, A.C., Yang, S.F., 1982. A simple method for determination of resistance to gas diffusion in plant organs. Plant Phys. 70, 21-23.

Diaz-Perez, J.C., 1998. Transpiration rates in eggplant fruit as affected by fruit and calyx size. Postharv. Biol. and Technol. 13, 45-49.

Farnham, D.S., Thompson, J.F., Marousky, A.M., 1975. Temperature management of cut roses during simulated transit. Floral Rev. 195, 26-28.

Gardigen van, P.R., Grace, J., Jeffree, C.E., 1991. Abrasive damage by wind to the needle surfaces of *Pinea sitchnsis* (Bong.) Carr. and *Pinus sylvestris* L. Plant Cell Environ. 14, 185-193.

Gordon, D.C., Percy, K.E., Riding, R.T., 1998. Effects of uv-B rediation on epicuticular wax production and chemical composition of four *Picea* species. New Phytol. 138, 441-449.

Grierson, W., Wardowski, W.F., 1978. Relative humidity effects on the postharvest life of fruits and vegetables. HortScience. 13, 570-574.

Hatfield, S.G.S., Knee, M., 1988. Effects of water loss on apple in storage. Int. J. Food Sci. Technol. 23, 575-583.

Hoang, M.L., Verboven, P., Baelmans, M., Nicolaï, M., 2004. Sensitivity of temperature and weight loss in bulk of chicory roots with respect to process and product parameters. J. of Food Engin. 62(3), 233-243.

Jackson, J.E., Sharples, R.O., Palmer, J.W., 1971. The influence of shades and within-tree position on apple fruit size, colour and storage quality. J. Hort. Sci. 46, 277-287.

Jenks, M.A., Ashworth, E.N., 1999. Plant epicuticular waxes: Function, production, and genetics. Hort. Rev. 23, 1-67.

Johansen, D.A., 1940. Plant microtechnique, McGraw-Hill, New York.

Kader, A.A., 1985. Storage systems. P49-53. In: A.A. Kader, R.F. Kasmire, F.G. Mitchell, M.S. Reid, N.F. Sommer, J.F. Thompson, 1985. Postharvest technology of horticultural crops. Cooperative extension of University of California, Division of Agricultural and Natural Resources. Special publication 3311.

Lendzian, K.J., Kerstiens, G., 1991. Sorption and transport of gases and vapours in plant cuticles. Rev. Environ. Contam. Toxicol. 121, 65-128.

Maguire, K.M., Banks, N., Lang, A., 1998. Harvest and cultivar effects on water vapour permeance in apples. In: E. J. Mitcham (ed.), Proc. 7th Int. Controlled Atmosph. Res. Conf. 2. 246-251, Davis, CA, USA. pp. 246-251.

Maguire, K.M., Banks, N.H., Opara, L.U., 2001. Factors affecting weight loss of apples. Hort. Rev. 25, 197-234.

Maharaj, R., Sankat, C.K., 1990. The shelf-life of breadfruit stored under ambient and refrigerated conditions. Acta Hort. 269, 411-424.

Naidoo, Y., Lawton, J.R., Barnabas, A.D., Coetzee, J., 1990. Ultrastructure and cytochemistry of *Squamulae intravaginales* of the marine angiosperm, *Halophila ovalis*. S.A. J. Bot. 56(5), 546 – 553.

Nobel, P.S., 1991. Cells and diffusion. In: Physicochemical and enviromental plant physiology. Academic Press, San Diego. p. 1-46.

Paull, R.E., 1999. Effect of temperature and relative humidity on fresh commodity quality. Postharv. Biol. and Technol. 15, 263-277.

Reid, M.S., 1991. Effects of low temperature on ornamental plants. Acta Hort. 298, 215-222.

Rinallo, C., Mori, B., 1996. Damage in apple (*Malus Domestica* Borkh) fruit exposed to different levels of rain acidity. J. of Hort. Sci. 71, 17-23.

Roth, I., 1977. Fruits of angiosperms. Gebruder Bontraeger, Berlin.

Roy, S., Watada, A.E., Conway, W.S., Erbe, E.F., Wergin, W.P., 1994. Low-temperature scanning of frozen hydrated apple tissue and surface organisms. HortScience. 29, 305-309.

Salisbury, F.B., Ross, C.W., 1985. Plant physiology. Wadsworth, Belmont, California. p. 85.

Sastry, S.K., 1985. Factors affecting shrinkage of fruits in refrigerated storage. Am. Soc. Heat Refrig. Aircond Eng. Trans. 91, 683-689.

Senti, F.R., Rizek, R.L., 1975. Nutrient levels in horticultural crops. HortScience. 10,243.

Tu, K, Nicolaï, B., De Baerdemaeker, J., 2000. Effects of relative humidity on apple quality under simulated shelf temperature storage. Scientia Hort. 85, 217-229.

Veraverbeke, E.V., Lammertyn, J., Saevels, S., Nicolaï, B.M., 2001. Changes in chemical wax composition of three different apple (*Malus domestica* Borkh.) cultivars during storage. Postharv. Biol. and Technol. 23, 197-208.

Wills, R.B.H., McGlasson, W.B., Graham, D., Tee, T.H., Hall, E.G., 1989. Effects of water loss and humidity. In: Postharvest: An introduction to the physiology of handling of fruit and vegetables. 3rd ed. New South Wales Univ. Press, Kensington. pp. 53-60.

Woods, J.L., 1990. Moisture loss from fruits and vegetables. Postharv. News and Inform. 1, 195-199.

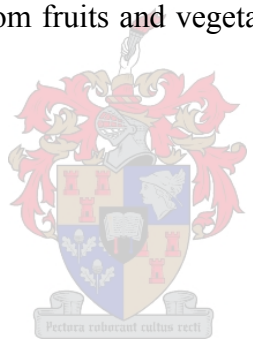

Table 1

The effect harvesting date on of weight loss (%) for ‘Packham’s Triumph’, ‘Beurré Bosc’ and ‘Forelle’ during the 2003 season.

Harvesting date	Weight loss (%)		
	‘Packham’s Triumph’	‘Beurré Bosc’	‘Forelle’
Early	3.39 a	4.1367 a	2.30 a
Late	1.35 b	1.2283 b	1.23 b
LSD	0.4657	0.4391	0.5950
Pr>f	<0.0001	<0.0001	0.0025

Means separated within columns using least significant differences (0.05).

Table 2

Significance levels (0.05) for the main effects on weight loss for ‘Packham’s Triumph’, ‘Beurré Bosc’ and ‘Forelle’ during the 2004 season.

Main effects	‘Packham’s Triumph’	‘Beurré Bosc’	‘Forelle’
Harvesting date	0.0013	0.2650	0.8277
Fruit size	0.0008	0.0447	0.0112
Harvesting date x Fruit size	0.0042	n/s	n/s

n/s = insignificant

Table 3

Effect of harvesting date on fruit firmness measurements of ‘Packham’s Triumph’, ‘Beurré Bosc’ and ‘Forelle’ taken on the days of sampling in 2003 and 2004.

Season	Harvesting date	Fruit firmness (kg)		
		‘Packham’s Triumph’	‘Beurré Bosc’	‘Forelle’
2003	Early	7.42 a	7.09 a	6.65 a
	Late	5.99 b	6.09 b	5.56 b
	LSD	0.4306	0.4405	0.3547
	Pr>f	<0.0001	<0.0001	<0.0001
2004	Early	6.74 a	6.28 a	6.90 a
	Middle	5.66 b	5.72 b	5.82 b
	Late	5.04 c	5.23 c	5.81 c
	LSD	0.538	0.391	0.504
	Pr>f	0.0005	0.0075	0.0003

Means separated by columns using least significant differences (0.05).

Table 4

Effect of fruit size on weight loss (%) of ‘Beurré Bosc’ and ‘Forelle’ after postharvest simulation during the 2004 season.

Fruit size	Weight loss (%)	
	‘Beurré Bosc’	‘Forelle’
Large	5.46 b	5.40 b
Medium	5.87 a	5.57 b
Small	5.88 a	6.47 a
LSD	0.3792	0.7341
Pr>f	0.0447	0.0112

Means separated within columns using least significant differences (0.05).

‘Packham’s Triumph’ showed significant interactions and main effects could therefore not be discussed on their own.

Table 5

The effect of harvesting date, fruit size and stem waxing on weight loss (%) of 'Packham's Triumph' after 12 days storage at 18 °C during 2004.

Main effects					
Harvesting date		Fruit size		% Stem waxed	
Early	3.23 a	Large	2.74 b	Control	3.49 a
Middle	3.03 b	Small	3.29 a	15 %	2.94 b
Late	2.77 c			50 %	2.78 b
				100 %	2.84 b
LSD	0.1736	LSD	0.1409	LSD	0.1993
Pr>f	<0.0001	Pr>f	<0.0001	Pr>f	<0.0001

Means separated within columns using least significant differences (0.05).

'Beurré Bosc' and 'Forelle' showed significant interactions and main effects could therefore not be discussed on their own.

Table 6

Significance levels (0.05) of harvesting date, fruit size and stem waxing treatment on weight loss of 'Packham's Triumph', 'Beurré Bosc' and 'Forelle'.

Season	Main effects	'Packham's Triumph'	'Beurré Bosc'	'Forelle'
2003	Harvesting date	<0.0001	<0.0001	<0.0001
	Waxing treatment	<0.0001	0.0112	0.0498
	Harvesting date* Waxing treatment	0.0079	0.0202	0.0045
2004	Harvesting date	<0.0001	<0.0001	<0.0001
	Fruit size	<0.0001	<0.0001	<0.0001
	Waxing treatment	<0.0001	<0.0001	<0.0001
	Harvesting date x Fruit size	n/s	<0.0001	<0.0001
	Harvesting date x Waxing treatment	n/s	0.0016	0.0016
	Fruit size x Waxing treatment	n/s	n/s	0.0167
	Harvesting date x Fruit size x Waxing treatment	n/s	n/s	n/s

n/s = insignificant

Table 7

Significance levels (0.05) for harvesting date and storage temperature of 'Packham's Triumph', 'Beurré Bosc' and 'Forelle' for respiration rates obtained during 2004.

	'Packham's Triumph'	'Beurré Bosc'	'Forelle'
Harvesting date	0.0309	0.0240	0.0015
Storage temperature	<0.0001	<0.0001	<0.0001
Harvesting date x Storage temperature	n/s	n/s	0.0017

n/s = insignificant

Table 8

Effect of harvesting date and storage temperature on the respiration rate for ‘Packham’s Triumph’ and ‘Beurré Bosc’ after 7 days of storage in 2004.

Harvesting date	‘Packham’s Triumph’ (mg/kg/h)	‘Beurré Bosc’ (mg/kg/h)	Storage temperature	‘Packham’s Triumph’ (mg/kg/h)	‘Beurré Bosc’ (mg/kg/h)
Early	6.06 b	5.48 b	0 °C	3.25 b	3.86 b
Middle	8.64 a	6.98 ab	15 °C	12.28 a	9.58 a
Late	8.59 a	7.70 a			
LSD	2.09	1.54	LSD	1.71	1.26
Pr>f	0.0309	0.0240	Pr>f	<0.0001	<0.0001

Means separated within columns using least significant differences (0.05).

‘Forelle’ showed significant interactions and main effects could therefore not be discussed on their own.

Table 9

Effect of harvesting date and storage temperature on the rate of dry mass (CHO) loss for ‘Packham’s Triumph’ and ‘Beurré Bosc’ after 7 days of storage in 2004.

Harvesting date	‘Packham’s Triumph’ (mg/kg/h)	‘Beurré Bosc’ (mg/kg/h)	Storage temperature	‘Packham’s Triumph’ (mg/kg/h)	‘Beurré Bosc’ (mg/kg/h)
Early	4.12 b	3.72 b	0°C	2.21 b	2.62 b
Middle	5.88 a	4.75 ab	15°C	8.35 a	6.52 a
Late	5.84 a	5.24 a			
LSD	0.0014	0.0001	LSD	0.0012	0.0009
Pr>f	0.0309	0.0240	Pr>f	<0.0001	<0.0001

Means separated within columns using least significant differences (0.05).

‘Forelle’ showed significant interactions and main effects could therefore not be discussed on their own.

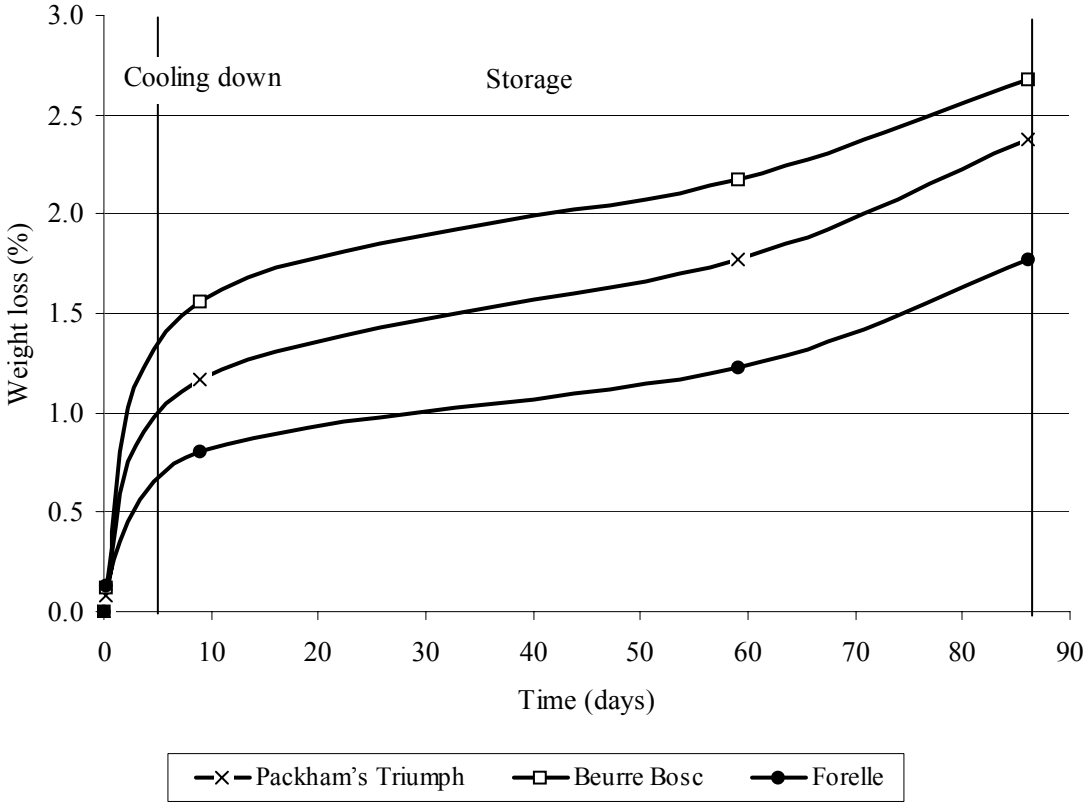

Fig. 1a. Weight loss (%) of 'Packham's Triumph', 'Beurrè Bosc' and 'Forelle' during the postharvest handling simulation of 2003.

Fig. 1b. Weight loss (%) of 'Packham's Triumph', 'Beurrè Bosc' and 'Forelle' during the postharvest handling simulation of 2003. A different set of fruit, than in Fig. 1a, was used to obtain these data.

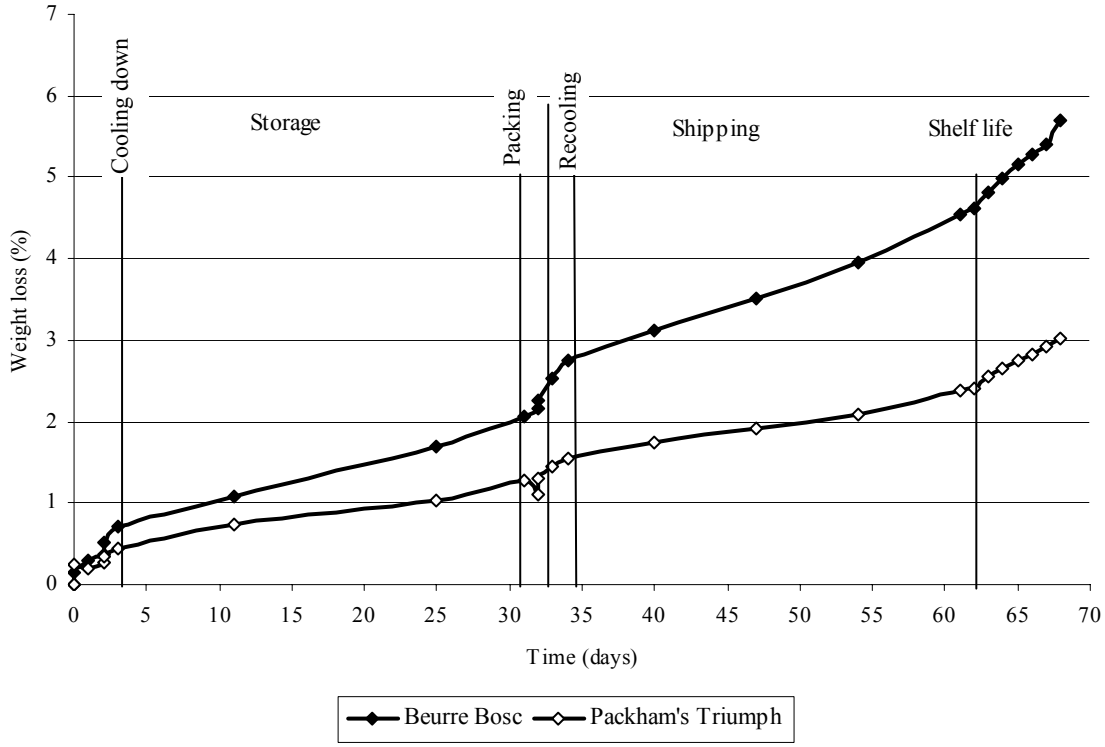

Fig. 2. Weight loss (%) of 'Packham's Triumph' and 'Beurrè Bosc' during the postharvest handling simulation of 2004.

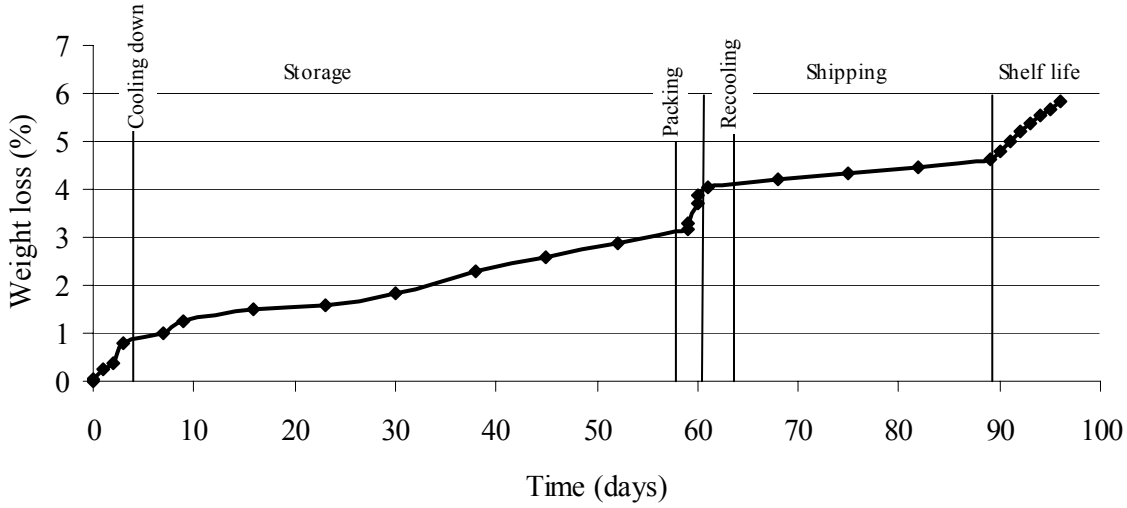

Fig. 3. Weight loss (%) of 'Forelle' during the postharvest handling simulation of 2004.

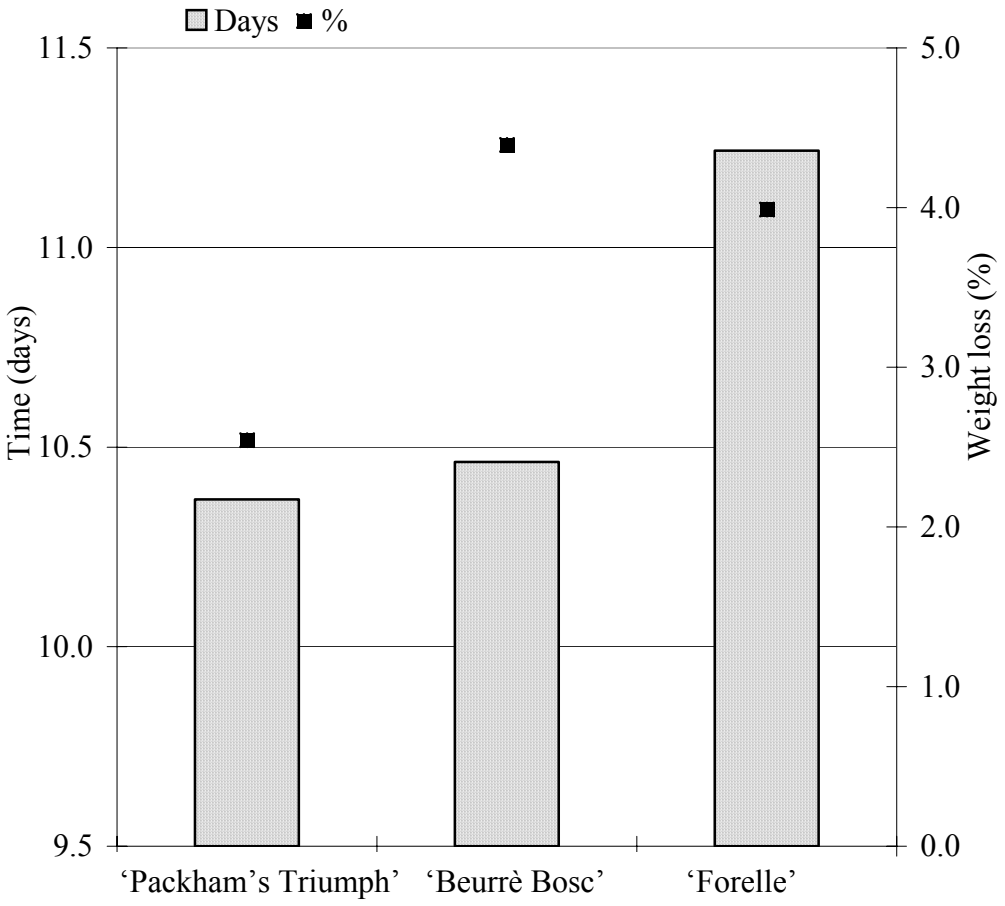

Fig. 4. Time (days) and degree (%) of weight loss needed before signs of shrivel became visual in untreated fruit.

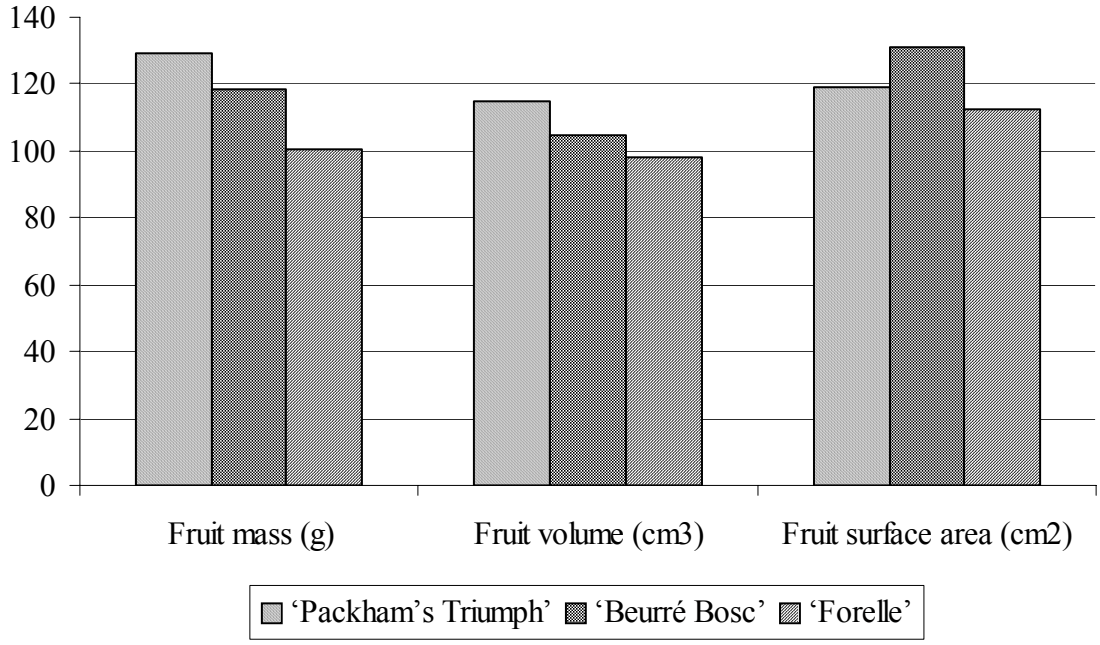

Fig. 5. Fruit dimensions for fruit classified as 'small'.

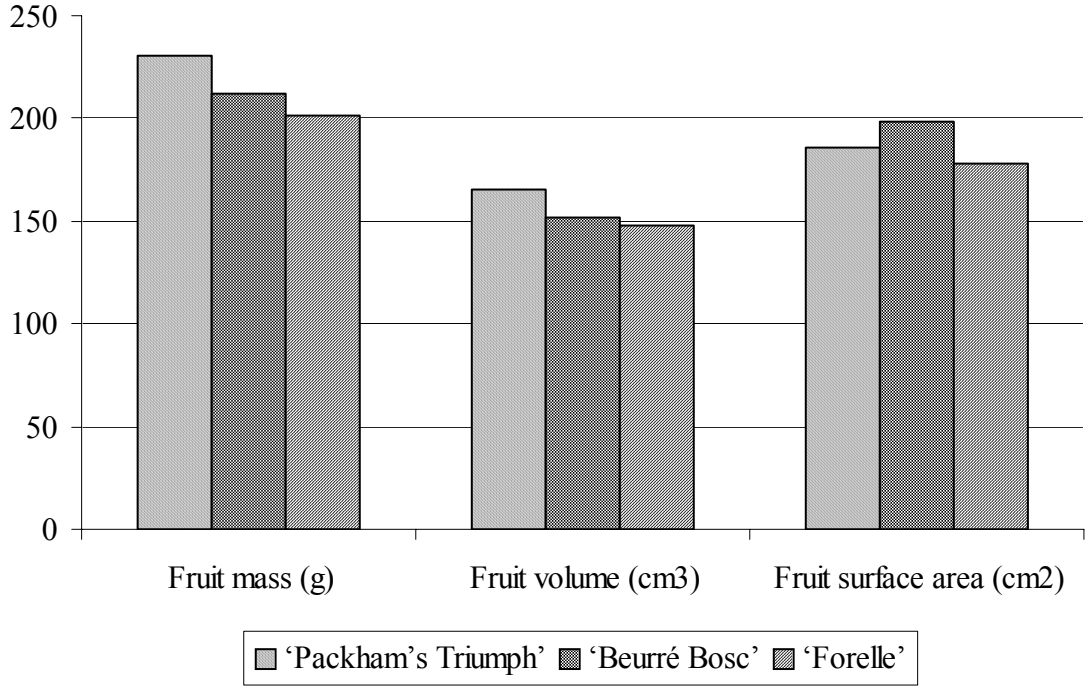

Fig. 6. Fruit dimensions for fruit classified as 'large'.

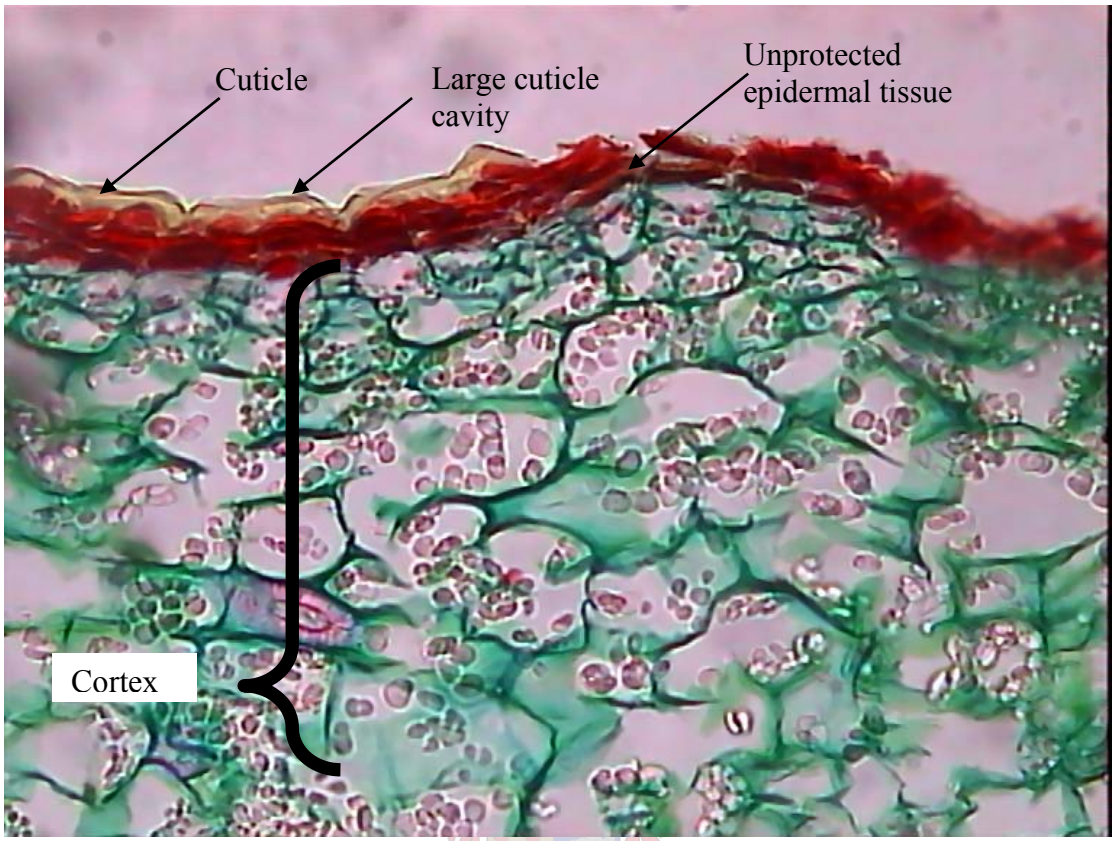

Fig. 7. Transection of epidermal tissue from ‘Packham’s Triumph’ (150 x magnification).

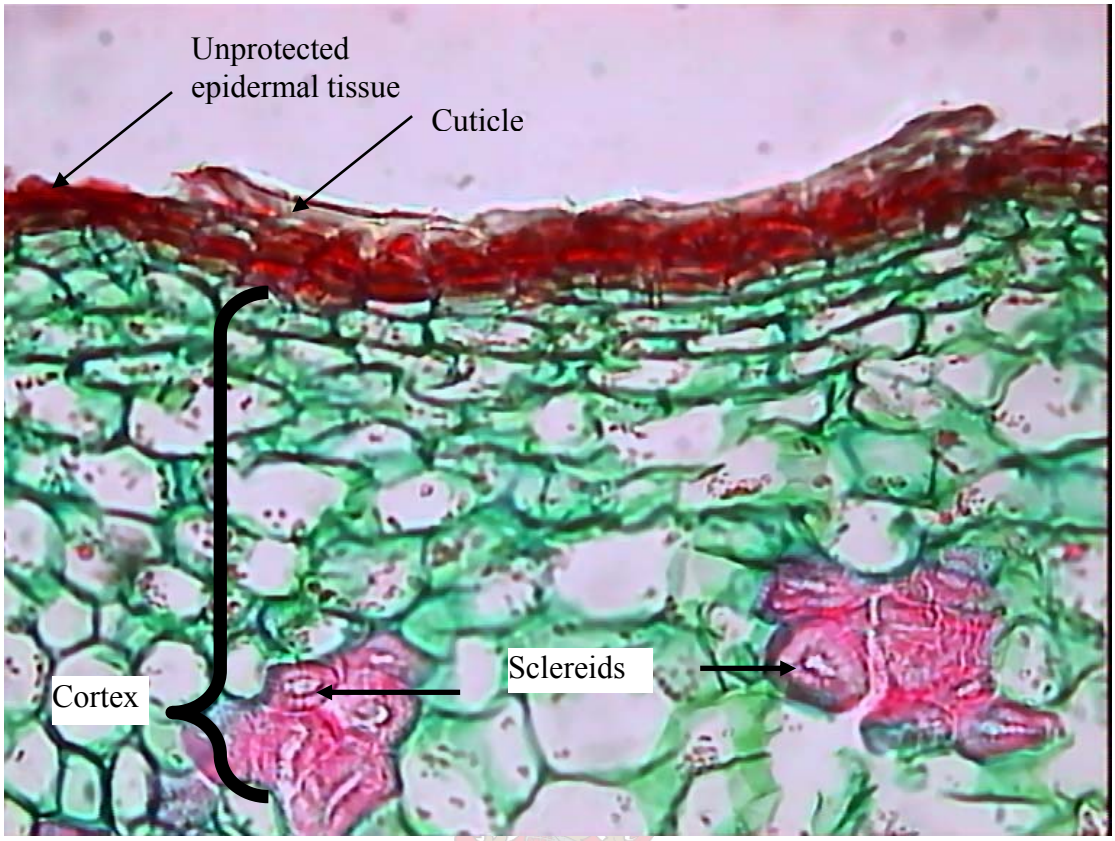

Fig. 8. Transection of epidermal tissue from ‘Beurrè Bosc’ (150 x magnification).

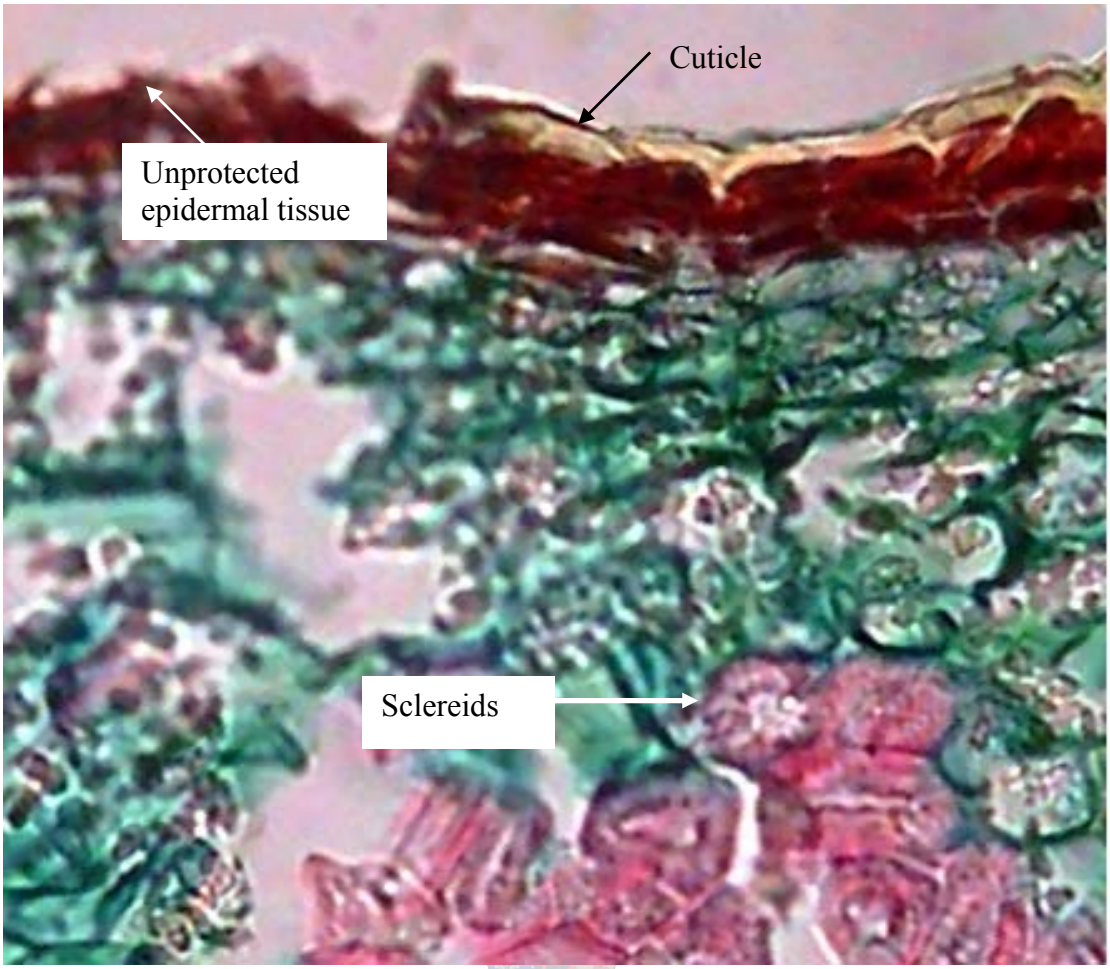

Fig. 9. Transection of epidermal tissue from ‘Forelle’ (150 x magnification).

ARTICLE II EFFECT OF HARVESTING DATE, FRUIT TEMPERATURE AND STORAGE DURATION ON FRICTION DISCOLOURATION OF PEARS.

ABSTRACT

The influence of variables (harvesting date, fruit temperature and storage duration) has been studied on the occurrence and susceptibility of friction discolouration in 'Doyenne du Comice' and 'Packham's Triumph' pears. Fruit sorting conditions were analysed and simulated during 2003. This provided for an industry related method in which laboratory scale simulations could be conducted during 2004. Friction discolouration proved to be most troublesome during 2003 opposed to results obtained during 2004. Throughout both years the incidence and severity of friction discolouration (FD) were higher in 'Doyenne du Comice' than in 'Packham's Triumph'. The number of significant interactions present illustrates the intricacies involved with explaining FD of fruit. Although no significant difference was found between the FD encountered at fruit temperature of 3 °C and 15 °C, discolouration was greater at the higher temperature. Unexpected, contradictory results were obtained for both cultivars in relation to storage duration. No conclusive results could be duplicated in terms of polyphenol oxidase (PPO) activity, total phenolic (TP) content and the occurrence of FD. However, it seems as if the availability of TP from the equatorial skin samples were regulated by PPO activity.

KEYWORDS: Friction discolouration, skin browning, belt burn, enzymatic browning, 'Doyenne du Comice', 'Packham's Triumph', polyphenol oxidase, total phenolics, impact recording device.

INTRODUCTION

Friction discolouration (FD) of pears is a very serious postharvest problem in the pear industry (Wang and Mellenthin, 1973, Mellenthin and Wang, 1974, Chen and Varga, 1996). The unsightly reduction of visual quality is one of the foremost causes for consumer discontent (Raese, 1989). Sommer et al. (1960) commented that such symptoms are difficult to comprehend and that the causes are seldom fully understood. FD is also referred to as skin browning, abrasion marks, belt burns and friction bruises (Smith, 1946). This disorder is

characterised by diffuse brown skin discolourations, especially at high or irregular fruit surfaces (Meheriuk et al., 1994). Such discolourations are induced by a number of mechanical injuries that fruit are subjected to during harvest, packing, transportation and marketing (Mitcham et al., 2001, Feng, 2004), followed by biochemical reactions that lead to browning (Jiménez-Atiénzar et al., 2004).

There has been reported that the timely harvesting of the fruit might influence the degree to which this disorder is experienced. Ambiguous descriptions of the effect that causes the discolouration leads to uncertainties within the literature. It is commonly accepted that both bruising and frictional forces give rise to the skin browning, at epidermal cell level, of pear fruit (Mitcham et al., 2001). Mitcham et al. (2001) also found that skin browning closely correlated with fruit firmness, where firmer fruit showed less browning. This, however, is in contrast with what was found by Kvåle (1979). More mature fruit, which will obviously be less firm, proved to be the least susceptible to discolouration. The method used to induce the damage needs to be questioned. Are the experiments designed to induce discolouration caused by frictional damage, or by bruising the fruit? Is the browning classified under FD or the collective term, skin browning, which accommodates both friction and bruising discolorations? The effect that harvesting date, or rather fruit maturity, has on the fruit's susceptibility to skin discolouration has been studied in 'Bartlett' (Mitcham et al., 2001), 'Doyenne du Comice' (Amarante et al., 2001) and an array of cultivars by both Kvåle (1979, 1988) and Amiot et al. (1995).

Fruit temperature is believed to primarily influence the fruit firmness and the biochemical properties of the pear, or more specifically, the amount of phenols present and the activity of the enzyme, polyphenol oxidase (PPO) that catalyses enzymatic browning. This is reflected by research done on 'Doyenne du Comice' by Amarante et al. (2001). The initial incidence of FD was found to be lower in colder (0 °C) fruit, but after adequate rising of the fruit core temperature (48 h at 20 °C), the increase in FD severity was found to be greater than what was found with warmer (10 °C) fruit. This demonstrates the inhibiting nature of low temperature on PPO activity. The effect of fruit firmness, which decreases with an increase in temperature and thereby influences cell turgidity, might also play a fundamental role in

determining FD occurrence. Fruit firmness and PPO activity are both factors that should be taken into consideration while trying to explain these phenomena.

Storage duration as a controllable component in the post harvest chain has been studied by a number of researchers (Mellenthin and Wang, 1974, Kvåle, 1988, Spanos and Wrolstad, 1990, Amiot et al., 1995, Mitcham et al., 2001). The influence of the increased maturity and effect of the biochemical, phenolic an enzymatic compounds, are primarily associated with the change in FD susceptibility of pears.

Sufficient and acceptable substrate, among others, is needed for enzymatic browning to take place (Goupy et al., 1995). These substrates come in an array of chemical compounds called phenols (Harborne and Simmonds, 1964). The availability of these compounds within the pear peel is fundamental in these biochemical reactions (Amiot et al., 1995). By studying the presence of the polyphenols in pear peel at different harvesting dates and after certain storage duration, an understanding of substrate availability can be gained. Alongside this, the understanding of the PPO activity is also vitally important in estimating pear susceptibility to enzymatic browning. Enzyme activity is known to influence the extent and degree of browning of pear peel (Gauillard and Richard-Forget, 1997).

This study was conducted to evaluate the different cultivars' susceptibility to FD in terms of harvesting date and fruit storage. The effect of fruit temperature at the time of injury was also questioned. Would it be best to handle fruit immediately after cold storage, whilst still cold, or would fewer discolourations be present if core temperature were to be increased? Further examinations of biochemical constituents believed to play a part in enzymatic browning were also examined. The effect of harvesting date and storage duration on total phenolic content and PPO activity in pear peel were evaluated.

MATERIALS AND METHODS

Season 1: 2003.

Friction discolouration simulation

An impact-recording device (IRD400 manufactured by Techmark, Inc., USA) was used to calculate the impact encountered on a commercial pear packing line at Kromco, Grabouw. Eleven transitions, handling and translocation situations were identified. The Max G values of these were calculated and used as reference during laboratory simulation.

A laboratory shaker (Gerhardt, RO 30, Bonn) was modified to simulate the action that fruit is subjected to during packing practices. By using the IRD, it was found that the shaker created comparable vibration or impacts at certain revolutions per minute (rpm's) to generate MaxG values similar to those found during packing practices. The shaker only rotated in the horizontal plane and the desired velocity was set manually. A corrugated cardboard box (280 x 370 x 80 mm) was lined with smooth transport belt, as used in packhouses, wherein the sampled fruit was placed. These fruit were then subjected to the specific treatment and the friction discolouration was calculated, by means of the skin-browning index (SBI), to determine the relationship between vibration velocities and friction discolouration.

Pears, cv. 'Packham's Triumph' and 'Doyenne du Comice', were harvested at the commercial farms, Monteith and Beaulieu, respectively, in the greater Elgin/ Grabouw area (Lat: 34,11°S and Long: 19,01°E). The harvested fruit were taken to Kromco and subjected to the different vibration treatments. Friction discolouration was evaluated 24 hours after the treatment by means of the SBI (Mitcham et al., 2001).

Skin browning index = [(Ax1 + Bx2 + Cx3 + Dx4 + Ex5) x 0.75 + (Fx0.25)] / Total # fruit

A = # pears with < 1 % brown area
B = # pears with 1-2 % brown area
C = # pears with 3-5 % brown area
D = # pears with 6-10 % brown area
E = # pears with > 10 % brown area
F = total value of brown colour intensity for all pears evaluated*

*Colour intensity is subjectively recorded on a 1-to-5 scale, with 1 being low intensity and 5 high intensity.

Each repetition comprised of 5 fruit, along with the IRD, being vibrated at a specific velocity for 2 minutes. The time duration was limited to 2 minutes because the IRD could not handle the higher number of impacts associated with the longer vibration treatment. Although the fruit samples were subjected to slightly lower impact values than those experienced on the packing line, the duration was longer than most of the transitions in the packing line. Velocities of 75, 85, 95 and 105 rpm were used during the 2003 season.

Fruit maturity

Each cultivar was harvested on three different dates. 'Doyenne du Comice': 15, 22 and 29 January and 'Packham's Triumph': 29 January, 5 and 12 February. Fruit underwent firmness evaluation on arrival at Kromco. Readings were taken on opposite, peeled sides of the fruit with a penetrometer (Southtrade, FT 327, Italy) fitted with an 8 mm tip.

Season 2: 2004

Friction discolouration simulation

Harvested fruit was placed into clear plastic sampling bags and transported on protective bubble wrap to the University of Stellenbosch. Fruit intended for cold storage was treated with a 2 % iprodione (dicarboximide, Aventis CropScience) solution to prevent decay during storage. Fruit firmness was determined at arrival at the University. Fruit intended for storage were packed in plastic bags and telescopic cartons, and taken to Hortec, Stellenbosch, and stored in the commercial size experimental cold stores.

During the 2004 season fruit were only vibrated at 105 rpm, which was found to correlate best with industry related conditions. Variables of fruit core temperature (3 and 15 °C) and cold storage duration at 0 °C (0, 1, 2 and 3 months) were used. Fruit was evaluated 24 hours after the simulation by the SBI method.

Undamaged fruit skin samples, peeled from the equatorial region from each of the cultivars at the different storage durations were obtained for laboratory analysis. Total phenol content and polyphenol oxidase activity was determined by means of spectroscopy.

Fruit maturity

Each of the cultivars was harvested on three different dates. 'Doyenne du Comice': 20, 25 and 30 January and 'Packham's Triumph': 30 January, 6 and 13 February. Fruit underwent firmness evaluation on arrival at Kromco. Readings were taken on opposite, peeled sides of the fruit with a penetrometer (Southtrade, FT 327, Italy) fitted with an 8 mm tip.

Storage duration

Fruit from each cultivar was subjected to the vibration simulation on arrival and after 1, 2 and 3 months of 0 °C storage. Stored fruit was treated with a 2 % iprodione (dicarboximide, Aventis CropScience) solution to prevent decay. Fruit was stored at 0 °C inside plastic bags and telescopic fruit cartons.

Fruit core temperature

To obtain the different fruit core temperatures, the fruit samples were taken out of storage 24 hours prior to the treatment and stored at the desired temperature. The plastic bags were removed from the fruit cartons to ensure better air movement and subsequent fruit temperature adjustment inside the carton. Fruit that underwent the vibration treatment without any storage were kept for 24 hours at the desired temperature (3 and 15 °C) to obtain the respective core temperature.

Enzyme (PPO) extraction and purification

The PPO extraction and analysis procedure was a modification of the methods used by Barrett et al. (1991) and Mitcham et al. (2001). All the steps, where possible, were carried out on ice to prevent increases in temperature and subsequent premature activation of the enzyme. One gram of finely ground pear peel, taken from the equatorial region, was added to 1 g polyvinylpolypyrrolidone (PVPP) and 9 ml of chilled extraction buffer (0.05 M phosphate, 1 M KCl, pH = 7). This mixture was placed in a refrigerator (8 °C) and stirred for 10 minutes. Subsequently it was filtered through one layer of cheesecloth. The filtrate was

centrifuged (14 000 x g) for 30 minutes at 4 °C. The supernatant was then filtered through a Whatmann #4 (Whatmann International Limited, Kent, Engeland) filter paper.

Determination of PPO activity

A blank contained 1.96 ml of the reaction buffer (0.2 M Phosphate, 0.1 M Citrate, pH = 6.5) as well as 0.44 ml of a catechol solution (0.5 M catechol in a 10 fold dilution of the reaction buffer). Each sample contained 0.2 ml extract, 1.76 ml reaction buffer and 0.44 ml of the catechol solution. Before the cuvette was placed inside the spectrophotometer is was thoroughly mixed by shaking vigorously for 3 seconds. The absorbance of each sample could then be followed at 420 nm over time using a Varian Cary 3C spectrophotometer (Varian Analytical Instruments, CA). A fresh catechol solution was made on each day of analysis, as it was thought that degradation might take place while exposed to light. The PPO activity of the enzyme (PPO) was calculated using the initial gradient (first 24 seconds) of the curve that was obtained. PPO activity is presented as the change in absorbance at 420 nm per gram pear peel per minute.

Extraction and measurement of total phenolics (TP) content

The phenolic compounds were extracted, partially purified and the total phenolic content was determined by means of a Folin-Ciocalteu (FCR) method, based on the methods used by Tsao and Yang (2003), Kim et al. (2003) and Mitcham et al. (2001). Fruit were peeled at the equatorial region and the skin samples were immediately placed into a –80 °C freezer. Freeze-dried samples were ground to powder under liquid nitrogen with a mortar and pestle and placed back into the –80 °C freezer until later analysis. To each sample of 1 g of powdered fruit skin, 10 ml of 80 % ethanol was added. The mixture was then stirred for 2 hours (at 8 °C) to allow the extraction of most of the phenolic compounds. The extract was filtered through a Whatmann #2 (Whatmann International Limited, Kent, England) filter paper.

A series of gallic acid solutions (30, 60, 100, 200, 300, 400, 500 mg/l) was prepared to serve as calibration standards. To each sample, consisting of 0.4 ml extract, 4 ml ddH_2O and 0.4 ml FC reagent were added. This was then shaken and left for 5 minutes. Thereafter, 4 ml Na_2CO_3 (7 %) and 1.2 ml ddH_2O was added. After a 90 minute incubation period, the

absorbance at 750 nm was measured against the standard curve using a Varian Cary 3C spectrophotometer (Varian Analytical Instruments, CA). Total phenolic content is expressed in mg gallic acid per 1 g pear skin. All samples were repeated in six replicates.

Statistical analysis

Statistical Analysis Systems (SAS), Enterprise Guide VI version 1.3 was used to determine the analysis of variance (ANOVA) and LSD values with a 5 % significance level. A complete randomised design was used for the data set. In the cases where interactions were present, contrasts were examined. All the treatments were done in 6 replicates.

RESULTS

Friction discolouration simulation

Increasing the velocity of the shaker led to higher impact (MaxG) values, as well as a higher number of impacts (Fig. 1). The SBI values followed the same trend, with higher velocities causing more damage in both 'Doyenne du Comice' (Fig. 2) and 'Packham's Triumph' (Fig. 3). The average MaxG values encountered for each vibration velocity, ranged from just above 9.3, for the slowest of the four treatments, to 10 G for the 105 rpm treatment. Test trials were done beforehand to determine these velocities. In the case of velocities lower than 75 rpm, the contact that fruit made with each other was very little. FD of an extremely minor degree, if any at all, was encountered on these fruit. Velocities of more than 105 rpm also created a problem due to the fact that there were too many impacts in the 2 minute period for the IRD to record. Therefore it was decided to use the range from 75 to 105 rpm with 10 rpm intervals for each treatment.

During the 2004 season it was decided to use only the 105 rpm treatment as it best correlated with relevant industry conditions. Each cultivars reaction to the different variables (harvesting date, fruit core temperature and storage duration) would therefore be evaluated after being subjected to a two minute, 105 rpm treatment. Although similar trends were evident in most cases, a great number of interactions was found between the variables (Table 1). This prevents the discussion of the different variables on their own.

Two variables (harvesting date and vibration velocity) were used during the 2003 season. Significant differences and interactions were found to be present in both cultivars (Table 1). The severity of FD was much worse in 2003 (Table 2) than 2004 (Table 3), especially in the case of 'Doyenne du Comice'. This particular cultivar also showed a greater degree of discolouration than 'Packham's Triumph' throughout all the simulations (Table 2 and 3).

Fruit maturity

Fruit firmness measurements for 2003 coincided with those of the fruit sampled in 2004 (Table 4). Fruit firmness significantly decreased as harvesting date increased (Table 4). This predictable phenomenon is noticeable in both years.

Storage duration

No conclusive results were obtained in terms of the effect of storage duration on fruit susceptibility to FD. Both cultivars showed significant interactions involving storage duration (Table 1). This was also found to be the case with PPO activity and TP content, although similar trends exist in some instances.

Fruit core temperature

Once again no significant differences were found between the two temperatures that were studied. However, the average occurrence of FD was higher at 15 °C (0.48 and 0.28) than at 3 °C (0.44 and 0.22) in both 'Doyenne du Comice' and 'Packham's Triumph', respectively (Fig 4).

PPO activity

PPO activity proved to be influenced by storage duration rather than by the specific harvesting date. This seems to be the case in both cultivars (Table 5). PPO activity was greater in 'Packham's Triumph' than in 'Doyenne du Comice' (Table 6).

Total phenolic (TP) content

The TP content was also affected by significant interactions between harvesting date and storage duration (Table 5). TP levels were typically higher in 'Doyenne du Comice' than in 'Packham's Triumph' (Table 6). The association between TP content and PPO activity at

certain storage durations is presented for 'Doyenne du Comice' (Fig. 5) and 'Packham's Triumph' (Fig. 6). Similar trends are noticeable in both cultivars. When PPO activity decreased, TP content increased. This trend is even more noticeable when considered in terms of harvesting date for 'Doyenne du Comice' (Fig. 7) and 'Packham's Triumph' (Fig. 8). Although not statistically analysed, no association could be drawn between encountered SBI values (upper x-axis on Fig. 5-8) and either PPO activity or TP content.

DISCUSSION

Friction discolouration simulation

Similar devices to the IRD have been used extensively for determination and evaluation of the impact endured by commodities during postharvest handling (Zapp et al., 1990, Sober et al., 1990). The occurrence and severity of FD increased as the fruit was subjected to more rigorous vibration treatments (Fig. 2 and 3). This can be expected since fruit injury is a prerequisite for enzymatic browning to take place (Meheriuk et al., 1994). By intensifying the impact on the fruit, a greater number of epidermal cells underwent injury (Slaughter et al., 1993) and subsequently the integrity of the cell membranes would have been lowered. This brought about the amalgamation of phenol compounds and PPO in the presence of oxygen, thereby allowing the enzymatically catalysed, oxidative process to take place (Haruta et al., 1999).

Similar discolourations were encountered by Berardinelli et al. (2005), while simulating transport vibration on pears. These discolorations consisted mainly of darkened streaks, without any bruising and deterioration to the underlying flesh, of the epidermal tissue. This only partially differed from what was found with the friction discolouration simulations. The manifestation of the discolouration, as found by Berardinelli et al. (2005) during transport, consisted only of isolated discolouration areas, whereas continues sections of discolouration were found during frictional discolouration simulation. This is attributed to the way in which the fruit were packed during transport. During the sorting, and simulation thereof, fruit are free to role onto either side and encounter frictional forces, rendering them injured and susceptible to enzymatic browning. This emphasize the importance of preventing vibration damage during transport by either tight fill packaging (Slaughter et al., 1993) or the use of

polyethylene bags (Slaughter et al., 1998), as well as the implementation of effective decelerator elements between transitions of fruit packing lines (Garcia-Ramos et al., 2003).

The degree of FD was much higher in 2003 than in 2004. The only difference in sampling/ handling procedures was that all the samples were collected and transported to Stellenbosch before 11h00 during 2004. This allowed the fruit to be handled before it experienced very high temperatures. During 2003, fruit were sampled and subjected to the treatments within a maximum of 2 hours after harvest. No discrimination was made in terms of time of harvest or fruit temperature. In some cases fruit core temperatures of above 25 °C were recorded. This made it necessary to investigate the effect of fruit temperature on FD during 2004.

Assessment of fruit discolouration occurred 24 h after simulated vibration of the pears. This period proved sufficient as a number of researchers (Kvale, 1988, Slaughter et al., 1993, Amarante et al., 2001, Mitcham et al., 2001) allowed for similar periods of time before assessment were made. Berardinelli et al. (2005) stored the fruit for 8 days, at 20 °C, before it was examined.

During 2003, 'Doyenne du Comice' scored almost double that of 'Packham's Triumph' in terms of SBI values. No record could be found of comparisons between 'Doyenne du Comice' and 'Packham's Triumph' in relation to their respective response to vibration treatments. Gorny et al. (2000) studied the difference in enzymatic browning of pear slices from different cultivars. Pear slices of 'Anjou' and 'Red Anjou' developed more intense discolourations than 'Beurre Bosc' and 'Bartlett'. Amiot et al. (1995) classified 'Doyenne du Comice' as highly susceptible to discolouration. The use thereof by Amarante et al. (2001) as research material underlines the susceptible nature of this particular cultivar.

A great variety of assessment methods have been used by researchers throughout the years. Most resently, Berardinelli et al. (2005) determined the actual size of the affected fruit skin, by removing it from the fruit, without ascribing the intensity of the discolouration. The method used during this trial, as also used by Mitcham et al. (2001) and Feng et al. (2004), make accomodation for both extend and intensity of discolouration. By using similar classification methods, comparable results are obtained during research.

During 2003 discolouration values were of the same magnitude, as ascribed by Mitcham et al. (2001) on 'Bartlett', than what was encountered on 'Doyenne du Comice' (Fig. 2). Feng et al. (2004) also recorded similar magnitudes of discolouration after a variety of treatments. The discolourations were far less during 2004 (Table 3) than in 2003 (Table 2). This was the case with both cultivars.

Harvesting date

Fruit firmness measurements proved that comparable fruit were used during both years (Table 4). Although 'Doyenne du Comice' was harvested a week later during 2004, with only 5 days between each harvesting date, maturity measurements proved that these fruit were only slightly less mature than those used during 2003. As would be suspected, fruit firmness significantly decreased as harvesting date was delayed and fruit maturity increased. This was also found by Amiot et al. (1995).

The effect of harvesting date proved to be substantial during both years in terms of FD occurrence in 'Packham's Triumph' and 'Doyenne du Comice' (Table 1). This can however only be discussed in combination with the velocity experienced due to significant interactions (Table 1). Amarante et al. (2001) found a decreasing linear relationship between fruit firmness and fruit temperature. Significant interactions illustrated the intricacies involving the effect of harvesting date on FD susceptibility during 2004. A number of significant interactions were found between FD and harvesting date, during this research, but are not presented. 'Doyenne du Comice' proved the most complex of scenarios, involving fruit temperature, storage duration and a three-way interaction involving both fruit temperature and storage duration.

For some reason, opposite results were obtained for the two cultivars during 2003 (Table 2). The second harvesting date resulted in the highest SBI score in the case of 'Packham's Triumph', but the lowest for 'Doyenne du Comice'. Although not experimentally evaluated, this might be due to temperature differences on that specific day. It was generally found that warmer fruit showed a higher susceptibility to FD than colder fruit. During 2004, the second

harvesting date again resulted in the highest SBI values for both cultivars (Table 3). Slaughter et al. (1996) found that late season 'Bartlett' pears were more susceptible to vibration damage than early or mid-season fruit. There is, however, evidence that supports the opposite (Raese, 1989, Mellenthin and Wang, 1974, Kvåle, 1988).

Storage duration.

Significant interactions with other variables (harvesting date and fruit core temperature) were found in both cultivars during 2004 (Table 1). When the averages for each storage duration are evaluated, an inverse reaction is illustrated by the two cultivars (Table 3), with SBI tending to increase for 'Packham's Triumph' and decrease for 'Doyenne du Comice' as storage duration increased. This reaction is somewhat unexplainable. From the literature it is evident that, as storage duration is prolonged, susceptibility to FD also increases (Mitcham et al., 2001). This is largely attributed to the accumulation of phenolic compounds present in the pear peel (Wang and Mellenthin, 1973, Mellenthin and Wang, 1974, Meheriuk et al., 1982, Kvåle, 1988), the decrease in fruit firmness (Kvåle, 1988, Amiot et al., 1995, Mitcham et al., 2001) and the loss of water from the epidermal cells (Amarante et al., 2001). Apparently this renders the fruit less turgid, which influences the cell membrane integrity negatively and makes the cell more susceptible to frictional damage. This specific response is somewhat reflected, although only very slightly, by 'Packham's Triumph' during 2004. The surprisingly low value recorded after 3 months of storage is unexpected.

Fruit core temperature.

No significant difference was found in relation to the two fruit temperatures that were evaluated (Table 1). However, higher average SBI values were recorded with warmer fruit (15°C) than with colder (3 °C) fruit (Fig. 4). A definite trend is visible when the two temperatures are compared. In retrospect, a higher evaluation temperature (20-25 °C) might have proved to reveal a significant increase in FD due to stimulation of enzyme activity by higher temperatures (Weemaes et al. 1998).

Amarante et al. (2001) found a quadratic relationship between fruit temperature and FD. No differences were found between different temperatures (0 and 20 °C) after 24 h storage at the respective temperatures. FD susceptibility increased linearly as fruit were kept at ambient

temperature after removal from cold storage. Although, during this research, fruit were allowed 24 h at room temperature (±20 °C) after vibration injury, similar resulted could not be duplicated. As the loss of water (Amarante et al., 2001) and ripening stage (Amiot et al., 1995) of fruit has been correlated to FD susceptibility, storage at ambient temperature should be avoided.

The FD trend that was observed, between 3 and 15 °C, coincide with what is suggested by Amarante et al., (2001). While colder, firmer fruit are more susceptible to bruising damage (Sekse and Opedal, 1993), warmer fruit seems more susceptible to FD. This is attributed to a greater degree of water loss, shrinking the skin and leaving it rougher (Amarante et al., 2001), and lowering turgor pressure, which renders it more tolerable to impact (Garcia et al., 1995). Thus, a clear definition needs to be done when discolouration is considered.

Polyphenol oxidase (PPO) activity.

Harvesting date, as a single variable, did not have any influence on the activity of PPO (Table 5). However, the combination of harvesting date and storage duration did prove to have significant interaction in terms of PPO activity. This is evident in both cultivars. No uniform change could be detected in PPO activity over the 3 months of storage of both cultivars (Table 6). The average values after the longest storage period (three months) was, however, significantly lower in both cultivars when compared to the shorter storage durations.

It is interesting to note that the activity of PPO extracted from 'Packham's Triumph' was higher than what was found to be the case with 'Doyenne du Comice' (Table 6), even though SBI values were lower (Table 3). This rhymes with what is expected from an enzyme, which lowers the activation energy and thereby catalyses the reaction without taking part in the reaction itself (Salisbury and Ross, 1992). Because evaluations were done 24 hours after simulation, and the discolouration occurs shortly after injury (Feng et al., 2004), the possible difference in reaction time cannot be commented on.

Total phenolic (TP) content.

Interactions between harvesting date and storage duration once again proved to be significant in both cultivars. As most papers report (Amiot et al., 1995), it was found that TP content of

'Doyenne du Comice' increased with an increase in storage period (Table 6). This was not found to be the case with 'Packham's Triumph' (Table 6).

Interesting assumptions can be made about the contributing factors and their respective importance to FD in both 'Doyenne du Comice' (Fig. 5 and 7) and 'Packham's Triumph' (Fig. 6 and 8). It seems as if PPO activity and TP content are generally inversely proportional to each other. Often, when PPO activity was low, a relatively high TP content was recorded. This emphasizes the fact that, when the oxidative process is suppressed by whichever means, the TP content will increase due to the slower conversion rate to quinones. This assosiation between TP content and PPO activity was also suggested by Mellenthin and Wang (1974). PPO activity and total phenol levels were determined from 'd'Anjou' pear peel, harvested on three different dates and after weekly intervals of storage duration. A complete inverse between the two constituents was observed, with TP levels being low at first and increasing during prolonged storage (8 weeks). In the case of 'Packham's Triumph' (Fig 6 and 8) it appears as if high SBI values were obtained when the PPO activity was relatively high, rather than a high TP content. However, this relationship is not as clearly illustrated in the case of 'Doyenne du Comice' (Fig 5 and 7). This possible relationship between PPO activity and FD is very difficult to explain. Although PPO activity is instrumental during the oxidative process that causes FD, this relationship seems highly unlikely. The extent of FD would more likely be related to available TP content within the tissue itself. The ability of enzymes to catalyse the same reaction over and over again makes it look as if it is not the limiting factor concerning FD. This becomes even more evident when considering that evaluation of fruit was done 24 hours after vibration treatment and fruit were therefore granted enough time for complete discolouration to take place.

The effect of cell membrane integrity, acting as barrier between the needed constituents, might be underestimated. Although TP content and PPO activity influence the reaction time and the intensity of discolouration, they still have to be liberated from the separate locations within the cell to take part in the reaction. This, and not so much the characteristics of the constituent, might influence the susceptibility more than initially thought.

CONCLUSION

Industry related handling conditions could be simulated by using the modified laboratory shaker and vibrating fruit at various velocities. Higher discolouration values were obtained at higher vibration velocities. This confirms that epidermal rupture, and cellular decompartmentalization, are required for enzymatic browning to occur.

Fiction discolouration was noticeably higher in 'Doyenne du Comice' than in 'Packham's Triumph'. Although not significantly different, discolouration was more severe in warmer (15 °C) than in colder (0 °C) fruit. This might be due to lower cell membrane integrity, or to the higher enzymatic activity at higher temperatures. Interdependency between PPO and TP could clearly be seen. When PPO activity was high the availability of TP was relatively low in the epidermal tissue that was analysed. Contradictory results were obtained for 'Doyenne du Comice' and 'Packham's Triumph' in terms of storage duration. A number of significant interactions, *inter alia* harvesting date and storage duration, confirmed the intricacies involved in explaining this postharvest disorder.

LITERATURE CITED

Amarante, C., Banks, N.H., Ganesh, S., 2001. Effects of coating concentration, ripening stage, water status and fruit temperature on pear susceptibility to friction discolouration. Postharv. Biol. and Technol. 21, 283-290.

Amiot, M.J., Tacchini, M., Aubert, S.Y., Oleszek, W., 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. J. Agric. Food Chem. 43, 1132-1137.

Barrett, D.M., Lee, C.Y., Lui, F.W., 1991. Changes in the activity and subcellular of PPO in 'Delicious' apples during controlled atmosphere storage. J. Food Biochem. 15, 185-199.

Berardinelli, A., Donati, V., Giunchi, A., Guarnieri, A., Ragni, L., 2005. Damage to pears caused by transport. J. Food Engin. 66 (2), 219-226.

Chen, P. M., Varga, D.M., 1996. Storage challenges with winter pears. WSU- TFREC Postharvest information network: Washington Tree Fruit Postharvest Conference, 1996 Proceedings, Storage challenges with winter pears. http://postharvest.tfrec.wsu.edu/PC96A.pdf.

Feng, X., Biasi, B., Mitcham, E.J., 2004. Effects of various coatings and antioxidants on peel browning of 'Bartlett' pears. J. Sci. Food Agric. 84, 595-600.

Garcia, J.L, Ruiz-Altisent, M., Barreiro, P., 1995. Factors influencing mechanical properties and bruise susceptibility of apples and pears. J. Agric. Eng. Res. 61, 11-18.

Garcia-Ramos, F.J., Ortiz-Canavate, J., Ruiz-Altisent, M., 2003. Decelerator elements for ramp transfer points in fruit packing lines. J. Food Engin. 59, 331-337.

Gauillard, F., Richard-Forget, F., 1997. Polyphenol oxidases from 'Williams' Pear (*Pyres communis* L, dv. 'Williams'): Activation, purification and some properties. J. Sci. Food Agric. 74, 49-56.

Goupy, P., Amiot, M.J., Richard-Forget, F.C., Duprat, F., Aubert, S., Nicolas, J.J., 1995. Enzymatic browning of model solutions and apple phenolic extracts by apple polyphenol oxidase. J. Food Sci. 60, 497-501.

Gorny, J.R., Cifuentes, R.A., Hess-Pierce, B., Kader, A.A., 2000. Quality changes in fresh-cut pear slices as affected by cultivar, ripeness stage, fruit size, and storage regime. J. Food Sci. 65(3), 541-544.

Harborne, J.B., Simmonds, N.W., 1964. In: Biochemistry of phenolic compounds, J.B., Harborne . Academic Press, London. pp. 79-91

Haruta, M., Murata, M., Kadokura, H., Homma, S., 1999. Immunological and molecular comparison of polyphenol oxidase in Rosaceae fruit trees. Phytochem. 50, 1021-1025.

Jiménez-Atiénzar, M., Cabanas, J., Gandía-Herrero, F., García-Carmona, F., 2004. Kinetic analysis of catechin oxidation by polyphenol oxidase at neutral pH. Biochem. and Biophys. Res. Comm. 319, 902-910.

Kim, D., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemical from various cultivars of plums. Food Chem. 81: 321-326.

Kvåle, A., 1979. FD of two pear cultivars in relation to date of harvest and phenolic compounds in the fruit. Acta Agric. Scand. 29, 29-32.

Kvåle, A., 1988. Skin discolouration of four pear cultivars in relation in to maturity, degree of ripening and duration of storage. Norwegian J. Agric. Res. 2, 139-142.

Meheriuk, M., Prange, R. K., Lidster, P. D. Porritt, S.W., 1982. Postharvest disorders of apples and pears. Agriculture Canada, Publication 1737/E.

Mellenthin, W. H., Wang, C. Y., 1974. FD of 'd' Anjou' pears in relation to fruit size, maturity storage and polyphenol oxidase activities. HortScience. 9, 592-593.

Mitcham, E.J., Feng, X., Biasi, B., 2001. Susceptibility to and the control of skin browning in 'Bartlett' pears. Report to Californian pear advisory board.

Raese, J.T., 1989. Physiological disorders and maladies of pear fruit. Hort. Rev. 11, 357-411.

Sekse, L., Opedal, M.L., 1993. Effect of temperature on bruising damage in 'Gravenstein' apples. Acta Hort. 326, 299-303.

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, fourth ed. Wadsworth Publishing Company, Belmont Calif. pp. 191-192.

Slaughter, D.C., Hinsch, R.T, Thomson, J.F., 1993. Assessment of vibrational injury to 'Bartlett' pears. Trans. ASAE. 36(4), 1043-1047.

Slaughter, D.C., Hinsch, R.T, Thomson, J.F., 1996. Vibration induced injury during transportation of pears. Tree Fruit Postharv. J. 7(1), 8-11.

Slaughter, D.C., Thompson, J.F., Hinsch, R.T., 1998. Packaging 'Bartlett' pears in polyethylene film bags to reduce vibration injury in transit. Trans. ASAE, 41 (1), 107-114.

Smith, E., 1946. Handling injuries on pears following cold storage. Proc. Am. Soc. Hortic. Sci. 47, 79-83.

Sober, S.S., Zapp, H.R. and Brown, G.K. 1990. Simulating packing line impacts for apple bruise prediction. ASAE Paper No. 89-6074. MI: ASAE.

Sommer, N.F., Mitchell, F.G., Guillou, R., Luvisi, D.A., 1960. Fresh fruit temperature and transit injury. Am. Soc. Hort. Sci. 76, 156-162.

Spanos, G.A., Wrolstad, R.E., 1990. Influence of variety, maturity, processing, and storage on the phenolic composition of pear juice. J. Agric. Food Chem. 38, 817-824.

Tsao, R., Yang, R., 2003. Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using HPLC. J. Chrom. 1018(1), 29-40.

Wang, C.Y., Mellenthin, W.M., 1973. Relationship of friction discoloration to phenolic compounds in 'd'Anjou' pears. HortScience. 8(4), 321-323.

Weemaes, C.A., Ludikhuyze, L.R., Van den Broeck, I., Hendrickx, M.E., Tobback. P.P., 1998. Activity, Electrophoretic characteristics and heat inactivation of polyphenol oxidase from apples, avocados, grapes, pears and plums. Lebensm-Wiss. U. Technol. 31, 44-49.

Zapp, H.R., Ehlert, S.H., Brown, G.K., Armstrong, P.R. and Sober, S.S. 1990. Advanced instrumented sphere (IS) for impact measurements. ASAE Paper No. 89-6046. St. Joseph, MI: ASAE.

Table 1

Significance levels (0.05) for treatment variables for ‘Packham’s Triumph’ and ‘Doyenne du Comice’ pears in 2003 and 2004.

Season		‘Packham’s Triumph’	‘Doyenne du Comice’
2003	Harvesting date	<0.0001	<0.0001
	Velocity	<0.0001	0.0010
	Harvesting date x Velocity	<0.0001	0.0005
2004	Harvest date	<0.0001	<0.0001
	Fruit temperature	0.0590	0.1589
	Storage duration	<0.0001	<0.0001
	Harvesting date x Temperature	n/s	0.0053
	Harvesting date x Storage duration	<0.0001	<0.0001
	Temperature x Storage duration	n/s	<0.0001
	Harvesting date x Temperature x Storage duration	n/s	0.0033

n/s = insignificant

Table 2

Effect of harvesting date and shaker velocity (revolutions per minute) on friction discolouration (quantified by means of the skin browning index) for 'Packham's Triumph' and 'Doyenne du Comice' during 2003.

	Skin browning index[z]	
Variable	'Packham's Triumph'	'Doyenne du Comice'
Harvest date 1	0.46 c	2.54 a
Harvest date 2	1.25 a	1.96 b
Harvest date 3	0.54 b	2.38 a
LSD	0.0695	0.1669
Pr>f	<0.0001	<0.0001
75 rpm	0.48 c	2.18 b
85 rpm	0.66 b	2.16 b
95 rpm	0.73 b	2.29 b
105 rpm	1.13 a	2.53 a
LSD	0.0803	0.1927
Pr>f	<0.0001	0.0010

Means within columns are separated using least significant differences (0.05).

[z] higher values indicate more severe skin browning.

Table 3

Effect of harvesting date and storage duration on friction discolouration (quantified by means of the skin browning index) for 'Packham's Triumph' and 'Doyenne du Comice' during 2004.

	Skin browning index[z]	
Variable	'Packham's Triumph'	'Doyenne du Comice'
Harvest date 1	0.18 b	0.43 b
Harvest date 2	0.35 a	0.59 a
Harvest date 3	0.21 b	0.36 b
LSD	0.076	0.0843
Pr>f	<0.0001	<0.0001
At harvest	0.10 c	0.73 a
1 month storage	0.23 b	0.41 b
2 months storage	0.41 a	0.28 c
3 months storage	0.24 b	0.42 b
LSD	0.0877	0.0974
Pr>f	<0.0001	<0.0001

Means within columns are separated using least significant differences (0.05).

[z] higher values indicate more severe skin browning.

Table 4.

Effect of fruit maturity (harvesting date) on fruit firmness for ‘Packham’s Triumph’ and ‘Doyenne du Comice’ during the 2003 and 2004 season.

		Fruit firmness (kg)	
Season		‘Packham’s Triumph’	‘Doyenne du Comice’
2003	Harvest 1	8.19 a	7.66 a
	Harvest 2	7.64 b	6.90 b
	Harvest 3	6.74 c	5.81 c
	LSD	0.4925	0.3116
	Pr>f	<0.0001	<0.0001
2004	Harvest 1	8.10 a	6.98 a
	Harvest 2	7.21 b	6.06 b
	Harvest 3	6.53 b	5.91 b
	LSD	0.6896	0.4145
	Pr>f	0.0003	<0.0001

Means within columns are separated using least significant differences (0.05).

Table 5

Significant levels (0.05) of treatment variables on polyphenol oxidase (PPO) activity and total phenolic (TP) concentration in 2004.

	'Packham's Triumph'		'Doyenne du Comice'	
	PPO (ΔA 420nm /g/min)	TP (mg gallic acid/g)	PPO (ΔA 420nm g/min)	TP (mg gallic acid/g)
Harvest date	0.4247	0.4911	0.3488	0.0003
Storage duration	<0.0001	0.2452	<0.0001	0.0341
Harvesting date x Storage duration	<0.0001	<0.0001	0.0493	0.0115

Table 6

Effect of harvesting date and storage duration on polyphenol oxidase activity (PPO) and total phenolic (TP) concentration during 2004.

Variable	'Packham's Triumph'		'Doyenne du Comice'	
	PPO (ΔA 420nm /g/min)	TP (mg gallic acid/g)	PPO (ΔA 420nm g/min)	TP (mg gallic acid/g)
Harvesting date 1	12.3 a	61.0 a	9.12 a	99.0 a
Harvesting date 2	13.0 a	59.2 a	9.79 a	89.6 b
Harvesting date 3	12.2 a	60.8 a	9.75 a	88.8 b
LSD	1.2776	3.2027	1.0188	5.227
Pr>f	0.4247	0.4911	0.3488	0.0003
At harvest	12.2 b	62.5 a	10.4 a	91.1 b
1 month storage	12.9 b	60.4 a	9.70 a	90.3 b
2 months storage	14.4 a	59.0 a	10.3 a	90.4 b
3 months storage	10.5 c	59.5 a	7.80 b	98.0 a
LSD	1.4752	3.6982	1.1764	6.0356
Pr>f	<0.0001	0.2452	<0.0001	0.0341

Means within columns are separated using least significant differences (0.05).

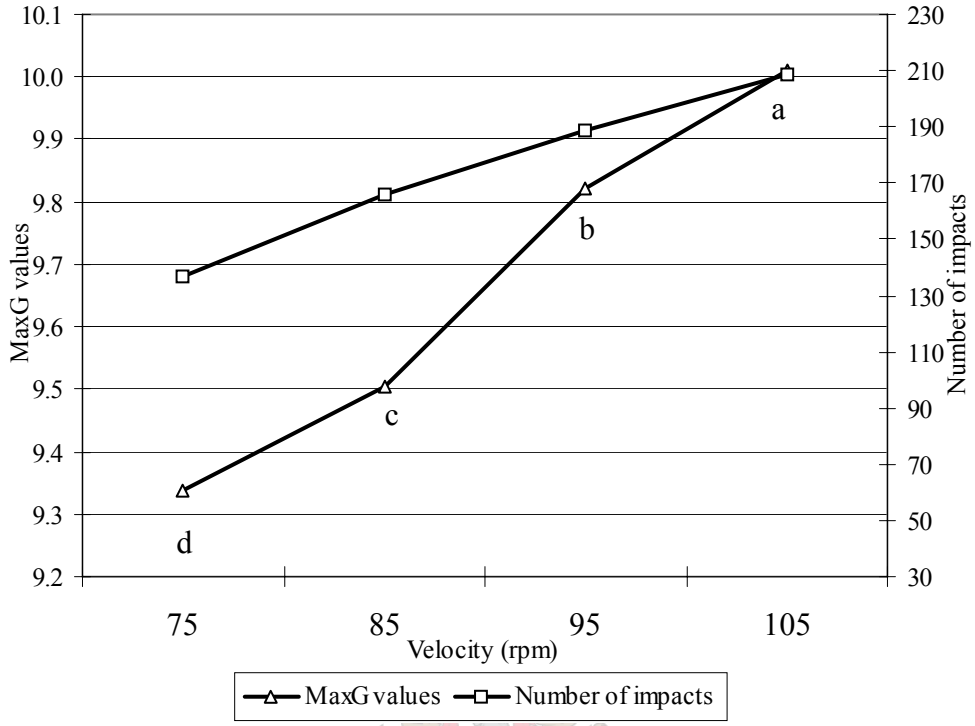

Fig. 1. Effect of shaker velocity (rpm) on the number and severity (MaxG) of the impacts, as recorded by an impact recording device (IRD).

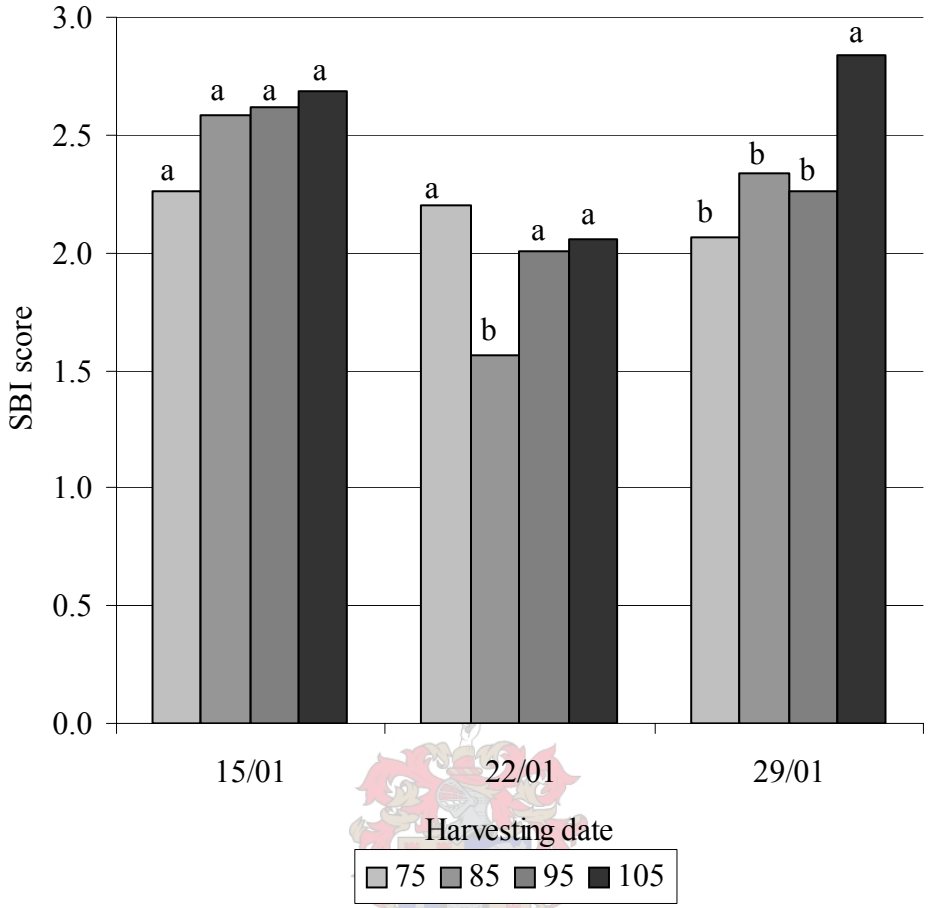

Fig. 2. Effect of fruit maturity (harvesting date) and shaker velocity on friction discolouration (quantified by means of the skin browning index) for 'Doyenne du Comice' during 2003.

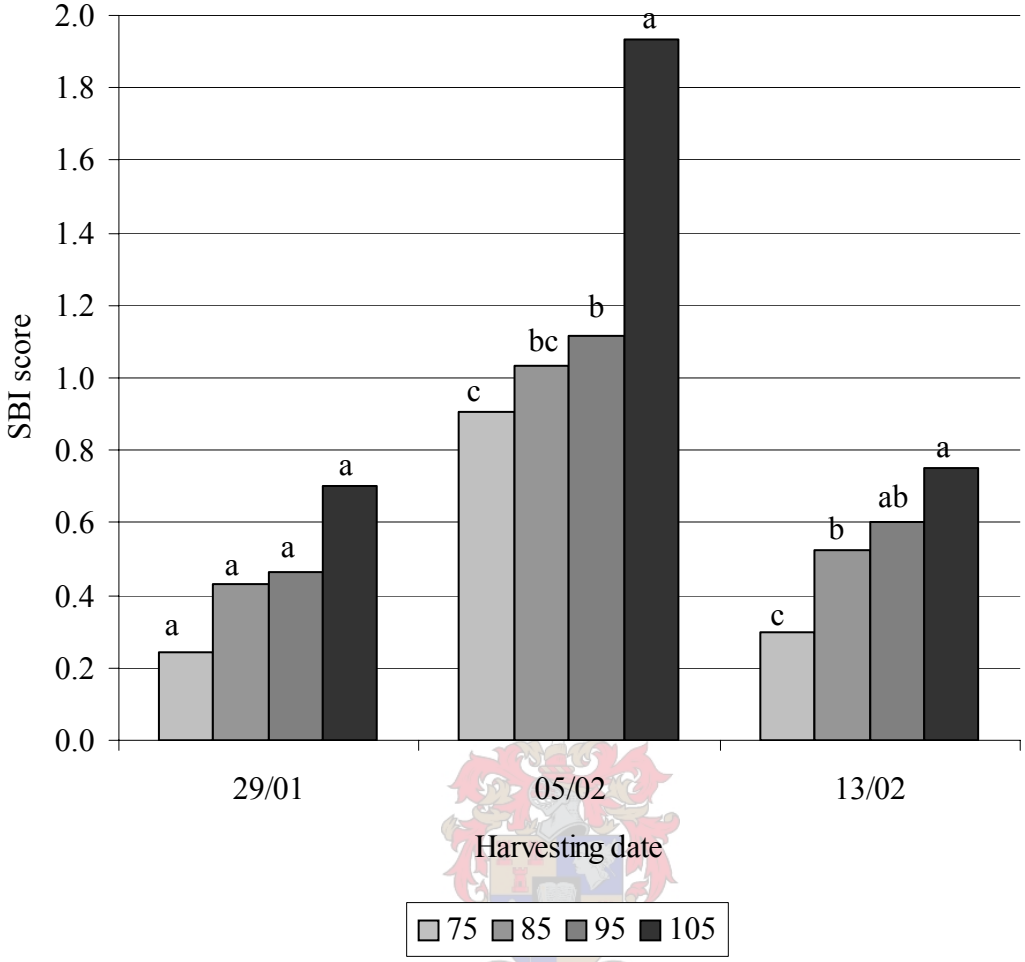

Fig. 3. Effect of fruit maturity (harvesting date) and shaker velocity on friction discolouration (quantified by means of the skin browning index) for 'Packham's Triumph' during 2003.

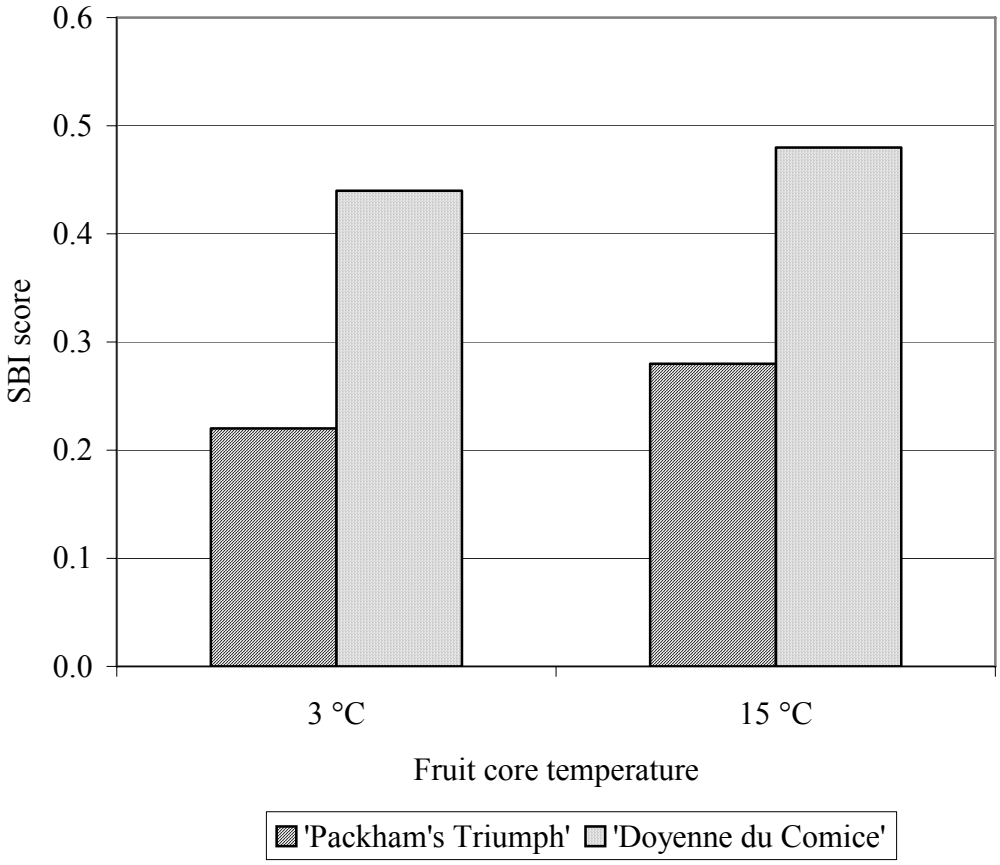

Fig. 4. Effect of fruit core temperature on friction discolouration (quantified by means of the skin browning index) during 2004.

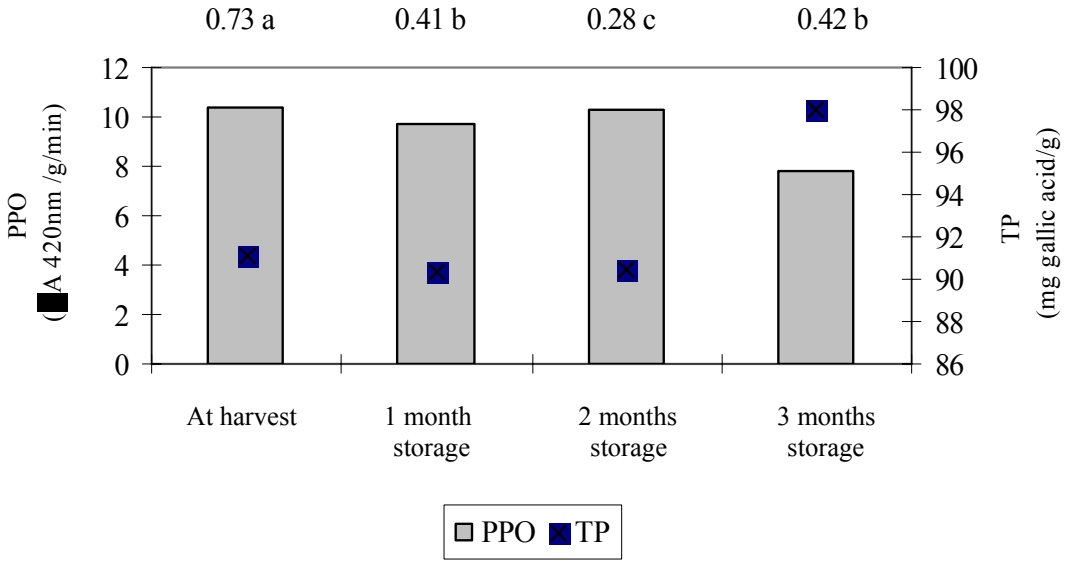

Fig. 5. Average polyphenol oxidase (PPO) activity and total phenolic (TP) content for 'Doyenne du Comice' at each storage duration during 2004. Friction discolouration (quantified by means of skin browning index) on upper x-axis.

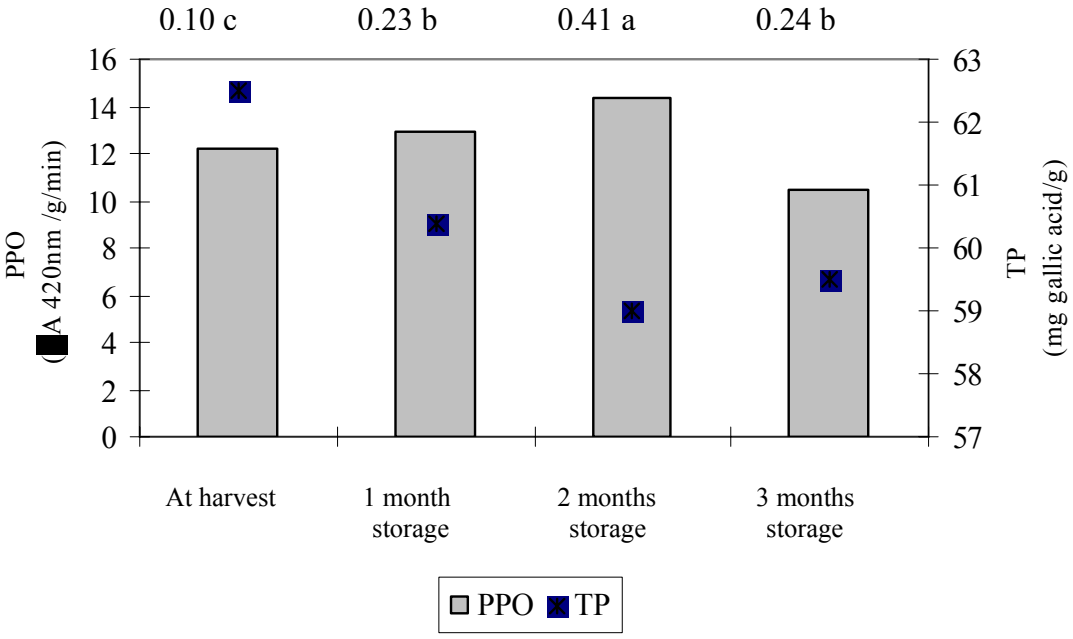

Fig. 6. Average polyphenol oxidase (PPO) activity and total phenolic (TP) content for 'Packham's Triumph' at each storage duration during 2004. Friction discolouration (quantified by means of skin browning index) on upper x-axis.

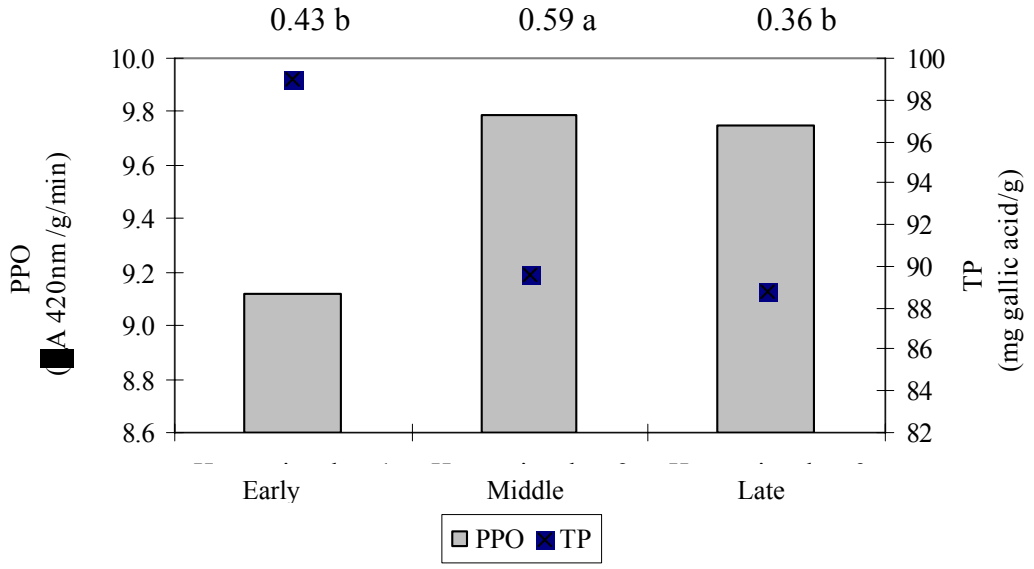

Fig. 7. Average polyphenol oxidase (PPO) activity and total phenolic (TP) content for 'Doyenne du Comice' at each harvesting date during 2004. Friction discolouration (quantified by means of the skin browning index) on upper x-axis.

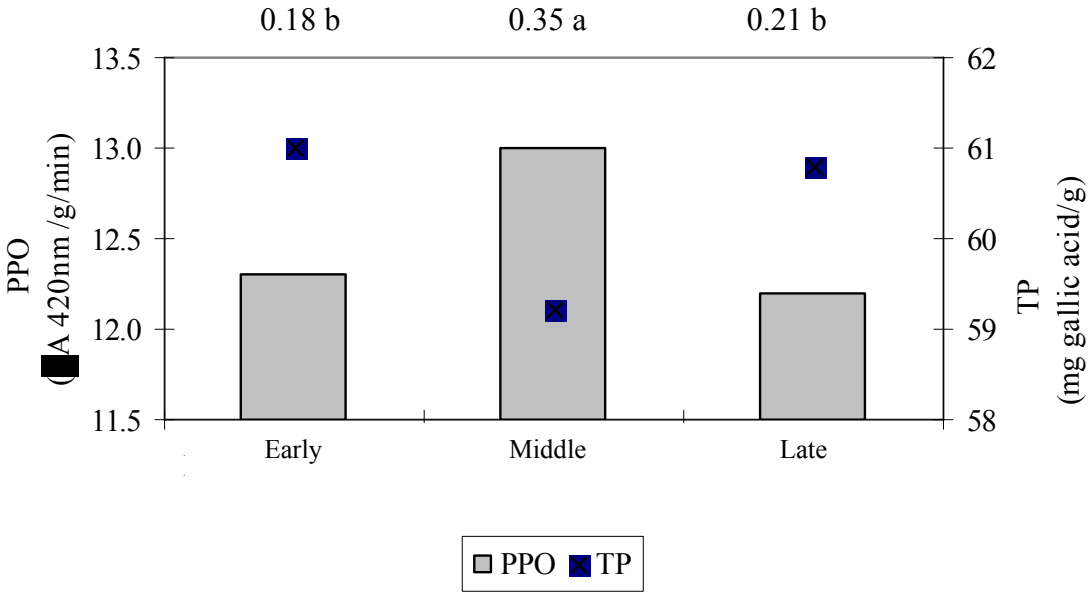

Fig. 8. Average polyphenol oxidase (PPO) activity and total phenolic (TP) content for 'Packham's Triumph' at each harvesting date during 2004. Friction discolouration (quantified by means of the skin browning index) on upper x-axis.

GENERAL DISCUSSION AND CONCLUSION

Identifying the critical stages during the postharvest handling of pears is fundamental in reducing the occurrence and severity of shrivelling and friction discolouration (FD). When dealing with highly susceptible cultivars, and not taking heed of preventative measures, large financial losses can be suffered. Although good general postharvest practices will always result in better fruit quality (Paull, 1999), there are, however, several procedures which can be implemented to encounter fewer losses due to shrivelling or friction discolouration.

The development of shrivelling, especially in the neck of pears, was studied in relation to variation of harvesting date, fruit size and storage duration. Periods during the postharvest handling of pears that proved to be most inducive to shrivelling were where storage temperatures greater than 0 °C were encountered. During these short periods, weight loss rates far exceeded that of the longer storage (0 °C) periods. This accentuates the importance of maintaining the cold chain and preventing unnecessary weight loss from pears. Unlike apples (5 %, Hatfield and Knee 1988), a loss of 2.5% resulted in visual shrivelling in 'Packham's Triumph' pears.

As also found in tomatoes (Shirazi and Cameron, 1993), the contribution of transpiration to total weight loss far exceeded that of respiration. Thus, when faced with the problem of shrivelling, the factors that promote transpiration should be addressed which will thereby lessen the extent of this disorder. The surface area to volume ratio is an important factor to consider as potential transpiration rates, and susceptibility to shrivel, are closely correlated to these fundamental properties. A large ratio, i.e. small fruit, and most often less mature, have a higher transpiration rate and would therefore be more susceptible to shrivel than larger, more mature fruit, as was found on peppers by Lownds et al. (1993). The contribution of the pedicel, as a route for fruit water loss, proved to be significant. By sealing of the fruit stem, as was done on eggplant by Diaz-Perez (1998), a noticeable reduction on the amount of weight loss experience by the fruit was observed.

Reducing the driving force and possible sinks during cold storage must always be strived for. The use of water soaked wooden fruit bins during prolonged cold storage has resulted in a lower incidence of shrivelling among pears (Griessel, pers. comm., 2004). Implementing the

use of plastic fruit bins during cold storage can provide similar advantages and present yet another solution for the reduction of this disorder.

The manifestation of FD on pears was more obvious in 'Doyenne du Comice' than in 'Packham's Triumph' through both years of research. This susceptibility of 'Doyenne du Comice' is supported by results of Amarante et al. (2001). FD, as an enzymatic, oxygen required, biochemical reaction is found to be very common in an array of commodities, including pears. As enzymatic browning is a defensive mechanism again possible infection, the epidermal cells are most prone to discolouration disorders. Unexpected contradictory results were obtained from the two cultivars in terms of storage duration. The general belief is that, as storage duration increases, FD becomes more problematic (Kvåle, 1988). This can be attributed to the availability of adequate substrate (phenolic compounds) and the condition of the epidermal cell membranes.

The influence of fruit core temperature, as a regulatory factor, cannot be ignored. It is well know that enzyme activity is influenced by temperature, and although not statistically proven, all evidence points towards temperature as a possible difference between the trials of 2003 and 2004. Enzymatic properties governed by temperature have also been reported by Amiot et al. (1995) on enzymes extracted from pear peel. At higher temperatures, the epidermal cells are more likely to have lost a greater degree of moisture, rendering their skin rougher and subsequently more susceptible to FD (Amarante et al., 2001).

The availability of phenolic compounds has been closely correlated to the enzymatic activity of PPO (Mellenthin and Wang, 1974). When PPO activity, extracted form epidermal cells, was high, the phenolic content was repeatedly relatively low. Therefore, after epidermal cells have undergone injury, substrate availability must surely be one of the main contributing factors for FD to occur.

It is paramount to implement the necessary pre- and postharvest procedures to minimize the occurrence of both shrivelling and FD. By identifying susceptible cultivars, and treating them accordingly, one can significantly reduce the losses that these disorders bring about. The focus of postharvest handling protocols for pears should be reducing the driving force, for

shrivelling, and preventing any kind of injury that would bring about oxidising conditions, for FD.

LITERATURE CITED

Amarante, C., Banks, N.H., Ganesh, S., 2001. Effects of coating concentration, ripening stage, water status and fruit temperature on pear susceptibility to friction discolouration. Postharv. Biol and Technol. 21, 283-290.

Amiot, M.J., Tacchini, M., Aubert, S.Y., Oleszek, W., 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. J. Agric. Food Chem. 43, 1132-1137.

Diaz-Perez, J.C., 1998. Transpiration rates in eggplant fruit as affected by fruit and calyx size. Postharv. Biol and Technol. 13, 45-49.

Griessel, H., 2004. Personal communication. Tru-Cape, Somerset West, South Africa.

Hatfield, S.G.S., Knee, M., 1988. Effects of water loss on apple in storage. Int. J. Food Sci. Technol. 23, 575-583.

Kvåle, A., 1988. Skin discolouration of four pear cultivars in relation in to maturity, degree of ripening and duration of storage. Norwegian J. Agric. Res. 2, 139-142.

Lownds, N.K., Barnabas, M., Bosland, P.W., 1993. Relationships between postharvest water loss and physical properties of pepper fruit (*Capsicum annum* L.). HortScience. 28, 1182-1184.

Mellenthin, W. H., Wang, C. Y., 1974. Friction discolouration of 'd' Anjou' pears in relation to fruit size, maturity storage and polyphenol oxidase activities. HortScience. 9, 592-593.

Paull, R.E., 1999. Effect of temperature amd relative humidity on fresh commodity quality. Postharv. Biol and Technol. 15, 263-277.

Shirazi, A., Cameron, A.C., 1993. Measuring transpiration rates of tomato and other detached fruit. HortScience. 28, 1035-1038.

APPENDIX A: SELECTED DATA OF ARTICLE I

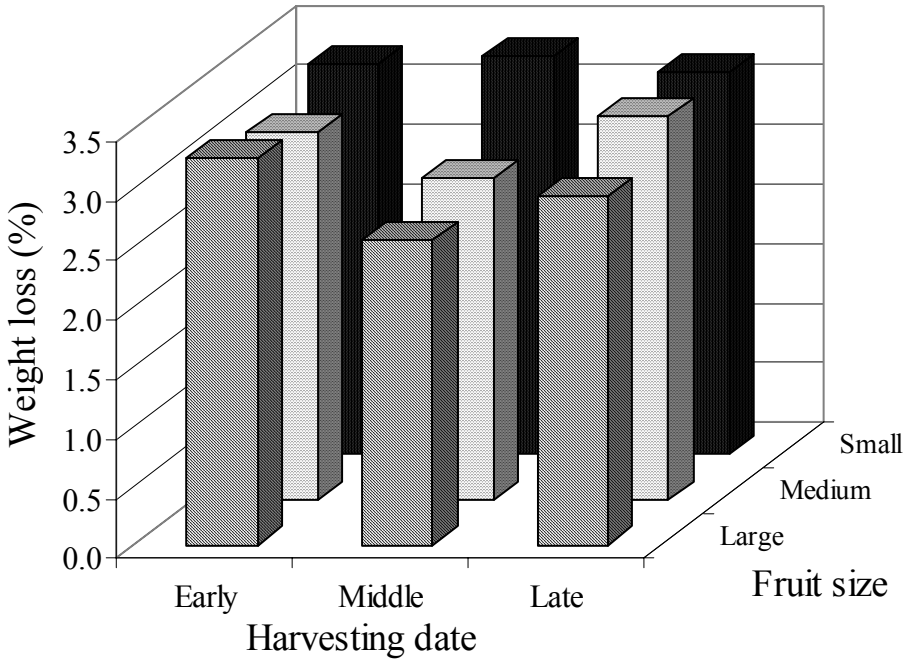

Fig. 1. Significant interaction between harvesting date (Q) and fruit size (L) on weight loss during 2004 postharvest simulation trial of 'Packham's Triumph'.
(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

Table 1a. Significance levels (0.05) for fig. 1.

	Harvesting date		
Fruit size	Early	Middle	Late
Small	a	a	ac
Medium	ad	ef	ab
Large	a	f	bcde

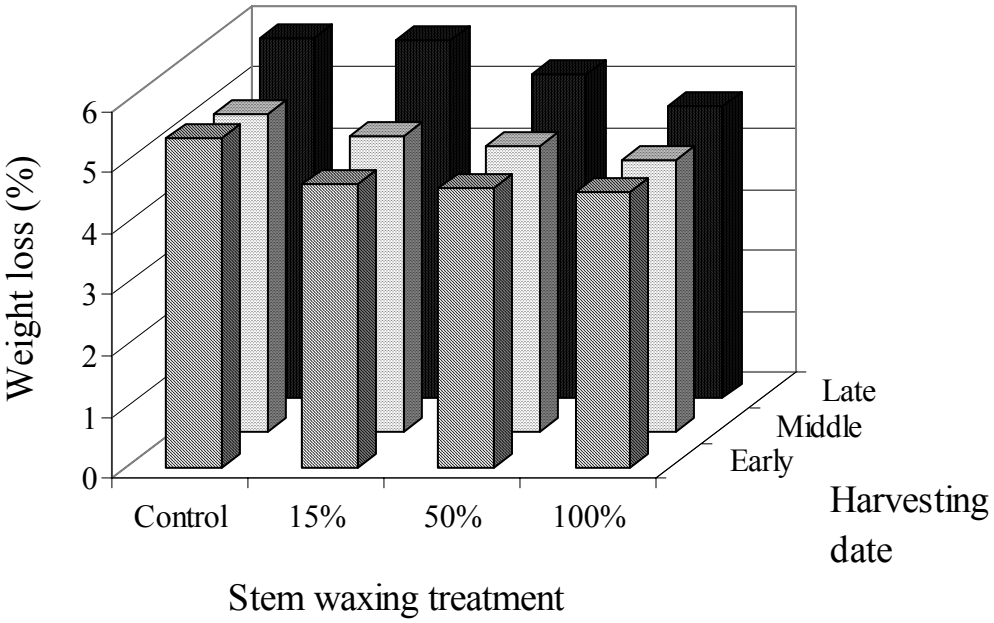

Fig. 2. Significant interaction between stem waxing treatment (Q) and harvesting date (L) on weight loss during 2004 stem waxing treatment trial of 'Beurrè Bosc'.
(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

Table 2a. Significance levels (0.05) for fig.2.

	Stem waxing treatment			
Harvesting date	Control	15%	50%	100%
Late	a	a	b	cdh
Middle	be	c	ce	defg
Early	b	cd	cg	defgh

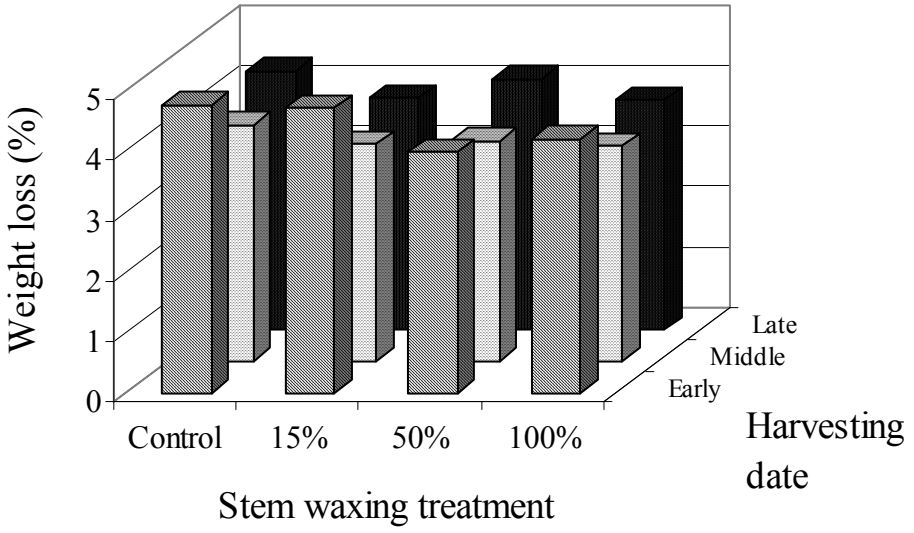

Fig. 3. Significant interaction between stem waxing treatment (Q) and harvesting date (L) on weight loss during 2004 stem waxing treatment trial of 'Forelle'.
(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

Table 3a. Significance levels (0.05) for fig. 3.

	Stem waxing treatment			
Harvesting date	Control	15%	50%	100%
Late	b	deg	bd	eh
Middle	cdef	fgh	fgh	gh
Early	a	a	be	bc

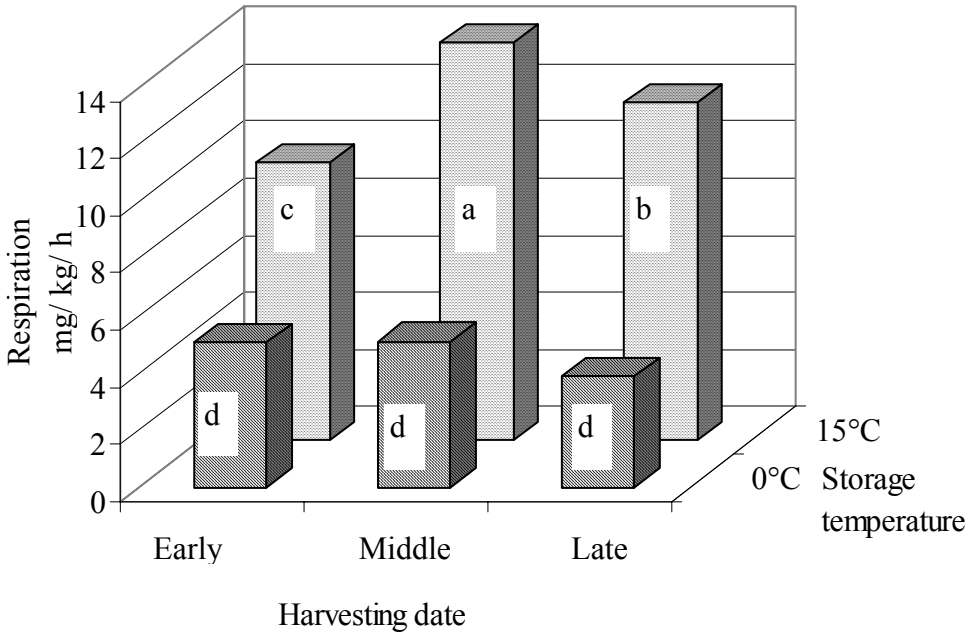

Fig. 4. Significant interaction between harvesting date (Q) and storage temperature on respiration rate during 2004 trial of 'Forelle'.

(Q) Refers to a quadratic association within interaction.

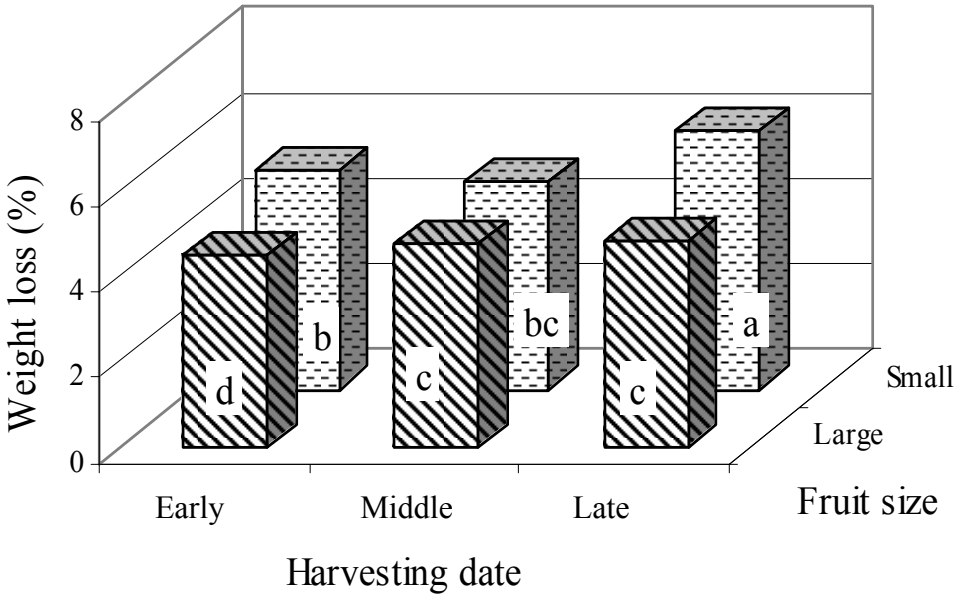

Fig. 5. Significant interaction between harvesting date (Q) and fruit size on weight loss during 2004 stem waxing trial of 'Beurrè Bosc'.
(Q) Refers to a quadratic association within interaction.

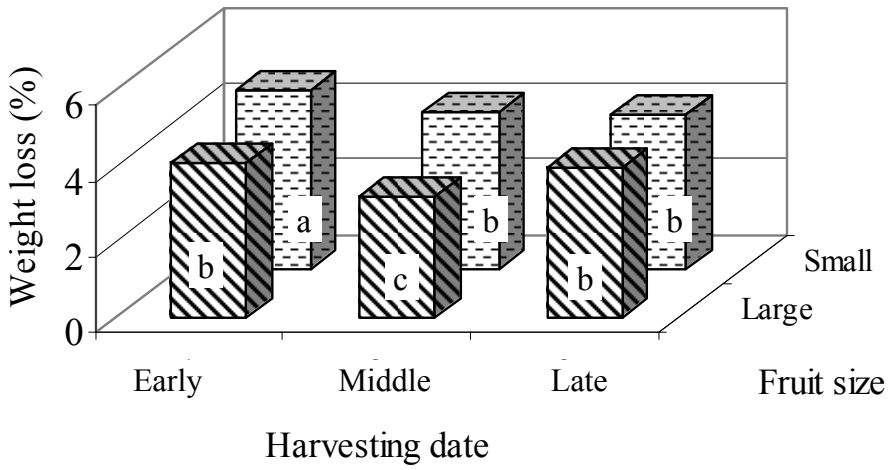

Fig. 6. Significant interaction between harvesting date (Q) and fruit size on weight loss during 2004 stem waxing treatment trial of 'Forelle'.

(Q) Refers to a quadratic association within interaction.

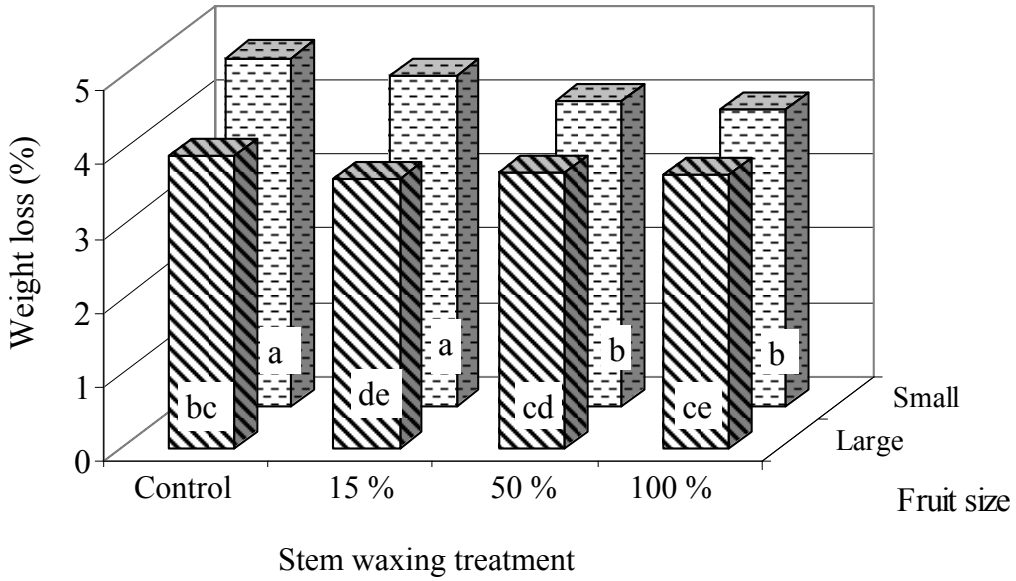

Fig. 7. Significant interaction between stem waxing treatment (L) and fruit size (Q) on weight loss during 2004 stem waxing treatment trial of 'Forelle'.
(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

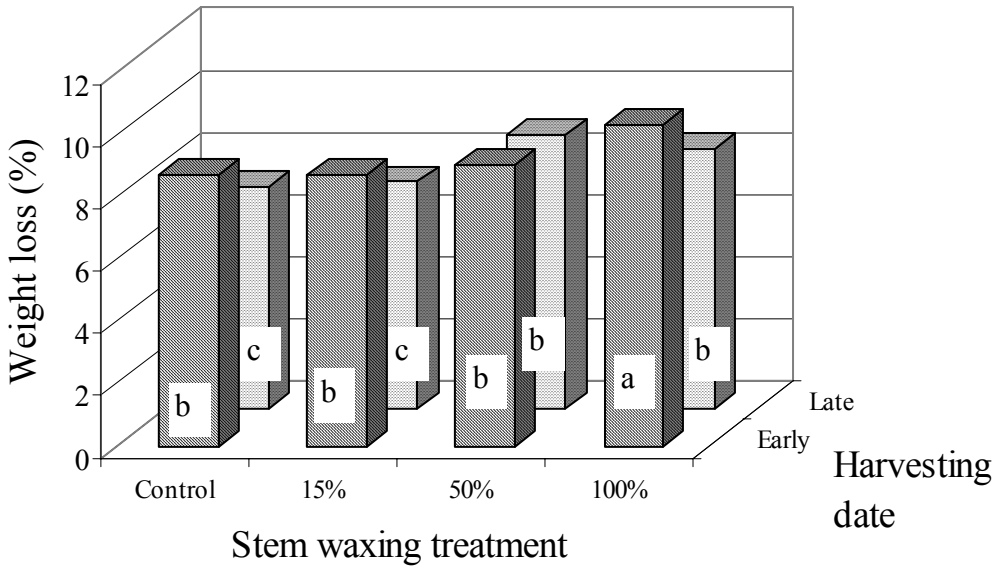

Fig. 8. Significant interaction between stem waxing treatment (L) and harvesting date on weight loss during 2003 stem waxing treatment trial of 'Packham's Triumph'.
(L) Refers to a linear association within interaction.

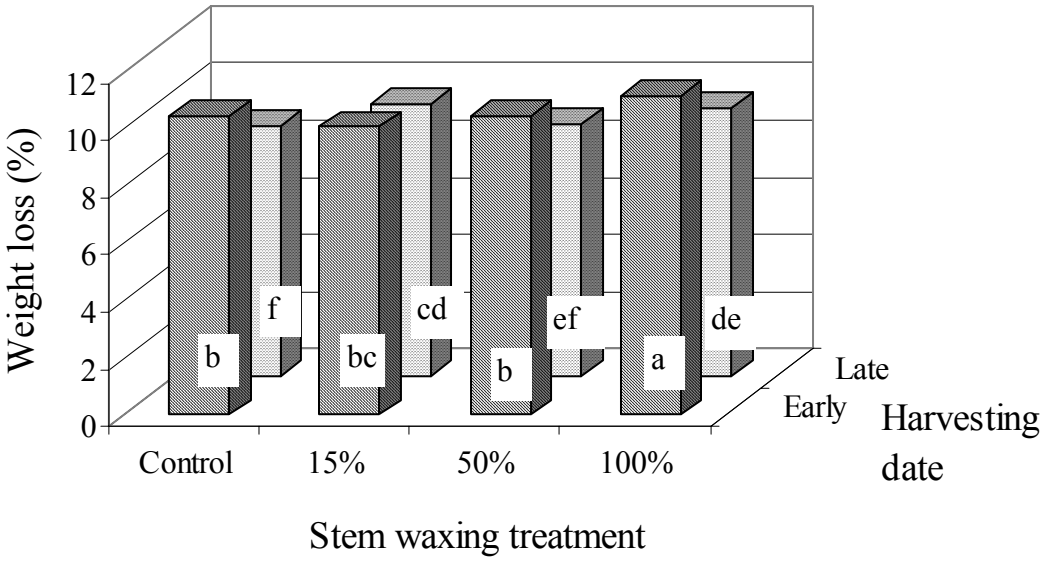

Fig. 9. Significant interaction between stem waxing treatment (L) and harvesting date on weight loss during 2003 stem waxing treatment trial of 'Beurrè Bosc'.

(L) Refers to a linear association within interaction.

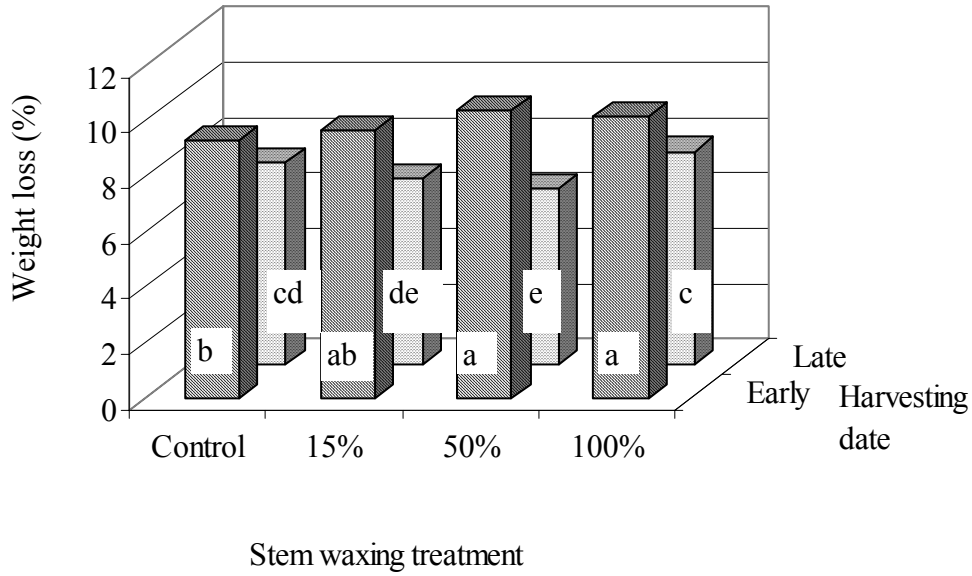

Fig. 10. Significant interaction between stem waxing treatment (L) and harvesting date on weight loss during 2003 stem waxing treatment trial of 'Forelle'.
(L) Refers to a linear association within interaction.

Fig. 11. Different shrivelling response of 'Packham's Triumph' pears to stem waxing treatment; control (left) and 100% (right). Photos were taken of comparable fruit, maturity and size, after identical storage conditions (18 °C) and duration (12 days).

Fig. 12. Different shrivelling response of ‘Beurrè Bosc’ pears to stem waxing treatment; control (left) and 100% (right). Photos were taken of comparable fruit, maturity and size, after identical storage conditions (18 °C) and duration (12 days).

APPENDIX B: SELECTED DATA OF ARTICLE II

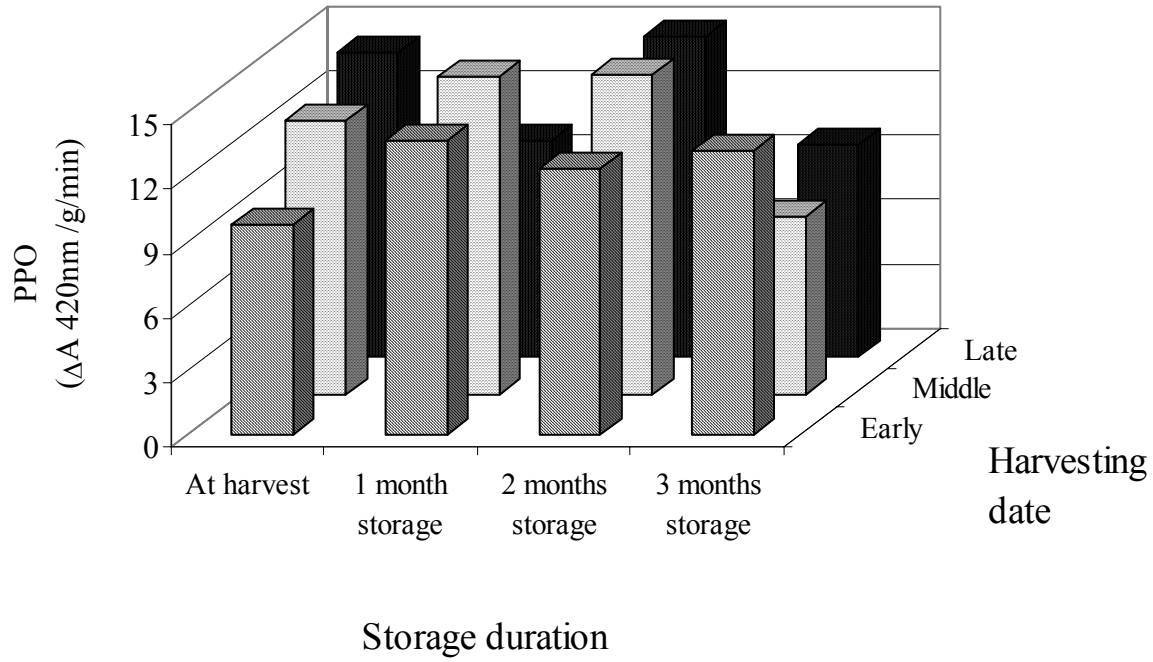

Fig. 1. Significant interaction between storage duration (Q) and harvesting date (Q) on polyphenol oxidase activity of 'Packham's Triumph' during 2004.
(Q) Refers to a quadratic association within interaction.

Table 1a. Significance levels (0.05) for fig. 1.

	Storage duration			
Harvesting date	At harvest	1 month storage	2 months storage	3 months storage
Late	ad	g	ac	g
Middle	bcde	ab	a	fg
Early	g	ae	bcdefg	bcde

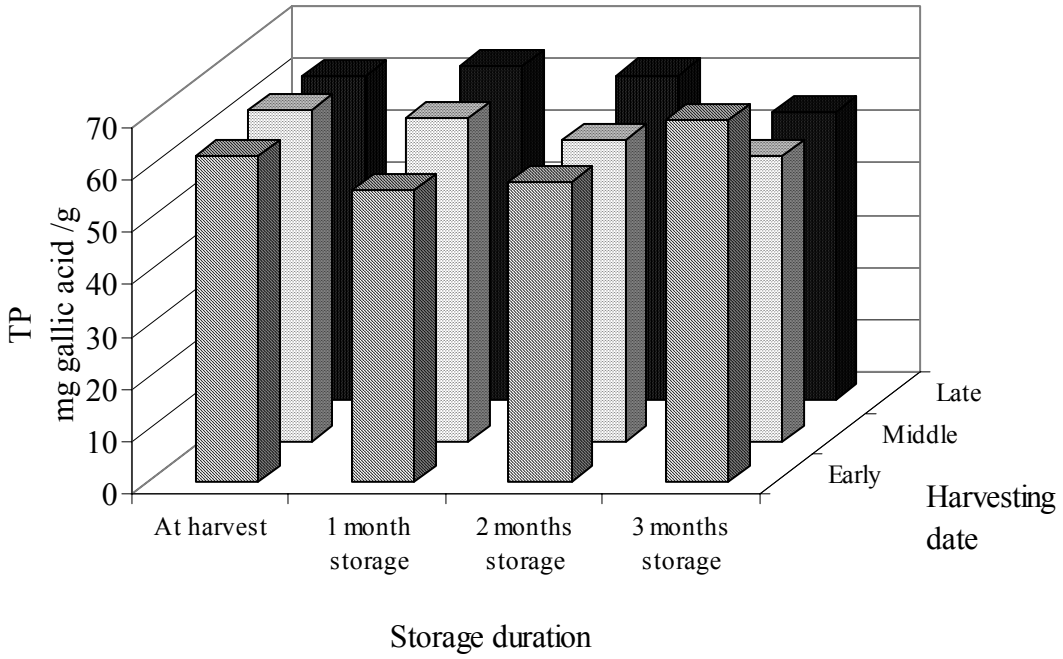

Fig. 2. Significant interaction between storage duration (Q) and harvesting date (L) on total phenolic content of 'Packham's Triumph' during 2004.
(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

Table 2a. Significance levels (0.05) for fig. 2.

	Storage duration			
Harvesting date	At harvest	1 month storage	2 months storage	3 months storage
Late	bcd	ab	bc	fg
Middle	ac	bce	bcf	fg
Early	bc	defg	cg	a

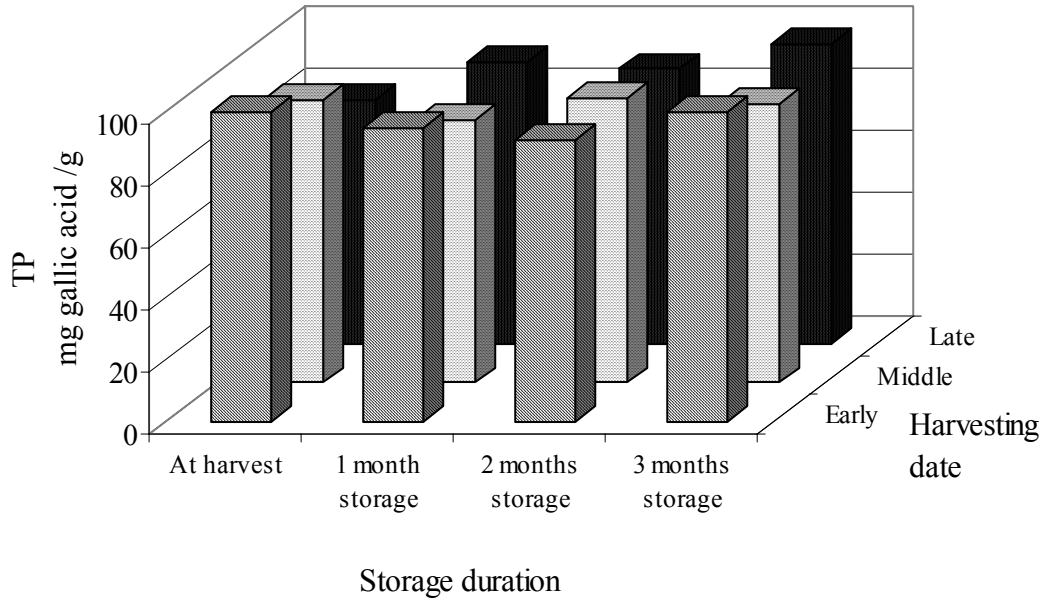

Fig. 3. Significant interaction between storage duration (Q) and harvesting date (Q) on total phenolic content of 'Doyenne du Comice' during 2004.
(Q) Refers to a quadratic association within interaction.

Table 3a. Significance levels (0.05) for fig. 3.

	Storage duration			
Harvesting date	At harvest	1 month storage	2 months storage	3 months storage
Late	j	cg	cj	ac
Middle	cf	defghij	ce	ci
Early	ab	bch	ch	a

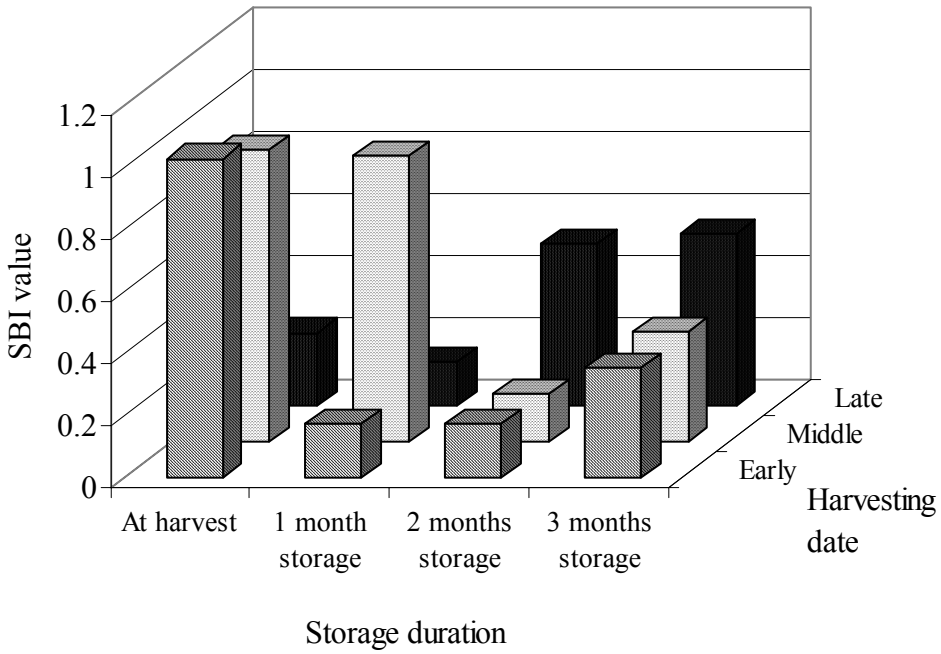

Fig. 4. Significant interaction between storage duration (Q) and harvesting date (Q) on friction discolouration (quantified by means of the skin browning index) of 'Doyenne du Comice' during 2004.

(Q) Refers to a quadratic association within interaction.

Table 4a. Significance levels (0.05) for fig. 4.

	Storage duration			
Harvesting date	At harvest	1 month storage	2 months storage	3 months storage
Late	de	e	bc	b
Middle	a	a	e	d
Early	a	e	e	cd

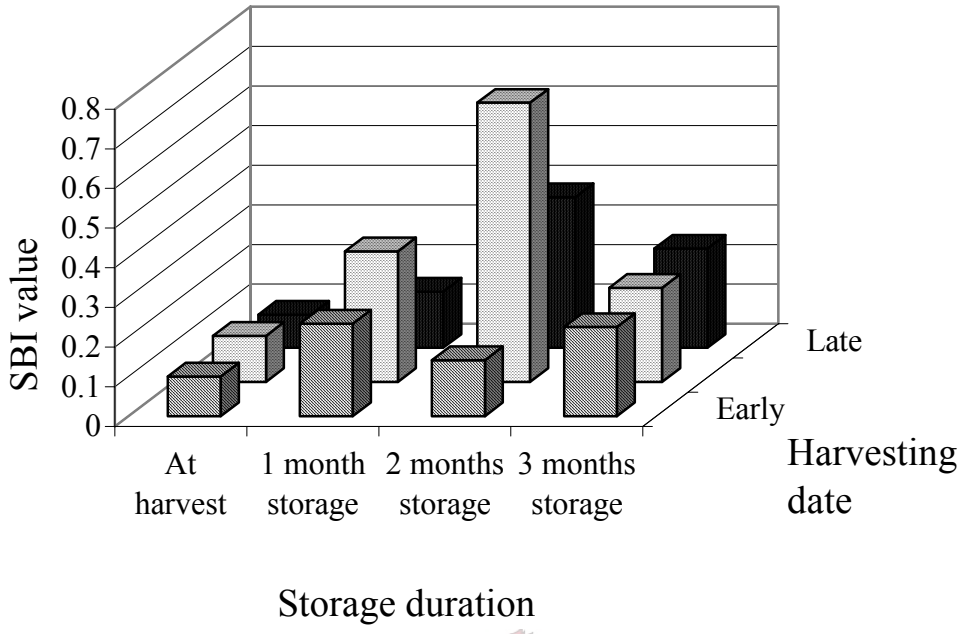

Fig. 5. Significant interaction between storage duration (Q) and harvesting date (Q) on friction discolouration (quantified by means of the skin browning index) of 'Packham's Triumph' during 2004.

(Q) Refers to a quadratic association within interaction.

Table 5a. Significance levels (0.05) for fig. 5.

	Storage duration			
Harvesting date	At harvest	1 month storage	2 months storage	3 months storage
Late	f	def	b	bd
Middle	def	bc	a	be
Early	def	bf	def	cdef

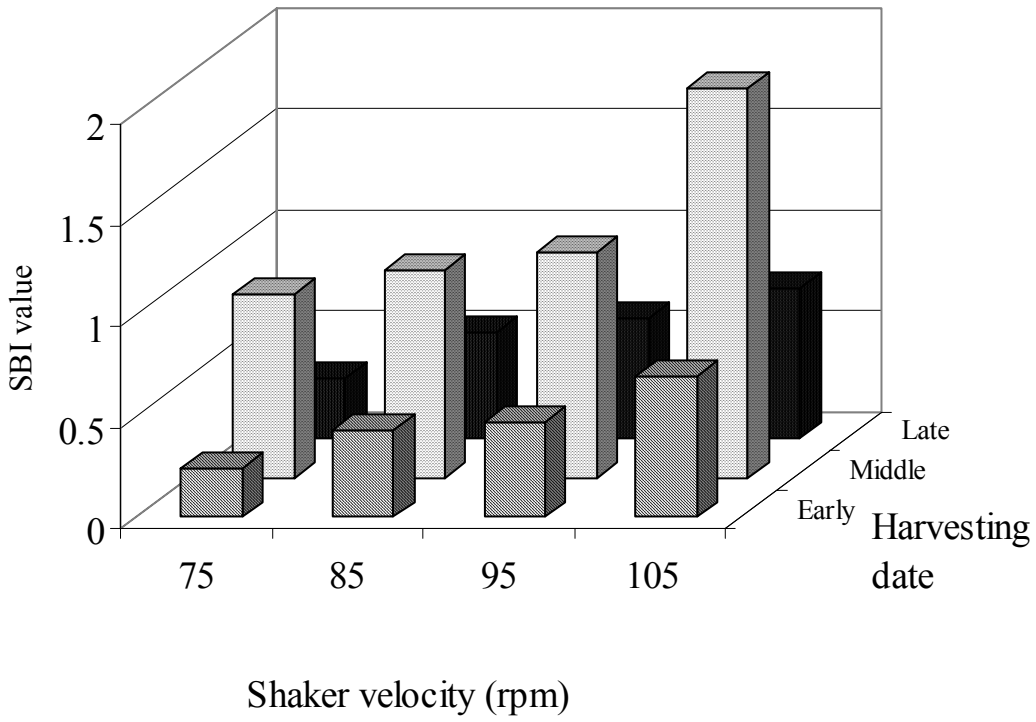

Fig. 6. Significant interaction between shaker velocity (revolutions per minute, Q) and harvesting date (Q) on friction discolouration (quantified by means of the skin browning index) of 'Packham's Triumph' during 2003.
(Q) Refers to a quadratic association within interaction.

Table 6a. Significance levels (0.05) for fig. 6.

	Shaker velocity (rpm)			
Harvesting date	75	85	95	105
Late	ij	fg	ef	d
Middle	c	bc	b	a
Early	j	ghi	fh	de

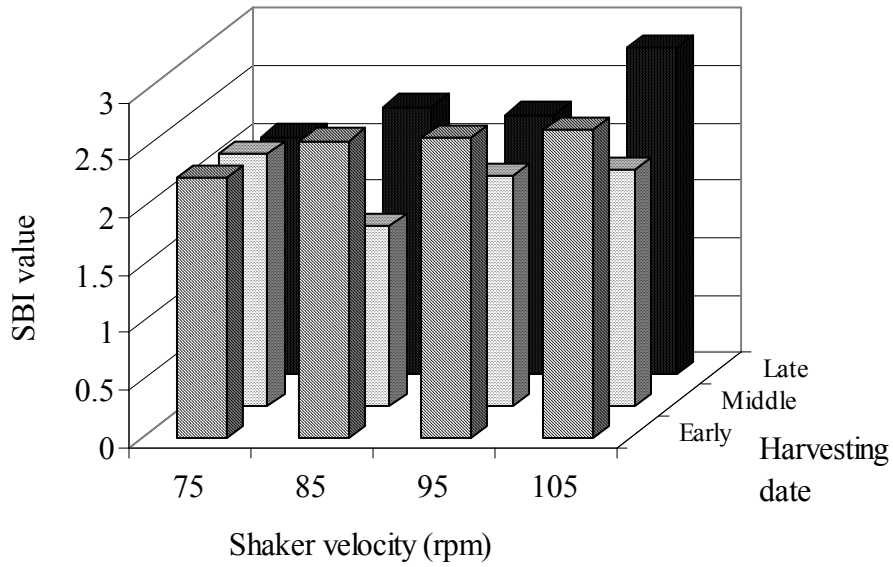

Fig. 7. Significant interaction between shaker velocity (revolutions per minute, Q) and harvesting date (Q) on friction discolouration (quantified by means of the skin browning index) of 'Doyenne du Comice' during 2003.
(Q) Refers to a quadratic association within interaction.

Table 7a. Significance levels (0.05) for fig. 7.

	Shaker velocity (rpm)			
Harvesting date	75	85	95	105
Late	def	bcd	cf	a
Middle	def	g	def	def
Early	ce	ac	ab	a

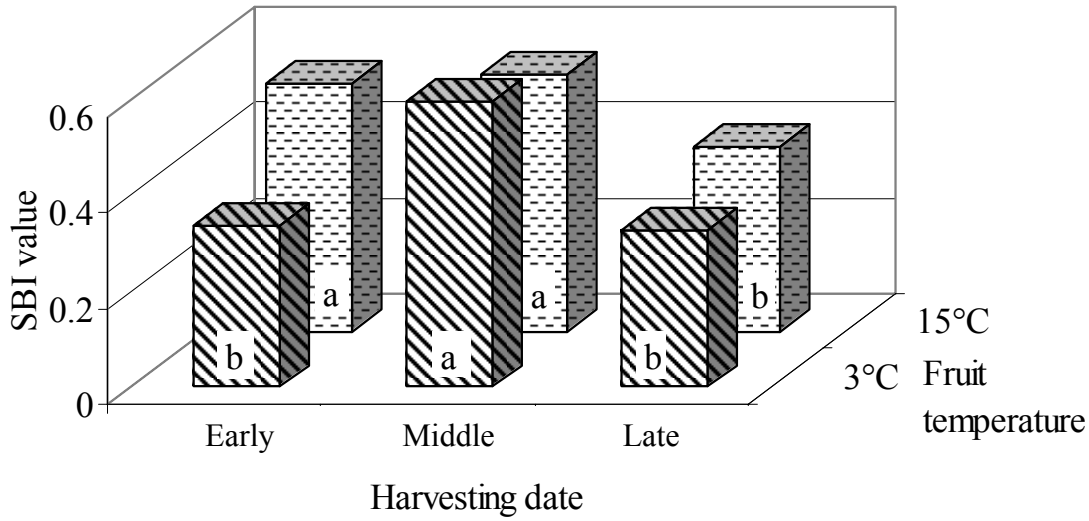

Fig. 8. Significant interaction between harvesting date (Q) and fruit temperature (L) on friction discolouration (quantified by means of the skin browning index) of 'Doyenne du Comice' during 2004.

(Q) and (L) refer to a quadratic and linear association, respectively, within interaction.

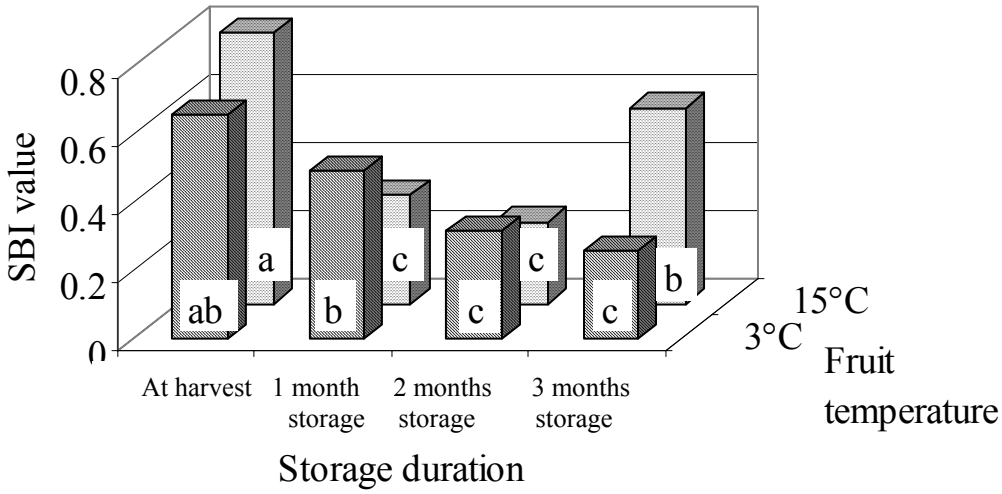

Fig. 9. Significant interaction between storage duration (Q) and fruit temperature on friction discolouration (quantified by means of the skin browning index) of 'Doyenne du Comice' during 2004.

(Q) Refers to a quadratic association within interaction.

Pectora roborant cultus recti