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COATING OF PEARS (VAR. 'PACKHAMS TRIUMPH') WITH KAFIRIN PROTEIN AND ITS EFFECT ON POSTHARVEST PHYSIOLOGY AND SHELF-LIFE

By

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Submitted in partial fulfilment of the requirements for the degree

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DECLARATION

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science & Technology at the University of Pretoria, is my work and has not previously been submitted by me for a degree at any other university or institution of higher education.

Sonya Buchner
November 2006

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ABSTRACT

Coating of pears (var. 'Packham's Triumph') with kafirin protein and its effect on postharvest physiology and shelf-life

By

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In order to reduce postharvest losses of exported Pome fruit and increase export revenue, export quality pears require a reduction in stem-end shrivelling and an extension in shelf-life, regardless of the presence or absence of refrigerated storage. A kafirin coating may fulfil these requirements during export and at the export destination, in retail and at fresh fruit markets. A two-phased approach was followed. During Phase 1, the physiological and biochemical behaviour of 'Packham's Triumph' pears were studied under ideal refrigerated (-0.5°C), temperature-abused (10°C) and typical ripening (20°C) conditions. These storage conditions were selected to simulate potential conditions during the export process. Phase 2 involved the development and application of a kafirin-based coating to increase the shelf-life of pears.

In Phase 1, two experiments were conducted concurrently on freshly harvested, uncoated pears. In Experiment 1, pears were stored at –0.5, 10 and 20°C and 95 to 98% RH for 42, 42 and 21 d respectively. An increase in storage temperature increased the metabolic activity of the pears and the rate of quality deterioration. Very few quality changes occurred in pears during storage at -0.5°C. Pears stored at 20°C ripened and became senescent in approximately half the time taken by pears at 10°C. However, fully ripe 'Packham's' pears from 10 and 20°C exhibited similar final

colour and firmness values. Stem-end shrivelling was exacerbated by storage at 20°C after only 4 days but not observed during storage at -0.5 or 10°C. In Experiment 2, 'Packham's Triumph' pears were stored at -0.5 and 10°C (95 to 98% RH) for 42 and 35 days, respectively before being ripened at 20°C for 7 days. Storage of pears at 10°C prior to ripening accelerated softening and yellowing in the pears, when compared to pears from -0.5°C storage. Storage duration prior to ripening at 20°C also resulted in pears of increasing softness and yellowness by the end of 7 days at 20°C. The effect of storage duration at -0.5°C was less severe on the ripening rate and intensity of softening and yellowing than storage at 10°C. Thus, storage at -0.5°C extended pear shelf-life and resulted in pears of better quality after ripening than storage of pears at 10°C.

In Phase 2, pears from Controlled Atmosphere (CA) storage were coated with a kafirin-based coating and stored at 20°C (35 to 45% RH) for 24 days. The ripening rate and the physiological behaviour and physico-chemical changes of pears used in Phase 2 were probably accelerated by 18 weeks under CA conditions and one week under RA conditions prior to the start of the shelf-life study. The kafirin coating did not retard ripening, which was probably already induced during storage before coating, but senescence in the coated pears was delayed by approximately 6 days. The rate of respiration, ethylene production, flesh softening and especially yellowing, was delayed by the coating. Coated and uncoated pears exhibited no growth of coliforms or lactic acid bacteria. Overall, coated pears had lower levels of aerobic mesophiles and yeast and mould growth than uncoated pears. Unfortunately, pear surface-shrivelling was intensified by the coating, probably due to the dehydrating action of the ethanol in the coating solution during dipping. However, the kafirin coating was able to extend pear shelf-life by delaying senescence and microbiological growth. The coating formulation may require a higher concentration of kafirin to increase its hydrophobicity and reduce pear shrivelling. The kafirin coating has possible potential to markedly extend the quality and shelf-life of 'Packham's Triumph' pears, provided that the pears are coated after minimal RA storage when pears are in the pre-climacteric phase.

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1. INTRODUCTION

The Southern African deciduous fruit industry is worth several billions of Rands in earnings, mainly from export. However, postharvest decay is a major limiting factor in successful fruit marketing and export. Foreign exchange earnings would increase if postharvest decay were reduced. Overseas consumers demand fruit of high quality, free from harmful chemical residues. Therefore new or alternative methods of disease control and maintenance of fruit quality are sought (Anon., 2003).

As one of South Africa's most popular pear varieties, 'Packham's Triumph' is exported in the largest quantities (Anon., 2005b). For this reason it is essential that the quality of the exported pears change as little as possible during export and retailing to ensure that the fruit reaches the European consumer in excellent condition. It is known that pears are harvested unripe but physiologically mature. In South Africa, fruit quality is primarily maintained by refrigerated storage of 'Packham's' pears at –0.5°C and 95% relative humidity (RH). In addition, the gaseous atmosphere around the pears may be altered in the form of controlled atmosphere (CA) storage for up to 6 months prior to export, and/or passive modified atmosphere packaging prior to and during export of the fruit. Such modified packaging is obtained by packaging pears in boxes that are lined with a polyethylene liner to protect pears against moisture loss and ripening by maintaining a fairly constant relative humidity around the fruit and restricting water vapour and gas exchange between the fruit and external atmosphere (Blanpied, 1990). However, polyethylene liners are not considered environmentally friendly and may contribute to packaging waste.

During exporting (shipping) and road transportation, a break in the cold chain may increase pear temperature from -0.5°C to between 2 and 5°C for a few days, but when the cold chain is reinstated, pears are cooled to -0.5°C again. However, fruit and vegetable markets or small retailers at the export destination may not refrigerate pears and hence expose them to conditions (10°C and regular atmosphere) inadequate to maintain pear shelf-life effectively. Such conditions would diminish the saleability of the fruit. 'Packham's Triumph' pears are also prone to skin shrivelling

as a result of moisture loss, especially during extended periods of cold storage (Coetzee, Logistics Manager, Kromco Limited, 2002 -personal communication). Prior to retailing, the pears may be ripened at 18 to 20°C for approximately three days to start the ripening process. (Moll, Marketing manager, Agrilink (Pty) Ltd, 2003 – personal communication). Eating ripeness (Eksteen and Ginsburg, 1977), characterised by the point of physiological ripeness of 'Packham's Triumph' pears, is only reached after seven days of ripening at 20°C (Amarante and Banks, 2002). On the retail shelves pears are often exposed to temperature abuse (10°C and above) because they are displayed without refrigeration, humidity control or modified packaging, which makes them susceptible to quality deterioration.

Edible coatings can act as a partial barrier to moisture and gas (CO_2 and O_2) exchange in fruit, provide physical protection during handling and transport and reduce packaging waste by offering an environmentally friendly alternative (Greener-Donhowe and Fennema, 1994). Carnauba wax coatings, in particular, reduced respiration rate and ripening in 'Packham's Triumph' pears by reducing the oxygen permeability of the fruit skin (Amarante and Banks, 2002).

The sorghum cereal industry in southern Africa is well developed and in excess of 30 000 tons of waste (protein-rich by-products) is produced annually (Taylor, J.R.N, University of Pretoria, 2002 - personal communication). Kafirin is an alcohol-soluble prolamin protein and is found in sorghum grain (Shull, Watterson and Kirleis, 1991).
A study by Buffo, Weller and Gennadios (1997) first suggested the use of sorghum kafirin in edible films and coatings. The protein-rich by-products from the sorghum industry would be an inexpensive resource, readily available for use in the production of edible coatings (Taylor, J.R.N, University of Pretoria, 2002 - personal communication) provided its ability to decrease moisture loss and retard ripening in fruit is satisfactory to fruit exporters.

2. LITERATURE REVIEW

Between harvest and consumption of export-quality pears, postharvest handling practices include sorting, storage and packaging (including coating with an edible coating); transport and export; ripening; and retailing. To fully understand the benefit that an edible coating may provide to fruit quality, the existing postharvest system, the reasoning behind it and its effect on fruit quality must be understood. The latter is important because physiological processes in the fresh fruit continue throughout its postharvest storage life. During each stage of the postharvest system, external factors impact on the physiological processes (i.e. respiration rate, ethylene production and moisture loss) of the pear. Furthermore, physico-chemical changes during ripening are often associated with quality factors such as skin colour, flesh firmness, acidity and sweetness. When an edible coating is included in the postharvest system, the efficiency of the coating may also be affected by postharvest factors and ultimately fruit quality may also be affected by the coating.

To understand the interaction between all the elements in this complex postharvest process the literature review is divided into the following sections:

- Botany and morphology of pears, with particular reference to the 'Packham's Triumph' cultivar
- Postharvest systems used in South Africa for export of 'Packham's Triumph' pears
- Postharvest factors affecting the shelf-life and quality of export fruits.
- Edible coatings used on pears

Where literature was not available for pears, literature on apples was used as they are both climacteric, deciduous fruits (Beattie and Wade, 1996).

2.1 Botany and morphology of pears

Pears are deciduous fruit from the rose family (*Rosaceae*) and fall under the pome fruit subfamily (*Pomoideae*) (Jackson, 2003). Two major commercially cultivated species within the genus *Pyrus* are *Pyrus communis L.* (the European pear) and *P.*

pyrifolia (the Asian pear). The latter is sometimes referred to as "Japanese" or "Oriental" pear, or "Nashi" (Anon., 2005b; University of Georgia, s.a.). It has the taste of a pear, being sweet and juicy, but looks like an apple and has a similar crisp, firm flesh when ripe (Anon., 2005b; University of Georgia, s.a.). Cultivars of the species *Pyrus communis* L. are grown in Europe and most of the "Western" world. These pears have "melting", juicy flesh when ripe (University of Georgia, s.a.). The cultivar 'Packham's Triumph', is a cross between the leading pear cultivar Williams 'Bon Chretien' ('Bartlett') and 'Uvendale's St Germain' (Jackson, 2003). It is harvested in February, and is a medium to large, unevenly shaped, light-green pear with conspicuous dark-green lenticels. The skin colour remains green or green-yellow when ripe (Birch, 1993).

2.1.1 Fruit anatomy

The core of pomes has a fleshy pith and beyond the core line is a cortex of flesh (Jackson, 2003). Figure 2.1 is a lengthwise section of a pear to illustrate the basic features of the pear anatomy. The shape of the pear fruit and the shape and length of its stem are often classified to describe the external character of each pear cultivar (Hedrick, 1925).

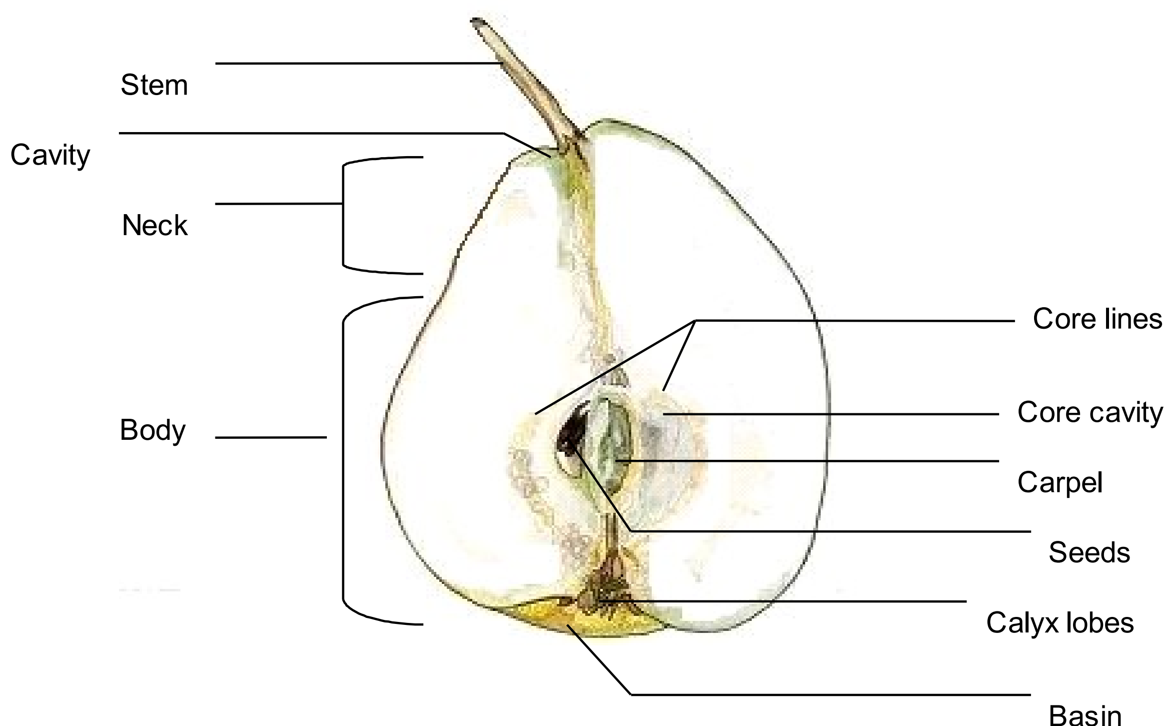


Fig.2.1. Lengthwise section of a pear (Hedrick, 1925)

'Packham's Triumph' pears are described by Jackson (2003) as being "obtuse pyriform". According to Hedrick (1925) the stem of apples and pears are longer in small fruits and shorter in large fruits. The stem is set in a depression at the proximal end of the pear, in a cavity. The skin in the cavity is sometimes russeted (reddish-brown colour) but may also be smooth. The core cavity is the open region of the core. The core consists of carpels and seeds. Carpels are closed cells that originate from leaves that are folded and united. Pome fruit may contain two to five carpels and each carpel contains two seeds (Hedrick, 1925).

The basin is distal to the cavity. It is the depression in which the calyx is set and sometimes is also referred to as the blossom-end. The calyx is a spiral of leaves that act as a protective covering to the flower, which is enfolded prior to fruit development. The lobes of the withered calyx remain on European pears. The neck is the part in which the stem is set and the inflated part that is crowned by the calyx is called the body (Hedrick, 1925).

2.1.2 Peel anatomy

Bell (according to Maguire, Banks and Opara, 2001) found that a generic fruit skin consists of four layers, namely: epidermal hairs (absent in mature fruit), cuticle, epidermis and hypodermis. The cuticle covers the outer epidermis but it does not cover the lenticels (Bell, according to Maguire *et al*., 2001). The cuticle may intrude between epidermal cells and even to the hypodermis (Esau, 1977).

In apples, the cuticle is a thick, bi-layered membrane consisting of two groups of lipid substances (Holloway, according to Maguire *et al*., 2001) namely an outer waxy layer (Fig. 2.2, i) and an inner cutin layer (Fig. 2.2, ii), each with different diffusion properties (Veraverbeke, Verboven, Scheerlinck, Lan Hoang and Nicolaï, 2003). Cutin forms the framework of the inner cuticle and is insoluble in polar solvents. Cutin is a three-dimensional polymer that consists of different long-chain substituted aliphatic acids. The outer part of the cuticle consists of waxes i.e. soluble cuticular lipids (SCL) that are soluble in polar solvents. These lipids can appear on the surface of the cuticle as epicuticular wax deposits to form a thick waxy layer, the most outer part of the cuticle (Holloway, according to Maguire, *et al*., 2001). Such

thick wax layers are typically found on apple, citrus and peach surfaces (Maguire, *et al.*, 2001).

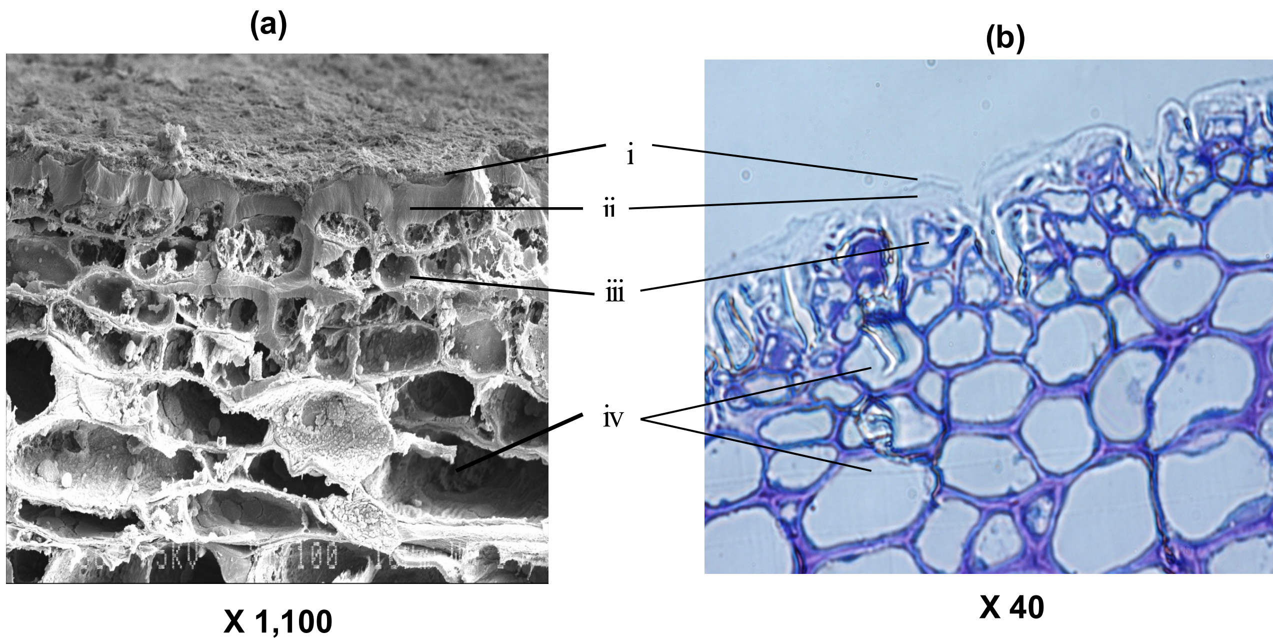


Fig. 2.2. Transverse section of the peel of a 'Packham's Triumph' pear, with underlying tissue as seen under (a) scanning electron microscope (SEM) and (b) light microscopy (40x), illustrating the (i) waxy cuticle layer, (ii) cutin layer of the cuticle, (iii) epidermal cells, (iv) cells of the hypodermis (own pictures)

SCL are also embedded in the cuticle as intracellular wax (Holloway, according to Maguire *et al.*, 2001). Soluble cuticular lipids not only distinctly affect the barrier properties of the cuticle to water but also act as the main barrier to water during transpiration (Maguire *et al.*, 2001). Their role in this regard appears complex. The structure and chemical composition of the SCL determine their resistance to water vapour transfer (Lendzian and Kerstians, according to Maguire *et al.*, 2001). Plant waxes were found to contain three different fractions or phases: a crystalline phase, solid amorphous phase and mobile amorphous phase (Reynhardt and Riederer, according to Maguire *et al.*, 2001). Riederer and Schreiber (according to Maguire *et al.*, 2001) subsequently found that waxy cutin contain impermeable flakes (the crystalline phase) embedded within a permeable matrix (the amorphous phase). Ultimately the impermeable flakes reduce the mobility of diffusing molecules through the amorphous phases (Schreiber, according to Maguire *et al.*, 2001).

Epidermal cells (Fig. 2.2, iii) are generally small, shallow, tubular shaped cells of similar size that form a continuous layer on the fruit surface. The hypodermis (also referred to as sub-epidermal tissue, Fig. 2.2, iv) consists of large, compact, thick-walled, parenchyma cells that have multilayered cell walls. A middle lamella consisting of pectins connect the cells (Esau, 1977). The epidermis and hypodermis give the fruit skin its toughness (Esau, 1977; Glenn and Poovaiah, 1987).

In apples, thin walled, large parenchyma cells with large intracellular spaces form the cortex of flesh (Glenn and Poovaiah, 1987). These parenchyma cells also make up the bulk of the tissue outside the carpels of a pear. Mealiness in ripe apples and pears results from the separation of these parenchyma cells and enlargement of the intracellular spaces (Esau, 1977). In addition, stone cells or sclereids are typical of 'Packham's Triumph' pear flesh (Fig. 2.3) and occur in clusters (Esau, 1977).

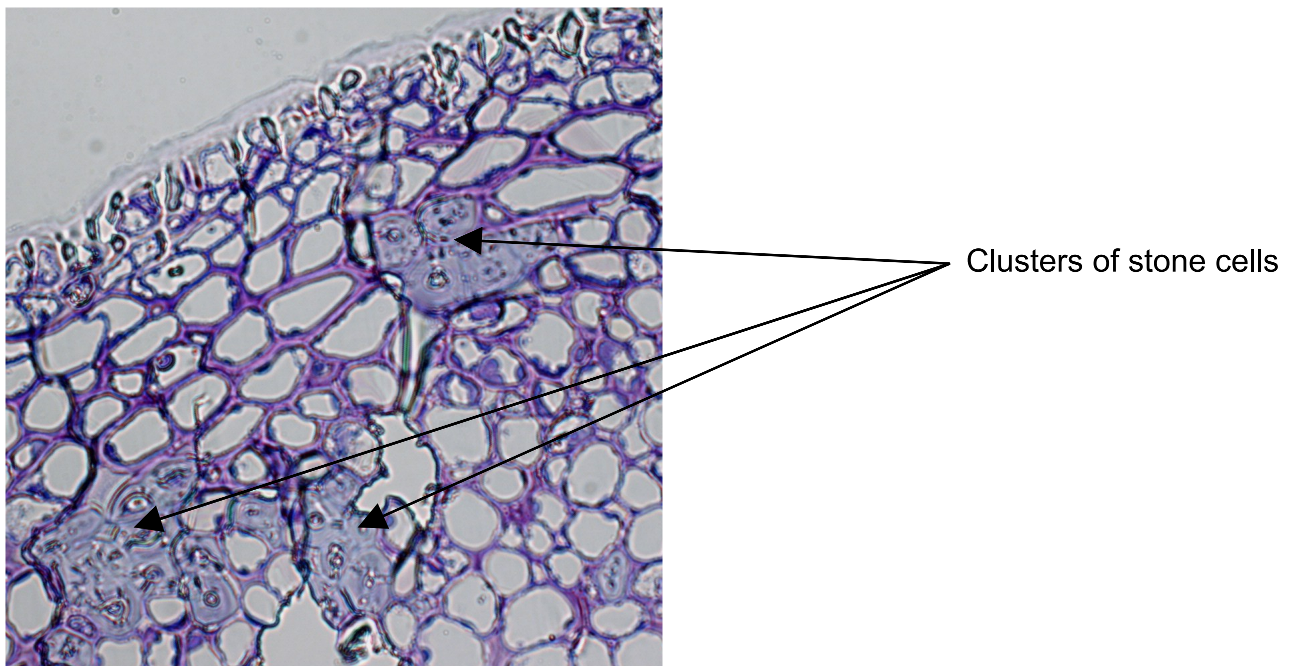


Fig. 2.3. Transverse section of fruit flesh of 'Packham's Triumph' pears under light microscope (x20) showing stone cells or sclereids (own picture)

2.2 Postharvest system to extend the quality of pears

After harvesting, pears are handled (cooled to –0.5°C), stored, sorted and packaged in such a way as to retain maximum fruit quality by retarding physiological processes in the fruit and, especially, the physico-chemical changes associated with ripening. Figure 2.4 illustrates the basic postharvest process, including the storage conditions used in South Africa to retain maximal pear quality prior to retailing. 'Packham's Triumph' pears are climacteric fruit (able to ripen off the tree). To reach the consumer in good quality, the pears are harvested physiologically mature but not eating-ripe (von Mollendorf, 1996), to ensure that they can endure postharvest handling, transport/ export and retailing (Kader and Barrett, 1996). Fruit quality is defined by postharvest researchers, producers and handlers as the degree to which certain attributes of the fruit, i.e. colour, firmness, organic acid and sugar content, are present (Shewfelt, 1999).

Pear maturation occurs preharvest while ripening occurs postharvest. Physiological maturity is the development stage that all fruits must reach before they are harvested and fruit can only mature while attached to the tree (Beattie and Wade, 1996). Pear maturity is determined by measuring flesh firmness, skin colour, seed colour, titratable acidity, total soluble solids and days from full bloom. Weekly tests are conducted from approximately six weeks prior to the theoretical optimum picking date of the specific cultivar (van der Merwe, 1996a). In South Africa, 'Packham's Triumph' pears are harvested form mid-February to early March (Coetzee, Logistics manager, Kromco Limited, 2002 - personal communication).

Ripening refers to the physiological changes that transform an inedible fruit into an edible one. Depending on the ripening behaviour of the fruit, ripening can occur on or off the tree. Fruits that mature into acceptable eating quality whilst attached to the tree exhibit non-climacteric ripening behaviour. Fruit that are able to ripen to acceptable eating quality off the tree exhibit climacteric ripening behaviour (Beattie and Wade, 1996).

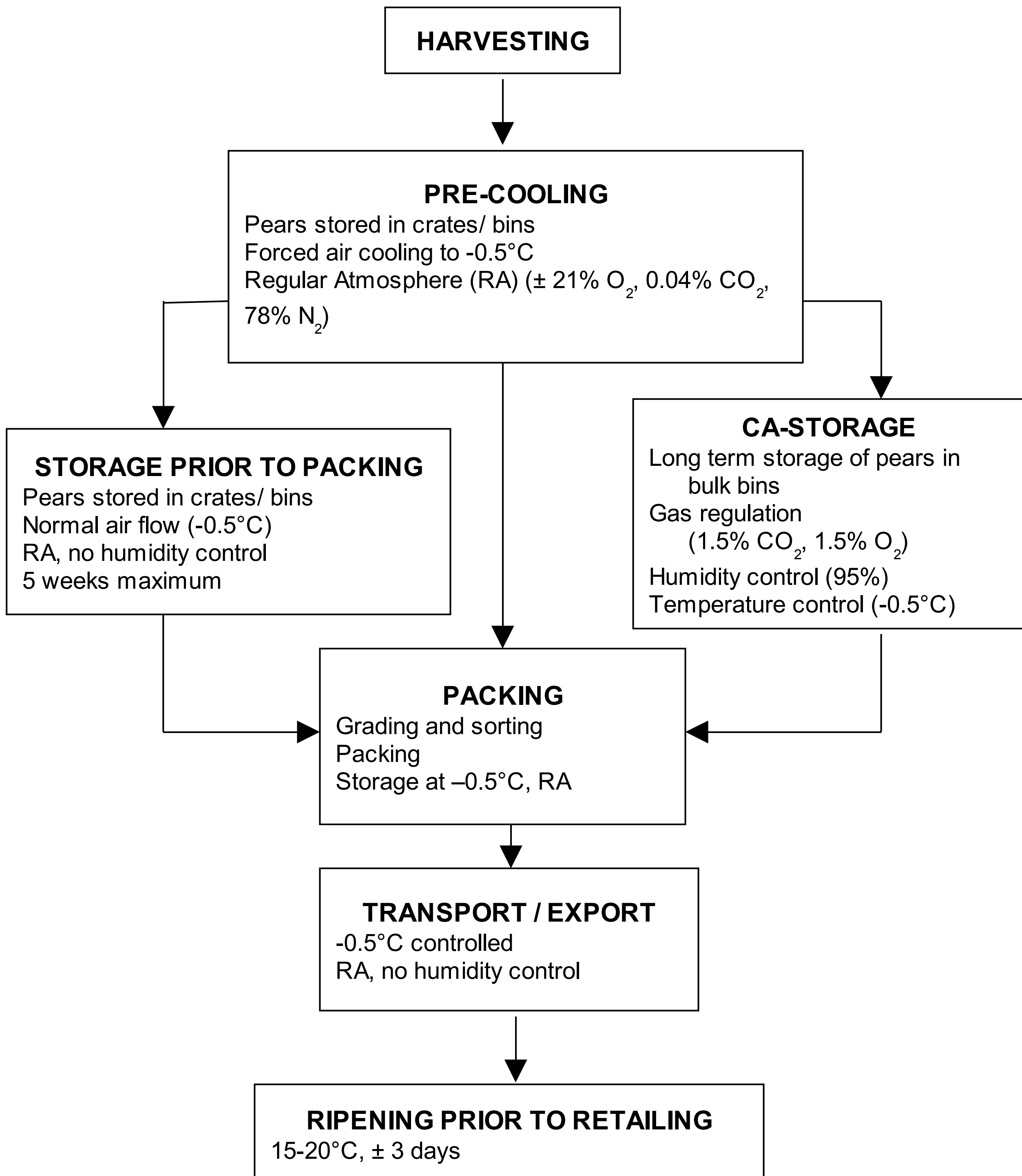


Fig. 2.4. Simplified pear postharvest handling system and the corresponding storage conditions used in South Africa to retain maximal pear quality prior to retailing (Coetzee, Logistics Manager, Kromco Ltd, 2002 and Crouch, Cape Span, 2002 – personal communications)

'Packham's' pears store well for long periods of time (Jackson, 2003). Depending on the demand for pears in retail, pears are either stored in regular atmosphere (RA) for

a few weeks or in CA for several months before they are packed, transported and exported (Fig. 2.4). The postharvest handling procedures (Fig. 2.4) will affect pear quality because the conditions under which pears are stored, packed, transported and exported will influence internal physiological processes and ultimately physico-chemical changes. Improper handling may bruise or injure the fruit, which may lead to browning, accelerated moisture loss, and increased respiration and ethylene production rates, microbiological contamination and ultimately fruit decay (Kader and Barrett, 1996).

During ripening of ‘Conference’ pears at 18°C, optimum physiological ripeness was reached sooner when the storage period, at 1°C under RA conditions, prior to ripening was extended (Henze, 1995). When storage at –1 to 0°C is prolonged (i.e. over-storage) pears may eventually loose the capacity to ripen normally at ambient temperatures (Blanpied, 1990) because pears become over-ripe (Crouch, Manager: Pome Fruit, Experico, October, 2006 - personal communication). In “over-stored” pears a yellow pear colour is sometimes accompanied by flesh softening, particularly in ‘Anjou’ and ‘Conference’ pears (Eksteen and Ginsburg, 1977), but not in ‘Bartlett’ and ‘Bosc’ pears (Blanpied, 1990). A later harvest date contributes to “over-storage” because it shortens the period from harvest to the stage where the pears are considered “over-stored” (Blanpied, 1990).

Once the pears have reached their export destination, ripening may be induced. Pears require temperatures of 15 to 20°C to ripen normally with good quality (Blanpied, 1990). In practice, pears are ripened at 18 to 20°C prior to retailing (Coetzee, Logistics Manager, Kromco Ltd, 2002- personal communication; Crouch, Cape Span, 2002 – personal communication). The fruit will continue to ripen during retailing. Ideally the aim is to have pears on the retail shelves for a short enough period so that it reaches the consumer in good condition with a few days of shelf-life left to ripen into acceptable eating quality. ‘Packham’s’ pears are characteristically large with a pale green skin that changes to “lime – yellow” when ripe (Jackson, 2003).

2.3 Postharvest factors affecting the shelf-life and quality of export pears

It is understood that several important preharvest factors such as harvest maturity, seasonal weather variations, heat waves, rainfall and preharvest sprays can affect the postharvest quality of pears (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication). However, for the purposes of this study the immediate focus is on the postharvest factors. External factors that influence fruit quality by affecting the physiological processes and subsequently the physico-chemical changes in pears are varied (Fig. 2.5).

2.3.1 Postharvest physiological processes that occur in pears

2.3.1.1 Transpiration

Transpiration is defined as the diffusion of water vapour through the cellular tissue of fruits to the atmosphere surrounding the fruit (Maguire *et al.*, 2001). Transpiration rate is dependant on the vapour pressure difference between the internal tissues of the fruit and the surrounding atmosphere. The vapour pressure difference is influenced by temperature and relative humidity (RH) (Kader and Barrett, 1996).

Pathways for transpiration

Prior to harvest, stomata (pores in the fruit skin) help regulate both water loss and carbon dioxide emission during active fruit growth, but water is forced to move through open lenticels or across the cuticle after harvest (Ben-Yehoshua, Burg and Young, 1985; Kerstiens, according to Veraverbeke *et al*., 2003) as stomata largely close at harvest (Ben-Yehoshua *et al.*, 1985) and lenticels act as stomatal replacements. Although transpiration through lenticels accounts for a small percentage of total transpiration in certain apple cultivars, moisture loss through the cuticle was 5 to 10 times more (Pieniazek, according to Maguire *et al.*, 2001). Packham's Triumph' pears also have lenticels (flat, tubular cells) but the cuticle does not cover these structures (Fig. 2.6).

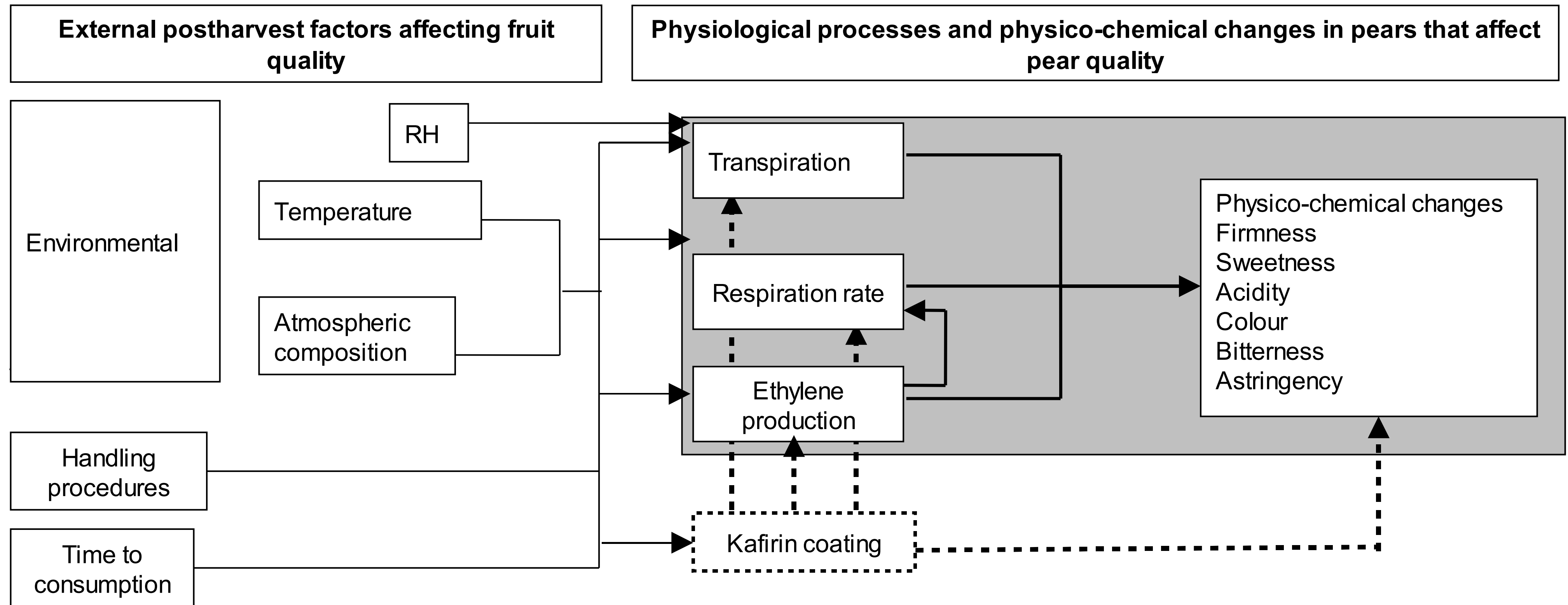


Fig. 2.5. The external postharvest factors that affect the shelf-life and quality of pears

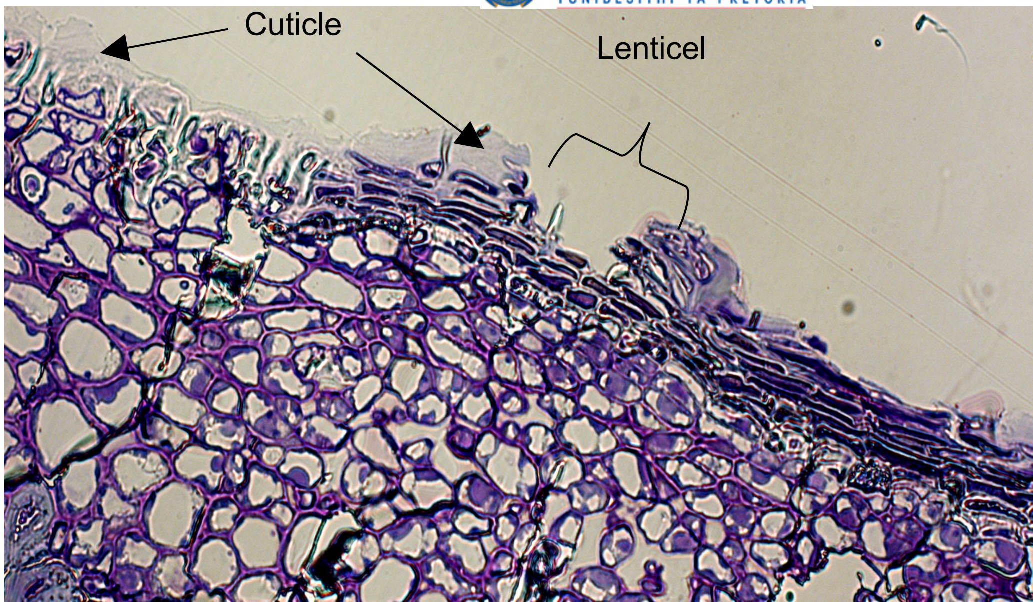


Fig. 2.6. Transverse section of ‘Packham’s Triumph’ pear skin under light microscope (x 20) illustrating the protection provided by the cuticle to the pear surface except over a lenticel (own picture)

The waxy phase of the cuticle has low water permeability (Baldwin, 1994). Consequently, the preferred pathway for water movement is through the liquid aqueous phase of the cuticle, where water conductance is much higher. Oxygen, CO_2 and C_2H_4 gasses are restricted from moving through the cuticle because their diffusivity in liquid water is 10^4 times less than in air (Ben-Yehoshua *et al.,* 1985).

Shrivelling

Postharvest shrivelling is a disorder that results from excessive weight loss in fruit and manifests as wrinkles on the fruit skin (Hatfield and Knee, 1988). According to Maguire *et al.* (2001) weight loss during respiration is partly from carbon (CO_2) but mostly through transpiration (moisture loss). In apples shrivelling can occur when weight loss is as little as 5% (Hatfield and Knee, 1988). Shrivelling in pears starts at the proximal (stem-end) and gradually expand towards the distal (calyx end) during storage under conditions of low humidity (Asakura, Muramato and Tanaka, 2001).

To reduce moisture loss in any fruit, factors that influence the ease with which water vapour can escape from fruit, otherwise known as the water vapour permeance, should be known and dealt with. The fruit skin, and in particular the cuticle, act as barriers to moisture during transpiration (Maguire *et al.*, 2001). Many factors influence water vapour

permeability of the cuticle, which in turn affects weight loss through transpiration and ultimately causes shrivelling.

According to Coetzee (Logistics Manager at Kromco Limited, personal communication, 2002) shrivelling is brought about by a number of factors such as maturity at harvest, environmental factors (temperature, relative humidity) and postharvest handling practices. At present, the most common way to prevent shrivelling is to store the fruit in 37.5µm polyethylene bags, which are placed in the fruit boxes at the time of packing. These bags result in a higher RH around the fruit and result in a lower water vapour deficit (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication).

Effect of maturity

'Picking pears well before or after optimum maturity usually results in faster shrivelling than pears picked at optimum harvest maturity. Pieniazek (according to Maguire *et al*., 2001) found that immature apples had a high permeance, which decreased as fruit approached harvest maturity. The reduced permeance is related to the quantity and structure of the soluble cuticular lipids (SCL) in the cuticle of immature apples (Woods, according to Maguire *et al*., 2001).

Similarly when apples are picked after optimum harvest maturity their water permeance progressively increases (Pieniazek, according to Maguire *et al*., 2001). Consequently fruit kept on trees for longer periods will have increased permeability. Jenks and Ashworth (according to Maguire *et al*., 2001) found that the wax crystals of the SCL in senescing tissues degrade due to weathering by wind, solar radiation and mechanical abrasion, together with the termination of wax production. Therefore fruit not harvested at optimum maturity have a greater chance of shrivelling due to increased permeance of the cuticle to water vapour.

Effect of temperature

Low temperature decreases the vapour pressure within the fruit and transpiration is also decreased by equilibration of fruit temperature and humidity with the environmental temperature and humidity. Long refrigerated storage periods can cause harmful excessive transpiration, even if the RH is high (Beattie and Wade, 1996). Moisture loss and shrivelling may thus occur during long periods in CA storage (6 to 9 months) (Coetzee, Logistics Manager, Kromco Limited, 2002 - personal communication).

During cold storage, micro-cracking of the cuticle is a disorder that increases the permeance of (Maguire *et al*., 2001) and subsequently moisture loss in apples as a result of the cracks being some 12 times more permeable to water vapour than the intact cuticle (Maguire, Lang, Banks, Hall, Hopcroft and Bennett, 1999). Similarly pears grown in the colder climates, like Ceres in South Africa, tend to shrivel earlier in the season (March) than in the warmer climate of Elgin where pears only start shrivelling in May because cold climates stress the pears more and cause water loss (Coetzee, Logistics Manager, Kromco Limited, 2002- personal communication).

Conversely a warm climate may also stress the fruit but this would result in the development of a thicker cuticle, which would reduce moisture loss (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication). However, an increased storage temperature increased fresh weight loss in pears, with the highest weight loss occurring in ‘Conference’ pears (Henze, 1995). Amarante, Banks and Ganesh (2001c) reported that increased temperatures caused the respiration rate of pears to increase, which contributed to moisture loss, and thus fresh weight loss. The moisture loss through respiration depended on the respiration rate of the pear cultivar as summer pears like ‘Bartlett’ have higher respiration rates (regardless of the temperature) than winter pears like ‘Bosc’, ‘Comice’ or Packham’s. However, the permeance of the cuticle of ‘Bosc’ and ‘Bartlett’ were higher, due to a network of cracks in the cuticle, than in ‘Packham’s’. Thus skin permeance combined with the vapour pressure difference caused by the increased temperature contributed much more to moisture loss than respiration.

Similarly the time of day for picking can place the pear under stress and cause shrivelling (Coetzee, Logistics Manager, Kromco Limited, 2002 - personal communication). This may be associated with increased temperatures during picking which may affect the fruit respiration rate as well as the permeance of the cuticle. Schonherr (according to Maguire *et al*., 2001) found that relatively high temperatures increased the moisture permeability of the cuticle and at temperatures above 45°C irreversible structural changes in the cuticle led to an increase in permeability (Maguire *et al*., 2001). Eckl and Gruler (according to Maguire *et al*., 2001) found that high temperatures caused a phase transition and reorientation of SCL which lead to the development of hydrophilic holes in the cuticle.

Effect of relative humidity

Transpiration is influenced by the relative humidity (RH) gradient between the fruit (100% RH in fruit intracellular spaces) and the surrounding atmosphere. Transpiration is decreased when the humidity of the surrounding atmosphere approaches the internal humidity of the fruit. Therefore storage of pears at high relative humidity (> 90 %) is vital to reduce transpiration (Baldwin, 1994; Fourie, 1996). Japanese pears stored in high RH (100%) appeared fresh, even after two months of storage at 2, 5 and 10°C respectively, while moisture loss at low RH (80, 78 and 72% RH) resulted in decreased juiciness and increased soluble solids content, regardless of the cold storage temperature. Moisture loss in the pear flesh concentrated the soluble solids. Pears stored at 2°C and 75, 79 and 90% RH exhibited shrivelling due to excessive moisture loss, while pears at 98 and 99% RH showed no shrivelling, after three months of cold storage (Asakura *et al.*, 2001). This behaviour is explained by the permeance of the hydrophobic cuticle, which increases as the RH of the air increases (Maguire *et al*., 2001). Lendzian and Kerstiens suggested that permeance of the hydrophobic cuticle increases because water coated pathways were created in the cuticle under high RH conditions as a result of the attachment of water molecules to polar groups within the cuticle (according to Maguire *et al*., 2001). Temperature and RH effects have already been discussed earlier. 'Packham's Triumph' pears are particularly prone to shrivelling and RH is generally maintained at 95% RH during CA storage (Crouch, Cape Span, 2002 - personal communication) to reduce moisture loss.

Postharvest handling practices

When the fruit is not handled with care after harvest bruising, cuts and stem-end punctures damage the fruit surface and may even expose the cellular material. The cellulose walls of the epidermis and hypodermis are highly permeable to moisture migration (Burton, according to Maguire *et al*., 2001), which would increase moisture loss and subsequently shrivelling (Maguire *et al*., 2001). Bruises and cuts may also put fruit under stress and according to Romani (1984) the fruit may respond with increased ethylene production and respiration rates, which in turn increases the rate of ripening and senescence. Fluid, leaking from the bruised tissue, and fruit flesh exposed by wounds or punctures are ideal areas for mould growth and other spoilage bacteria. The microbiological spoilage ultimately leads to fruit decay (Heard, according to Martin-Belloso and Soliva-Fortuny, 2006), which renders the pears unfit for sale and consumption.

2.3.1.2 Respiration

Aerobic respiration

Pears respire to maintain cytoplasmic biochemical processes and membrane integrity of cells and tissues (Maguire *et al*., 2001). On the tree the fruit is provided with carbohydrates and other organic compounds from leaves for active synthesis of cellular materials. After harvest, stored reserves become the only source of energy for maintaining cellular functions. During aerobic respiration energy is obtained by the oxidation of reserves (typically carbohydrates, fats, proteins or organic acids) in the presence of oxygen (O_2), which results in the formation of by-products such as carbon dioxide (CO_2) and water (Eksteen and Ginsburg, 1977). During aerobic respiration the rate of carbon loss is directly proportional to the respiration rate and hence the production of CO_2 (Maguire *et al*., 2001).

The energy derived from respiration drives the metabolic functions and physico-chemical reactions such as changes in skin colour and fruit flesh firmness (Eksteen and Ginsburg, 1977). The respiration pattern of climacteric fruits is a good indicator of their metabolic activity and physiological age (Eksteen and Ginsburg, 1977) because the occurrence of ripening and senescence manifests as sudden changes in the respiratory behaviour (Saltveit, s.a.). This makes the measurement of respiration rate a useful guide in predicting the potential storage life of produce (Wills, McGlasson, Graham and Joyce, 1998).

At physiological maturity of climacteric fruit the respiration rate is at a minimum, referred to as the pre-climacteric minimum (Eskteen and Ginsburg, 1977). At the commencement of ripening, a climacteric fruit is characterised by a dramatic increase in ethylene production followed by a sharp increase in the rate of respiration (von Mollendorf, 1996). This respiratory increase is known as the climacteric and it coincides with physiological and biochemical changes in the fruit that occur simultaneously but which are not necessarily co-dependent (Von Mollendorff, 1996). The respiration rate increases until a climacteric maximum (considered to be the point of eating ripeness) is reached after which senescence sets in, and is characterised by a rapid decline in fruit quality (Eskteen and Ginsburg, 1977). ‘Packham’s Triumph’ pears generally reach the climacteric maximum after seven days of ripening (Amarante, Banks and Ganesh, 2001a; Amarante and Banks, 2002).

Effect of temperature on respiration

Findlay and Combrink (1996) reported general respiration rates ranging between 30 to70, 8 to12, and 3 to 7 mg CO_2 kg^{-1}. h^{-1} for pears stored at 20, 10 and 0°C, respectively. Fruit stored at 0°C have a respiration rate six to ten times slower than that of fruit stored at ambient temperatures (20 to 26°C) (van der Merwe, 1996a). The optimum storage temperature for most pears cultivars is –0.5°C (Findlay and Combrink, 1996; van der Merwe, 1996a; Anon, 2005a). Respiration rate is at its slowest close to the freezing temperature of pears (–1 to –2°C). The exact freezing point of the pear depends on the soluble solids content of the fruit (Findlay and Combrink, 1996). It is well known that when the sugar or salt content of a food product is increased, the freezing point of the product is lowered. For the same reason the freezing point of a fruit with higher soluble solids content and thus a higher sugar content, will be lower. The optimum refrigerated storage temperature for 'Packham's Triumph' pears is –0.5°C to produce the slowest respiration rate without freezing the pears (Anon., 2005a).

Anaerobic respiration

If O_2 is limiting, which can occur under certain packaging, coating and storage conditions, the tissue may undergo anaerobic respiration. Glucose is converted to pyruvate, which is metabolised to either lactic acid or acetaldehyde, and the latter is converted to alcohol and CO_2 during fermentation (Wills, Lee, Graham, McGlasson and Hall, 1981). To compensate for the low energy production during anaerobic respiration, the flow of substrate through the initial steps of the cycle is increased which ultimately results in an increased formation of CO_2 (Saltveit, s.a.).

Sites for gas exchange in the fruit

Oxygen (O_2), carbon dioxide (CO_2) and ethylene (C_2H_4) gas diffuse through pores in the fruit skin (stomata) even when pores are partially closed, as often occurs after harvest (Ben-Yehoshua, Burg and Young, 1985). Gas exchange may also occur through the lenticels, which arise in some fruit after the stomata stop functioning (Kolattukudy, 1980).

2.3.1.3 Ethylene

Synthesis

Yang and Hoffman (according to Macrae, Robinson and Sadler, 1993), found that ethylene (C_2H_4) is one of the many volatile substances produced by fruit during ripening, and has

been identified as an endogenous ripening hormone. Ethylene production is the first indication that fruit has reached physiological maturity (the climacteric minimum) as the ethylene concentration increases just before the climacteric (the onset of ripening). The minimum C_2H_4 production rate required to trigger a rise in respiration rate (the climacteric) is less than 1 μL kg^{-1}. h^{-1} (Macrae *et al*., 1993).

Ethylene is synthesised from methionine (an essential amino acid) via the intermediates *S*-adenosyl methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang and Hoffman, according to Knee, 1990). Methionine is first converted to SAM, which in turn is fragmented to ACC and Methylthioadenosine (MTA) (Wang, 1990) by the ACC-synthase enzyme. ACC synthesis is possible in the absence of O_2 but ACC fragmentation to C_2H_4 has a strict O_2 requirement (Knee, 1990). Therefore it is said that low O_2 levels are more effective in delaying C_2H_4 production than high CO_2 levels, while the latter is effective in inhibiting C_2H_4 action (Kader, 1989).

Action during fruit ripening

In the pre-climacteric phase, fruit tissue is highly resistant to the action of C_2H_4. Prior to the climacteric minimum, an inhibitor may prevent C_2H_4 production, or a complimentary factor for C_2H_4 may have to develop within the plant before C_2H_4 has the desired effect on the fruit. As the fruit matures, resistance to C_2H_4 decreases. It is speculated that this process is controlled by endogenous C_2H_4 as fruit has to reach a certain level of sensitivity to C_2H_4 before it reacts. Therefore it is said, that the important factor in fruit development is not a change in the concentration of C_2H_4, but in the sensitivity of the tissue to it (Von Mollendorf, 1996). The dramatic increase in C_2H_4 production at the commencement of ripening is the result of an exponential increase in C_2H_4 concentration in reaction to a small amount of endogenous C_2H_4 (von Mollendorf, 1996).

The mechanisms involved in fruit ripening are complicated. According to a review by Alexander and Grierson (2002), C_2H_4 controls the expression of genes for the synthesis of certain enzymes related to ripening (like poly galacturonase and β-galactosidase responsible for flesh softening during ripening). Ripening starts in one area of the climacteric fruit and spreads to adjacent areas as C_2H_4 diffuses between cells, thereby integrating ripening throughout the whole fruit. Gene regulation is, in fact, classified as ethylene- dependant or independent and certain ripening-related genes (such as those for softening) are more sensitive to low C_2H_4 levels than others. In fact, Lelièvre *et al.*

(according to Agar, Biasi and Mitcham, 2000a) proposed that fruit softening was very sensitive to low levels of C_2H_4 and more so than colour change.

Although this practice is not followed in South Africa (Crouch , Manager: Pome Fruit, Experico, 2006 - personal communication), pears can be treated with ethylene after harvest as a substitute for ethylene development during cold storage, to ensure that the pears ripen uniformly and promptly when ripened for sale and consumption (Mitcham and Thompson, 1998). The external C_2H_4 initiates endogenous C_2H_4 production and induces ripening in the freshly harvested, early-season pears that will not ripen normally after harvest upon transfer to 20°C (Agar *et al.*, 1999, according to Agar *et al.*, 2000a). External C_2H_4 treatment of pears after export is used for the same reason in order to facilitate the export of green, firm fruit without risking bruising of half-ripened fruit during export (Agar *et al.*, 2000a).

Effect of temperature and RH

Cold treatment is known to promote the synthesis of C_2H_4 in pears (Murayama, Satoh, Ohta and Fukushima, 1995). In fact cold treatment is needed for normal ripening of winter pears such as 'Packham's Truimph' (Richardson and Gerasopoulos, 1993; Maage and Richardson, 1998). For pears to develop into an edible product, independent of the tree, C_2H_4 synthesis is needed to trigger ripening (Beattie and Wade, 1996; von Mollendorf, 1996). At physiological maturity, winter pears are not yet capable of sufficient auto-catalytic (stimulating its own) C_2H_4 synthesis to sustain ripening. Each cultivar has a specific time requirement at 0°C storage to stimulate the production of enough ethylene (Maxie and Ginsburg, 1974; Richardson and Gerasopoulos, 1993) to allow normal ripening of pears to commence immediately after removal from cold storage (Maxie and Ginsburg, 1974). In South Africa only 'Forelle' undergoes a mandatory cold storage period of eight weeks prior to export. For other cultivars like 'Packham's' the cold storage duration to which pears are exposed during transport and export (during shipping) is reported to be sufficient to stimulate C_2H_4 production (Crouch, Manager: Pome Fruit, Experico, 2005 - personal communication).

During short term storage (4 weeks) of 'Williams Bon Chretien' pears at -1.1°C, the pears ripened faster (4 days instead of 5 days) as a result of the cold stimulation of C_2H_4. The fruit possessed sufficient amounts of C_2H_4 to ripen immediately on removal from cold storage (Maxie and Ginsburg, 1974). During long term storage (9 months) of 'Packham's'

pears, Truter and Combrink (1993) found fruit to be significantly less firm and more yellow than control fruit after storage at -0.5°C followed by ripening for one week at 20°C. Quality changes were the result of C_2H_4 production during the long storage period.

Conversely after nine months storage at a higher temperature (4°C), 'Packham's' pears ripened unevenly and fruit quality (firmness, soluble solids content and titratable acidity) were found to be more affected by storage temperature than C_2H_4 levels (Truter and Combrink, 1993). Latter findings are supported also by Henze (1995) whereby an increased storage temperature accelerated the colour change in pears from green to yellow. The effect on fruit quality was most probably the result of the increase in temperature that increased the respiration rate, which resulted in an increased C_2H_4 production rate and ultimately an increased fruit metabolism. As a result of the temperature effect on C_2H_4 some packhouses or shipping containers use C_2H_4 absorbers (or scrubbers), such as potassium permanganate, to remove C_2H_4 (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication).

2.3.2 Physico-chemical changes in pears

The following physico-chemical changes in pears are often related to a change in fruit quality, especially during pear ripening.

2.3.2.1 Fruit firmness

The main components of fruit cell walls are cellulose, hemicelluloses and pectins (Macrae *et al*., 1993). The middle lamella is a section in the cell wall that separates adjacent cells and serves as a bonding agent for cells. It is rich in pectic compounds, (von Mollendrof, 1996). The precursor for these pectic compounds is the water-insoluble polymer called protopectin. It is cross-linked to other polymer chains through calcium bridges. These calcium-pectate complexes strengthen the cell walls and structural components, resulting in a firm fruit texture. Strengthening of the cell components may delay or prevent the loss of cell integrity and delay the activity of calcium-dependant enzyme activity (Wills *et al*, 1998). During ripening fruit softening is a result of the partial dissolving of the cell walls, including the middle lamellae by the action of enzymes that break down pectins and cellulose (Macrae *et al.*, 1993). During ripening protopectin, which is hydrolysed into soluble pectin, is the principle factor concerned in the softening of pears (Kadam, Dhumai and Shinde, 1995). The ripening enzymes pectin-methyl esterase (PME), polygalacturonase (PG) and cellulase are present in ripe fruit and responsible for the

changes in the pectic compounds. PME is present throughout fruit development and is responsible for de-esterification of pectin. PG-activity is low in unripe fruit, but increases exponentially during ripening and is responsible for hydrolysation of pectins. Cellulase is responsible for hydrolysis of cellulose in the cell walls of fruit (von Mollendorf, 1996).

2.3.2.2 Flavour (taste and aroma)

Sweetness

The main sugars responsible for sweetness in fruit are the monosaccharides glucose and fructose and the disaccharide sucrose (Macrae *et al.*, 1993). Fructose is the sugar present in the largest amount in pears followed by glucose, sorbitol, sucrose, xylose, galactose and arabinose (Kadam *et al*., 1995).

During ripening the total amount of soluble solids tend to increase. Protopectin is broken down to pectin, starch is almost completely converted to sugars and sorbitol is converted to fructose (Kadam *et al*., 1995). Starch hydrolysis increases the sucrose levels and sucrose is in turn hydrolysed to glucose and fructose. However, the total amount of sugars in pears is not the sole result of starch hydrolysis (Jackson, 2003).

Acidity

Organic acids play an important role in the sugar:acid ratio which influences the flavour of fruit. Malic acid is the major organic acid present in pears. Various amounts of citric, tartaric and oxalic acid may also be found (Kadam *et al*., 1995).

During fruit ripening there is a decline in organic acids because they are hydrolysed for energy during respiration or converted to sugars (Wills *et al*., 1998). The result is that the sweetness becomes more pronounced during fruit ripening (Jackson, 2003). In fact, titratable acidity and soluble solids are considered to be the best indicators for fruit ripeness and taste (Pesis, Dvir, Feygenberg, Arie, Ackerman and Litcher according to Toğrul and Arslan, 2004).

Organic acids are considered to be a reserve source of energy to fruit and are metabolised to the greatest extent when fruit ripen (Fourie, 1996; Wills *et al.*, 1998). During ripening and senescence organic acids become the major energy source because organic acids

have a higher ratio of O_2 molecules per carbon atom and thus require less O_2 than carbohydrates or fatty acids for the production of CO_2 during respiration (Wills *et al.,* 1981).

Astringency and bitterness

Phenolic compounds may also affect the taste of pears. Astringency and bitterness are often associated with the skin of some pear cultivars and are attributed to the presence of phenolic substances (tannins). Polyphenolics (polymeric phenols) of high molecular weight tend to be astringent while polyphenolics of low molecular weight tend to be bitter (as reviewed by Kadam *et al*., 1995). During ripening the phenolic content of pears decreases (Kadam *et al*., 1995) as a result of oxidation by polyphenol oxidase and through coupled oxidation reactions (Amiot, Tacchini, Aubert and Oleszek, 1995).

Flavour compounds

Aromatic esters that give pears their distinctive flavour are produced during ripening. The principle volatile compounds in ripe pears are ethyl, propyl, butyl, and hexyl acetates which account for 70.6% of the volatiles (Kadam *et al*., 1995).

2.3.2.3 Colour

Climacteric fruit shows rapid loss of green colour upon ripening. The loss of green colour (de-greening) is due to chlorophyll degradation as a result of pH changes, oxidative systems and chlorophyllases enzymes. During ripening de-greening is mainly associated with chlorophyll degradation and synthesis and/ or unmasking of pigments ranging from yellow to red (Wills *et al.*, 1998).

Carotenoid pigments are responsible for the yellow to orange colours of fruit and vegetables (Fourie, 1996). Carotenoids are synthesised during the developmental stages of the plant but remain masked by the presence of chlorophyll. Following degradation of chlorophyll, the carotenoid pigments become visible. With other tissues, carotenoid synthesis occurs concurrently with chlorophyll degradation (Wills *et al*., 1998). In apples the yellow of the underlying carotenoids are revealed by chlorophyll degradation (Lurie, 1998). As apples are also pome fruit it is expected that the revealing of carotenoids may also occur in pears.

The phenolic compounds in pears affect discolouration of the pear flesh. The browning potential of a fruit is dependant on its total phenolic content and the activity of

polyphenoloxidase (PPO) (von Mollendorf, 1996), otherwise know as polyphenolase (Kadam *et al*., 1995). PPO activity increases as fruit ripens. Normally, phenolic compounds are separated from these enzymes by cell membranes, but when the fruit is damaged (bruised or cut) membranes are broken. Enzymes come into contact with phenolics and react in the presence of O_2 (von Mollendorf, 1996). The browning reaction involves oxidation of ortho-diphenols in the presence of PPO to ortho-quinones, which then polymerise to form brown-coloured pigments (Kader, 1989). Pears are rich in the phenolic compound chlorogenic acid, which is the substrate for PPO in browning reactions (Kader, 1989).

2.3.3 The effect of atmospheric composition on fruit quality

Controlled atmosphere (CA) and modified atmosphere (MA) storage are used as supplements to refrigeration in order to preserve postharvest quality of fresh fruit and vegetables (Brody, 1989).

2.3.3.1 Controlled atmosphere (CA) storage

The gas composition at regular atmosphere (RA) in a cold store is 21% O_2, 0.04% CO_2 and about 78% nitrogen (van der Merwe, 1996b). No information could be found on the ethylene concentration of a cold store under RA conditions. In conventional CA storage the O_2 concentration is reduced to approximately 5% by blowing nitrogen into the chamber. As the fruit respire normally the O_2 concentration eventually reaches 2 to 3% at which stage the fruit is considered to be under CA storage conditions (van der Merwe, 1996b). The CO_2 level is kept at 0.5%. In the event that the carbon dioxide concentration becomes too high, air is blown into the chamber and the excess CO_2 is removed by scrubbers (Coetzee, Logistics Manager, Kromco Limited, 2002 – personal communication). Ethylene scrubbers may also be used in CA stores to maintain the ethylene concentration at below 1 mg/kg. However, it appears to be mainly used for apples to extend their storage life, retain firmness and reduce superficial scald (Van der Merwe, 1996b). 'Packham's Triumph' pears are stored at –0.5°C and the recommended gas regimes for CA storage are 1.5% O_2 and 1.5% CO_2. Under these conditions, pears will have a storage life of up to 9 months (van der Merwe, 1996b).

Other forms of CA storage include Low Oxygen (LO) and Ultra Low Oxygen (ULO) storage. Oxygen concentrations of 1.5% (LO) and 1% (ULO) are used during storage of

'd'Anjou' pears to extend fruit quality and storage life and inhibit superficial scald (Calvo, Salvador, Sanchez, 2000). Internal breakdown and softening in fruit are also prevented by LO and ULO storage (Van der Merwe, 1996b).

Effect on respiration

The respiration rate and thus fruit metabolism is dependent on the availability of O_2. When O_2 levels are reduced to between 1.5 and 2.5%, pear respiration and the time taken to reach the climacteric peak as well as the associated processes of senescence are delayed (Eksteen and Ginsburg, 1977). It seems to be so effective that lowering of the O_2 concentration to 2% decreased the respiration rate of apples by 64% (van der Merwe, 1996b).

However, if the O_2 concentration drops below 1%, often referred to as hypoxia, aerobic respiration may be replaced by anaerobic respiration or fermentation and the subsequent production of alcohol and acetaldehyde. As a result fruit will develop a distinctive alcoholic taste (van der Merwe, 1996b).

Unfortunately prolonged CA storage has its drawbacks. Pears sometimes fail to ripen after prolonged cold storage due to the loss of capacity of the tissue to synthesise ripening enzymes (Wang, 1990). Additionally, according to Kader (1989), 'Bartlett' pears stored in CA subsequently ripened slower at 20 to 25°C than fruit stored in air. This is in contrast to findings by Crouch (Manager: Pome Fruit, Experico, 2006 - personal communication) that pears from CA storage tend to ripen faster than pears from RA storage.

Effect on ethylene production

Generally when O_2 levels are reduced to below 8%, ethylene production of fresh fruit decreases and sensitivity to ethylene is reduced. Oxygen levels of 2.5% halves ethylene production and retards ripening (Burg and Burg, according to Kader, 1986). When O_2 is absent or when plant tissue is under anaerobic conditions, ethylene biosynthesis ceases. However, when the fruit is transferred back to air, there is a rapid increase in ethylene production. Low O_2 storage or anaerobic treatment of fruit causes accumulation of the ethylene precursor ACC, which is converted back to ethylene very rapidly in the presence of O_2 (Wang, 1990).

Elevated CO_2 concentrations can also suppress ethylene production. It was found that when pre-climacteric apples were treated with elevated CO_2 levels (20 to 30%) and low O_2 (1 to 3%) at 15 to 20°C, there was an initial inhibition of ACC synthesis, which was followed by a substantial accumulation of ACC (Wang, 1990). This is due to an inhibition in ethylene synthesis, suppression of general metabolism and the delay in the onset of ripening. Consequently, all reactions associated with ripening are delayed, including an increase in respiration rate, auto-catalytic ethylene production, rapid acid catabolism, synthesis of ripening enzymes, as well as softening and changes of pectic substances in the cell wall (Wang, 1990). It was found, however, that ethylene production was suppressed to a greater extent in fruits held in low O_2 atmospheres than in those subjected to elevated CO_2 concentrations (Kader, 1989).

Effect on quality attributes

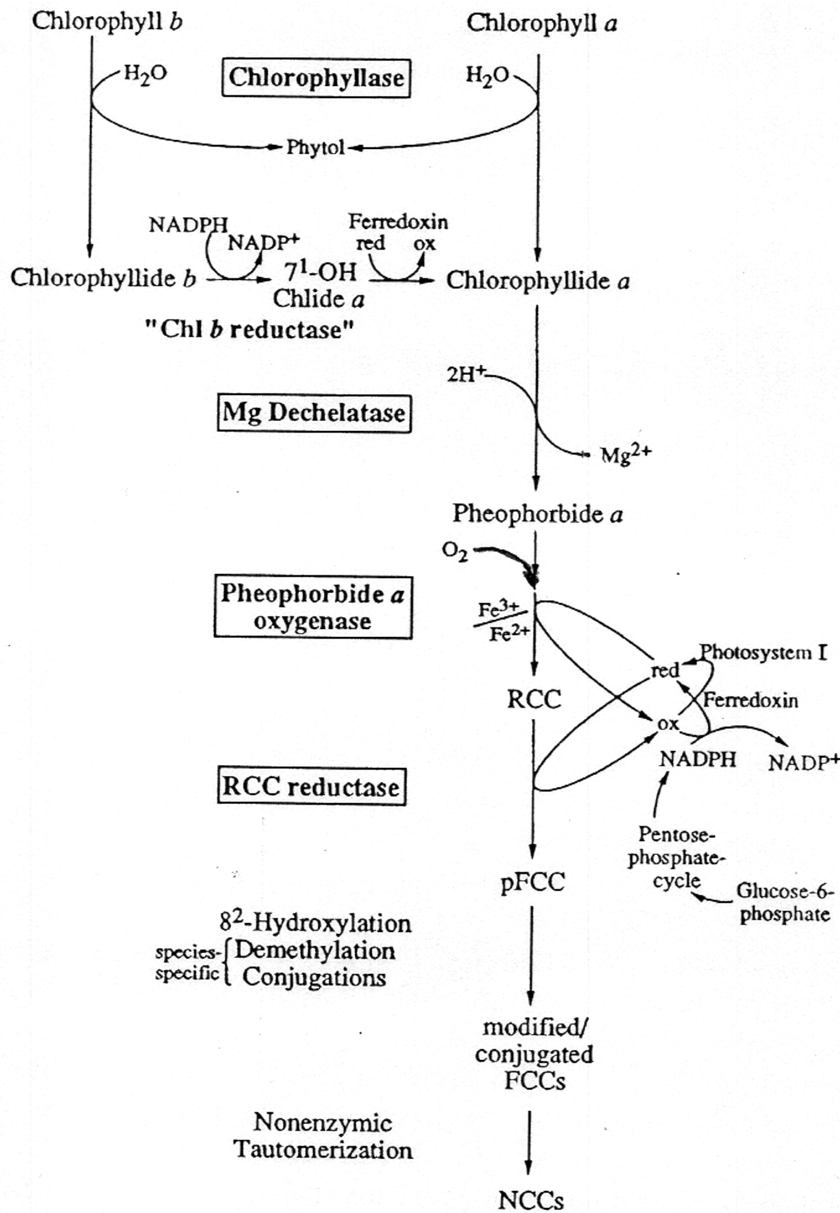
During ripening of pears at 20°C the texture and colour are affected differently by the atmospheric composition. Pears stored at high CO_2 concentrations (20%) remained significantly firmer over a period of six days (Kader, 1989). This relationship between firmness retention and CO_2 is supported by Blanpied and Hansen (according to Wang, 1990) who found that there was a linear relationship between firmness loss and CO_2 levels but not between firmness loss and O_2 levels in pears. Kader (2003) reported that the activity of cell wall degrading enzymes which cause fruit softening was retarded by CA conditions.

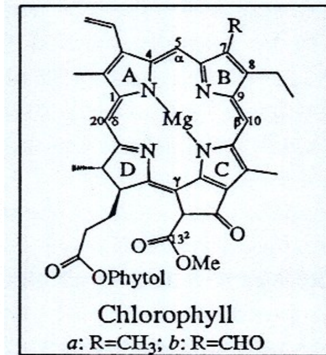
Apples and pears stored in CA retained more organic acids than fruit stored in air. Furthermore, there is a reduction in the loss of acidity in CA-stored apples and pears due to the reduced loss of malic acid. Refrigerated, CA storage of apples and pears resulted in reduced catabolism of organic acids and thus higher acid retention due to a decreased respiration rate and also to the fixation of CO_2 into the organic acids (Wang, 1990).

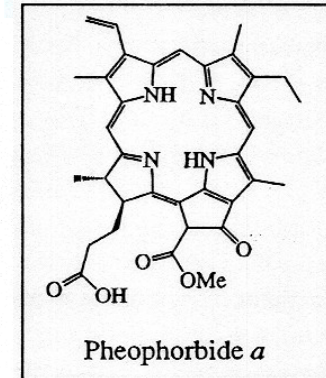
The loss of chlorophyll and the biosynthesis of carotenoids and anthocyanins are slowed down in fruits and vegetables kept in CA conditions (Kader, 1986). De-greening in 'Bartlett' pears stored in low O_2 atmospheres, was much slower than those stored under high CO_2 levels (Kader, 1989). Similarly, Amarante, Banks and Ganesh (2001b) found that de-greening was highly dependant on the internal O_2 concentration whereas textural changes were more sensitive to an increase in the CO_2 concentration.

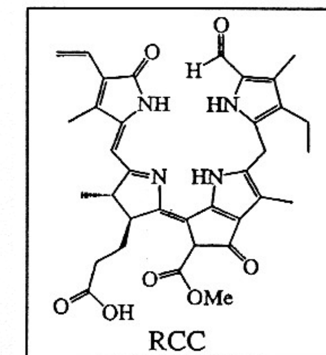
The effect of low oxygen concentrations on the retardation of de-greening was not explicitly described by any of the authors with similar results. However, Matile, Hörtensteiner and Thomas (1999) reviewed the chlorophyll degradation process and explained it according to the "pheophorbide-*a*-oxygenase (PaO) pathway" of chlorophyll breakdown in senescent leaves. This process is illustrated in Figure 2.7 along with the chemical structures of chlorophyll and intermediary catabolites. The initial enzyme involved in degradation of chlorophyll (Chl) and catalysis of the hydrolysis of ester bonds to yield chlorophyllide (porphyrin moieties) and phytol, is chlorophyllase (Chlase). This is regarded as the initial step of breakdown. Amounts of phytol and chlorophyllide have only been detected in trace amounts. This suggested that the process that actually caused de-greening followed swiftly once phytol and the central Mg-atom from chlorophyllide a, was removed.

The ring-opening step in the pathway responsible for yielding the first colourless product (pFCC, pre-fluorescent chlorophyll catabolite) is catabolised by pheophorbide-a-oxygenase (PaO), to yield RCC (red-coloured chlorophyll catabolite) from pheophorbide-a, and RCC-reductase to yield pFCC (Matile *et al*., 1999). It is clear from Figure 2.7 that this step requires O_2. Therefore, it may be the step in chlorophyll degradation that is most influenced by low O_2 concentrations during CA conditions. Hence, unless colourless products like pFCC are formed, pears will not de-green nor will the carotenoids be revealed.









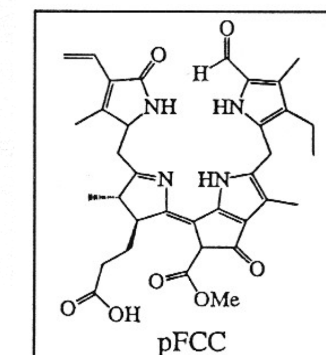


Fig. 2.7. The “pheophorbide a oxygenase (PaO) pathway” of chlorophyll breakdown in senescent leaves, including the chemical structures of chlorophyll and the intermediary catabolites Pheophorbide-a, RCC and pFCC (Matile *et al*., 1999)

2.3.3.2 Modified atmosphere (MA) storage

Mechanism of MAP

Passive modified atmosphere packaging (MAP) is used on 'Packham's' pears during transport and export. Pears are packed in polyethylene bags (when destined for the South African market) or in boxes lined with a polyethylene bag (when destined for export) (Coetzee, Logistics Manager, Kromco Ltd, 2002- personal communication; Crouch, Cape Span, 2002 – personal communication). Polyethylene bags allow pears to generate a modified atmosphere within the packaging during respiration (Blanpied, 1990) by restricting outward movement of water vapour and CO_2 while inward movement of O_2 is restricted to allow sufficient aerobic respiration (Beatie and Wade, 1996). As the fruit respires the carbon dioxide concentration gradually increases, while the O_2 concentration decreases. The permeability of the polyethylene bag determines the final gas concentrations (van der Merwe, 1996b).

This form of MAP is used for short-term storage of pears during transport, export and retailing (Fonesca, Oliveira and Brecht, 2002) but the benefits of MAP are lost upon opening the packaging (Van der Merwe, 1996b). Furthermore the polyethylene bags generate much waste packaging.

Interestingly, according to Crouch (Manager: Pome Fruit, Experico, 2006 - personal communication), pears will strictly not be under passive MAP conditions unless the polyethylene bag has been formally sealed i.e. with a cable tie. In South Africa the bags are only folded over the stack of packed pears inside the box. Alternatively, in the case of thrift packs, the packs have large perforations (6 mm in diameter). This means that depending on how well the bags in the boxes have been folded, the atmosphere composition may vary. The result is that fruit may be of mixed maturities, which is at times a problem in South African export fruit.

2.3.3.3 The disadvantage of CA and MA storage

Despite the advantages of CA and MA storage, there is the danger of the development of excessively high levels of CO_2, which leads to a physiological disorder called brown heart or core browning. The disorder manifests as internal flesh browning around the core of the pears (Fig. 2.8) with translucent lighter spots in the flesh. The spots increase in size and eventually dry out to form cavities of various sizes (Eksteen and Ginsburg, 1977).

Fig. 2.8. Internal browning of the flesh of a Rosemarie pear (with thanks to Crouch (Manager: Pome Fruit, Experico, 2006)

Any factor that is able to increase fruit respiration and thus the production of CO_2 may be able to promote core browning (Streif, Xuan, Saquet, and Rabus, 2001). According to Lammertyn, Verlinden and Nicolaï (2000), late picked pears, delayed cooling prior to CA storage and high temperatures seem to be the main factors responsible for brown heart formation in pears. The first symptoms of the disorder in pears occur when the CO_2 in the atmosphere around the pears exceeds 2%, while pears that are more resistant to this disorder, such as the cultivar 'Bon Chretien', will only develop gas injury at a CO_2 concentration of 4 to 5%. The degree of gas injury increases with an increase in CO_2 concentration (Eksteen and Ginsburg, 1977) and low O_2 levels during storage (Mitcham, 2002).

2.4 Edible coatings used on pears

Edible coatings have the potential to act as a partial barrier to moisture and gas. As a result the rate of transpiration and gas (CO_2 and O_2) exchange in fruit is reduced (Greener -Donhowe and Fennema, 1994). The coating creates a modified atmosphere and could be considered as a form of MAP. The result is a reduction in respiration rate and fruit metabolism. Edible coatings also provide physical protection to fruit during handling and transport, allow incorporation of additives (like nutrients or anti-microbials) and reduce packaging waste by offering an environmentally-friendly alternative (Greener-Donhowe and Fennema, 1994) without detracting from the natural appearance of the fruit.

2.4.1 Mechanism of an edible coating as moisture and gas barrier

The preferred path of moisture movement from the fruit to the atmosphere is through a liquid aqueous phase in the cuticle (Ben-Yehoshua *et al*., 1985). Hydrophobic edible films based on waxes (Amarante *et al*., 2001b) and zein protein (Park, Chinnan and Shewfelt, 1994; Bai, Alleyne, Hagenmaier, Mattheis and Baldwin, 2003) have proven to be successful in retarding moisture loss in pears by covering the cuticle (Amarante *et al*., 2001b).

The edible coating modifies the fruit's internal atmosphere by increasing CO_2 and decreasing O_2 concentrations, typical of MAP (Park, 1999). Gasses diffuse primarily through pores (lenticels) in the fruit skin. Lenticels are blocked when fruit are coated with wax, which restricts the transport of gasses (O_2 and CO_2) and ethylene (Ben –Yehoshua *et al*., 1985). Amarante *et al*. (2001b) and Amarante, *et al.* (2001a) found that waxes delayed ripening (in terms of reducing respiration and ethylene production rates, retaining flesh firmness, green colour, acidity and soluble solids) in coated pears by blocking the pores. As reviewed by Baldwin, Nisperos-Carriedo and Baker (1995) polysaccharide-based coatings exhibit good gas barrier properties for reducing the ripening rate but their hydrophilic nature makes them poor moisture barriers.

2.4.2 Factors that affect coating efficiency

The effectiveness of the edible coatings to extend the shelf-life of pears is influenced by factors relating to the edible coating and factors intrinsic to the fruit. Factors relating to the coating include the type of coating, coating application temperature and coating thickness. The type of coating and its thickness in turn affect the barrier properties of the coating. Intrinsic fruit factors include pear maturity, pear temperature at the time of coating and the pear skin surface.

2.4.2.1 Coating-related factors: Types of edible coatings used on pears

To understand the rationale behind the use of kafirin protein as a pear coating, the effect that other pear coatings have on the physiological changes in the fruit during refrigerated storage and ripening, should be explored.

Coatings are often based on the following substances:

- Polysaccharides including cellulose, carrageenan, sucrose polyesters
- Lipids including waxes and oils
- Resins like shellac
- Proteins from soy, whey, casein, gelatin and cereal proteins like zein (from maize), kafirin (from sorghum) and gluten (from wheat)

To improve the characteristics and capabilities of a coating, a composite is often used by combining one or more of the above mentioned substances. The major advantages and disadvantages of lipid-, polysaccharide-, resin- based pear coatings are outlined in Table 2.1.

Lipid-, polysaccharide- and resin-based coatings

Lipids and resins may be combined with several substances, including each other, to improve the characteristics of the coating. Lipid based coatings often contain waxes such as carnauba wax or oils (Table 2.1). Carnauba wax is a vegetable wax obtained from the carnauba tree (Anon, 2005c). It is available commercially under the names of Primafresh Wax® and Capsicum / Zucchini Wax® and its effect on pear quality has been examined extensively (Drake, Cavalieri and Kupferman, 1991; Drake, Fellman and Nelson, 1987; Amarante *et al.*, 2001a; Amarante *et al.,* 2001c; Amarante and Banks, 2002).

Amarante *et al.* (2001a, 2001c) found that carnauba wax delayed ripening by modification of the internal gas concentrations of the fruit. The effect of the different internal gas concentrations on fruit colour and texture is, however, quite different. Amarante *et al.* (2001a) found that the wax coating modified the internal O_2 more than the internal CO_2 concentration. The lower O_2 concentration was the main reason for the delayed ripening. Subsequently the coating delayed colour change during ripening (20°C) more than softening because de-greening at 20°C was oxygen dependant and only small changes in the O_2 concentration are needed to delay colour change.

To extensively delay softening during pear ripening at 20°C, the O_2 concentration must be extremely low. For that reason pears may soften during ripening without changing colour. Extensive softening inhibition during ripening may require excessively high coating concentrations, which may induce anaerobic respiration or physiological disorders in the pears (Amarante *et al.,* 2001a).

Table 2.1.

Comparison of the major advantages, mechanisms of action and disadvantages of lipid-, polysaccharide-, and resin- based pear coatings

Coating type	**Coating material**	**Advantages and mechanisms of action**	**Disadvantages**	**References**
Lipid	Carnauba wax	Good water barrier properties due to low moisture permeability, reduced respiration rate and moisture loss, retained colour and flesh firmness		Amarante *et al.*, (2001a), Amarante *et al.* (2001c), Amarante and Banks (2002) Baldwin, Burns, Kazokas, Brecht, Hagenmaier, Bender, Pesis (1999).
Lipid	Stripped corn oil emulsion	Retarded skin de-greening, flesh softening and reduction in titratable acidity during cold storage. Retarded ethylene production and action. Delayed ripening		Ju and Curry (2000)
Resin	Shellac	Low gas permeability, delays ripening	Low gas permeability causes anaerobic respiration, whitens on contact with moisture	Baldwin *et al.* (1999), Bai *et al.* (2003)
Resin /lipid combination	Shellac / Carnauba mix (Johnfresh™)	Low O_2 permeability, reduced respiration and moisture loss rate, retained skin colour, soluble solids content (SSC) and flesh firmness		Baldwin *et al*. (1999) Sümnü and Bayindirli (1994), Drake *et al.* (1991)
Lipid / polysaccharide combination	Soybean oil	Retarded quality loss through lowering of moisture and gas permeability	High concentrations of carboxymethyl cellulose excessively reduced O_2 permeability, resulting in anaerobic respiration	Toğrul and Arslan (2004)
Polysaccharide	Sucrose polyester - based	Low gas permeability, modified fruit internal atmosphere, retarded respiration, retained skin colour, flesh firmness, acidity and SSC	Ineffective moisture barrier	Meheriuk and Lau, according to Drake *et al.* (1987), Köksal, *et al.* (1994), Sümnü and Bayindirli (1994)
Polysaccharide	SPE and CMC blend	Reduced fungal infections and retarded spread of rot, relating to decreased O_2 and ethylene levels and increased CO_2 levels		Bancroft (1995), Bancroft, Herregods, Nicolai, Jager, Roy (2000)

Wax coating mixtures often contain a mixture of carnauba wax and shellac (Table 2.1). Shellac is a resin that is secreted by a female beetle in order for its eggs to adhere to tree bark (Klahorst, 1999). Examples of commercially available shellac combination coatings include Shield-Brite and Johnfresh™. The success of Johnfresh™ in reducing the respiration climacteric peak of "Ankara" pears more effectively than different concentrations of polysaccharide–based coatings (i.e. Semperfresh) is attributed to the low O_2 permeability of Shellac (Hagenmaier and Shaw, according to Sümnü and Bayindirli, 1994; Bai *et al.*, 2003).

Oils also form part of lipid-based coatings and may retard quality loss in pears (Ju and Curry, 2000; Toğrul and Arslan, 2004) (Table 2.1). Polysaccharides are often included in lipid based coatings to increase the gas barrier properties of the coating. The inclusion of carboxymethyl cellulose (CMC) in a soybean oil coating resulted in a reduced respiration rate and reduced moisture loss in pears (Table 2.1).

Sucrose polyester (SPE) coatings are polysaccharide-based and typically contain sucrose esters of fatty acids, sodium CMC and monoglycerides of fatty acids (Sümnü and Bayindirli, 1994). SPE coatings are hygroscopic in character, which may cause their water vapour permeability to be higher than that of commercial waxes. SPE coatings are therefore not the ideal coating to significantly reduce moisture loss (Köksal, Dumanoglu and Tuna, 1994; Sümnü and Bayindirli, 1994).

Moisture condensation on SPE-coated fruit may also make the fruit feel 'slippery', when the fruit are removed from storage (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication). Although green colour retention in coated pears (Drake *et al.*, 1991) has been attributed to reduced internal levels of O_2 and increased levels of CO_2 brought about by the coating, this may not be the mechanism for colour retention in SPE-coated pears. Sümnü and Bayindirli (1994) related colour retention in SPE-coated pears (that had higher respiration rates) to the penetration of the coating into the fruit affecting the chloroplast structure in the fruit. The only supporting evidence for this theory is by Frenkel, Klein and Dilley (1969) who found that injecting a sugar-alcohol solution into the central cavity region of 'Bartlett' pears, resulted in retarding respiration although flesh softening, chlorophyll breakdown and ethylene synthesis were not dramatically affected. However, when pears were

injected with water, ethylene synthesis as well as colour and textural changes were inhibited. It was concluded that the extent to which solution-infiltration inhibited ripening depended on the osmotic balance of the solution, because a favourable osmotic balance was required for ripening to occur.

Protein-based coatings

Proteins have good film forming ability but most proteins are hydrophilic in nature and coatings from such proteins do not sufficiently resist moisture permeation (as reviewed by Baldwin *et al*., 1995). Generally, compared to other edible polymer films, the O_2 permeability of protein films is low (Miller and Krochta, 1997). There is a shortage of literature on the effect of protein coatings on pears, in particular. For this reason the effect of protein coatings on climacteric fruit in general, will be reviewed. The effect of different protein-based coatings on the quality of different climacteric fruit is outlined in Table 2.2.

Table 2.2.

The effect of protein-based coatings on the quality of different climacteric fruits, compared to that of their uncoated counterparts

Protein	Fruit type	Effect on coated fruit	References
Soy protein isolate	Kiwi fruit	Retarded respiration rate, reduced moisture and acidity loss, retained flesh firmness	Xu *et al*.(2001)
Whey protein isolate	'Fuji' apples	Lowered internal O_2 concentrations and increased internal CO_2 concentration	Cisneros-Zevallos and Krochta (2003)
Zein	Apples and tomatoes	Retarded respiration rate, reduced moisture loss, retained skin colour and acidity	Bai *et al*. (2003), Park *et al.* (1994)

Hydrophilic proteins

The effect of a soy protein isolate (SPI) composite coating on kiwi fruit quality is given in Table 2.2. The composite soy-coating (Table 2.2) consisted of soy protein isolate, stearic acid and pullulan. Uncoated fruit exhibited rotting and fermentation towards the end of the 37d storage period at room temperature while the shelf-life of the

coated kiwifruit were extended to about three times that of its normal, expected shelf-life (Xu, Chen and Sun, 2001). However, the coating effectiveness was not only attributed to the soy protein. Pullulan (an extracellular microbial polysaccharide) is considered to be an effective O_2 barrier (Kaplan *et al*., according to Krochta and De Mulder-Johnston, 1997). In fact, when casein protein and stearic acid were combined in an edible coating, the latter reduced surface dehydration of peeled carrots, because the combination of the protein with stearic acid increased the moisture resistance of the coating (Avena-Bustillos, Cisneros-Zevallos, Krochta and Saltveit, 1994).

Whey protein isolate (WPI) is considered to be a good gas barrier (Table 2.2) but, as is the case with other hydrophilic biopolymer films, the RH of the environment affects its barrier properties (McHugh and Krochta, according to Cisneros-Zevallos and Krochta, 2003). This is because as RH increases the moisture from the environment acts as a plasticiser in the film, which increases gas permeability (Gontard *et al*., according to Cisneros-Zevallos and Krochta, 2003). Similarly the storage of WPI-coated apples at 20°C revealed that resistance of the coating to gas transfer increased as RH decreased and at 70 to 80% RH anaerobic respiration was induced in the coated apples due to excessively low internal O_2 levels (Cisneros-Zevallos and Krochta, 2003).

Hydrophobic proteins

Prolamin proteins are soluble in aqueous alcohol mixtures and include the proteins gluten, zein, kafirin, avenin and hordein in wheat, maize, sorghum, oats and barley, respectively. Zein protein was characterised by Shewry and Mifilin (1985) as containing high concentrations of the hydrophobic amino acids leucine, proline and alanine, which are characteristic of a typical prolamin protein. Zein coatings not only act as gas barriers to retard ripening but, being hydrophobic in nature, also reduce moisture loss (Table 2.2). A zein coating on apples, stored at 20°C and 50% RH, prevented shrivelling as a result of reduced moisture loss (Bai *et al*., 2003). However, the zein coating imparted some whitening to the coated fruit surface when moisture condensated on the hydrophobic coating after removal from cold storage. Whitening of the coating appeared to be dependant on the zein and plasticiser content in the coating, but whitening was minimal when the coating formulation

contained 10% zein and 10% plasticiser. Most importantly zein modified fruit internal atmosphere. The quality of zein coated apples was extended in a similar way to what was achieved by a commercial shellac coating (Bai *et al*., 2003). Thus, the gas permeability of the zein coating must have been similar to that of shellac. Shelf-life of zein coated apples was extended longer than that of the uncoated fruit or what was achieved by a commercial carnauba wax coating on the apples (Bai *et al*., 2003). Thus the gas permeability of the zein coating must have been better than that of carnauba wax.

Kafirin, the sorghum prolamin protein, is similar to zein (Shull *et al*., 1991). However, kafirin is less soluble in ethanol, more hydrophobic and exhibits a larger percentage of intermolecular disulphide cross-linking after heating in the presence of water (Duodu, Taylor, Belton and Hamaker, 2003). The increased hydrophobicity and degree of protein cross-linking of kafirin have been suggested to contribute to the lower digestibility of sorghum protein when compared to that of maize proteins (Duodu, Nunes, Delgadillo and Belton, 2003). Studies by Buffo *et al.*, (1997) and Gao, Taylor, Wellner, Byaruhanga, Parker, Mills and Belton (2005) indicated that sorghum kafirin may be suitable for use in edible films and coatings although this assumption may be premature as only free standing films were tested in the study. In addition kafirin is not known to be allergenic (Lopata, HOD; Division of Immunology, University of Cape Town, 2005 – personal communication; Skerrit, according to Gao *et al.*, 2005) which increases its suitability for edible coatings.

According to Buffo *et al.* (1997) kafirin may be an alternative to zein in film forming applications because (as found by Shull *et al.*, 1991) kafirin is similar to zein in molecular weight, solubility, structure and amino acid composition. In addition kafirin films with water vapour barrier properties similar to that of zein films were successfully produced by Buffo *et al. (*1997). Subsequently much work has been conducted on the film forming properties of kafirin. Gao *et al*. (2005) described extraction and drying conditions to obtain kafirin that will produce films of similar sensory quality and water vapour transmission rates to commercial zein. Byaruhanga, Erasmus and Taylor (2005) described how microwave heating could modify and improve the functional properties of kafirin films including the reduction of film water vapour permeability by more than a third. The O_2 permeability of kafirin

films can also be decreased by the addition of hydrolysable and condensed tannins (Emmambux, Stading and Taylor, 2004). There appears to be sufficient evidence of the similarities between kafirin and zein protein and films, the film forming ability of kafirin, and how to improve the functional properties of kafirin films. As a coating is effectively a thin film, kafirin protein should be able to form and perform well as an edible coating.

2.4.2.2 Coating-related factors: Application temperature

Coating application temperature may affect the ripening rate of pears. Compared to uncoated and waxed pears (dried at 60°C) drying waxed pears at 0°C increased texture retention during ripening (Drake *et al.*, 1991). When dried at 0°C, the wax coating increased the internal CO_2 concentration more than the reduction in internal O_2 concentration. The increased CO_2 concentration delayed ripening at low temperatures and firmness retention was delayed to a greater extent during cold storage than colour change because textural changes are more sensitive to changes in the CO_2 concentration than colour change (Amarante *et al.*, 2001a).

Conversely hot-dried waxed pears exhibited better colour and firmness retention and longer shelf-life than uncoated pears, although the ethylene concentration in the heated fruit was higher than that of the uncoated fruit during ripening (Drake *et al.,* 1991). Maxie and Ginsburg (1974) reported that temperatures above 30°C stop ethylene production or the loss of pear sensitivity to the gas, as it is known that fruit development is not only a change in the concentration of ethylene, but in the sensitivity of the fruit tissue to it (Von Mollendorff, 1996). Ethylene production is reversibly inhibited by temperatures above 40°C due to the loss of ACC-oxidase that converts ACC to ethylene (Paull and Jung Chen, 2000) but this inhibition is reversed when fruit are removed from heat after which the level of ethylene rises to higher levels than in non-heated fruit (Lurie, 1998). Texture retention in the hot-dried pears may have been a result of the coating as well as the drying temperature, because the synthesis of cell wall hydrolytic enzymes (polygalacturonase) is inhibited by high temperatures, resulting in slower softening at 30 to 40°C than at 20°C. On returning fruit to 20°C the softening rate increases, but it was less than in non-heated fruit (Lurie, 1998).

The cold-dried pears retained colour as a result of the low drying temperature, while colour retention in the hot-dried pears was a result of the presence of the wax coating only (Drake *et al.,* 1991). This is supported by Lurie (1998) and Paull and Jung Chen (2000) whereby heat treatment (35 to 40°C) of apples accelerated chlorophyll degradation. Apples and pears are similar in that chlorophyll degradation reveals the yellow of the underlying carotenoids already present (Lurie, 1998). Respiration rate, which affect ripening rate, is not only affected by the coating. High temperatures initially increase respiration rate but the rate drops to near or below that of non-heated fruit when heat is removed (Paull and Jung Chen, 2000).

Apples were coated with respectively carnauba wax, shellac wax and zein protein (Bai *et al*., 2003). The coated and uncoated fruit were heated at 50°C for five minutes, to dry the coating. Coated fruit exhibited firmer texture and higher titratable acidity than non-coated fruit (Bai *et al*., 2003), implying that the coating, and not the heat treatment, is the determining factor in delaying ripening. However, caution should be taken when relating textural changes in apples to textural changes expected in pears. Polygalacturonase (PG) is chiefly responsible for flesh softening in 'Bartlett' pears through decreasing pectin content and neutral sugar content (arabinose and galactose) in the cell walls (ElRayah Ahmed and Labavitch, 1980a, b). However, apples contain a different form of this enzyme than pears. Apples remain firmer longer than pears because apples contain only exo-PG, which hydrolyses the pectin chain from one end only, while endo-PG (typically in pears) randomly hydrolyses the pectin chain (von Mollendorff, 1996).

2.4.2.3 Coating-related factors: Coating thickness

Coating thickness will affect both gas and moisture permeability of the edible coating. Selection of the correct coating thickness is crucial to reap the full benefit of the edible coating while still enabling the fruit to ripen normally. An edible coating may be a good moisture barrier but the gas barrier properties may not be optimal due to the different pathways for moisture and gas diffusion. The moisture permeability of the coatings may depend on the concentration of wax (Amarante *et al*., 2001b) and zein (Bai *et al*., 2003). This may pose a problem in the use of coatings to delay both moisture loss and ripening because high concentrations of zein coating on tomatoes

(Park *et al.*, 1994) induced anaerobic respiration, resulting in the production of off-flavours and excessive moisture loss.

Effect on gas permeability

The blocked pores of the fruit skin decrease gas exchange (Ben Yehoshua *et al.,* 1985). The solids concentration of the coating solution affect coating thickness and thus gas permeability (as reviewed by Cisneros-Zevallos and Krochta, 2003). Amarante *et al*. (2001a, 2001b) found that the effectiveness of the wax coating in delaying ripening increased as the coating concentration increased. Wax concentrations as low as 5% (v/v) to up to 40% (v/v) significantly suppressed ripening of ‘Packham’s Triumph’ pears but at concentrations above 40% (v/v) coatings provided no additional benefits when compared to fruit coated with a 40% (v/v) coating (Amarante and Banks, 2002). Similarly, the shelf-life (at 20°C) of coated pears increased as the sucrose polyester coating concentration increased because gas permeability decreased (Köksal *et al.*, 1994). Sümnü and Bayindirli (1994) found that low concentrations (0.5%, w/v) of Semperfresh did not delay pear ripening whereas higher concentrations (1.0 to 1.5% w/v) were very effective.

However, excessively thick coatings may adversely affect pear ripening. According to Amarante and Banks (2002), Smock found in 1935 that apples and pears treated with a very thick wax layer may have failed to ripen at room temperature due to the low gas permeability of the thick coating. More specifically, coating thickness can affect normal colour change during ripening (storage at 20°C). Thick SPE coatings (1.2 and 1.5%, w/v) caused uneven colour change in ripening pears (Van Zyl, Torman and Von Mollendorff, according to Sümnü and Bayindirli, 1994). ‘Packham’s Triumph’ pears coated with high carnauba wax concentrations (20 to 40%, v/v) also exhibited skin blotchiness (uneven de-greening) during ripening because the thick coating modified O_2 concentrations in the fruit to a great extent (Amarante and Banks, 2002). As a result, de-greening, which is sensitive to small changes in O_2 concentrations, was retarded (Amarante *et al.*, 2001a). Skin blotchiness may have been the result of unevenness in the coating (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication) with the thicker parts of the coating impairing colour change the most and causing the blotchiness.

Excessive coating deposits also cause the formation of alcoholic off-flavours. Excessive coating deposits are impermeable to gasses resulting in the build-up of high levels of CO_2 and the induction of anaerobic respiration (Amarante *et al.*, 2001a; Park *et al.*, 1994). Park *et al.* (1994) found that alcohol was produced during anaerobic fermentation in ripening tomatoes when coated with a zein coating concentration of 27% (66 µm thick). In pears, elevated CO_2 levels (internally or in the atmosphere) during storage cause the physiological disorder brown heart (also known as core breakdown) (Kadam *et al.*, 1995). However, 'Packham's Triumph' pears do not develop this core breakdown, regardless of the coating concentration used or the ripening stage of the fruit at the time of coating with wax (Amarante *et al.*, 2001a; Amarante and Banks, 2002) because the low respiration rate of 'Packham's' pears during cold storage does not deplete O_2 and CO_2 is not increased to damaging levels (Amarante *et al.*, 2001c).

In addition it was reported that 'Packham's Triumph' pears tolerated hypoxic conditions well (Amarante *et al*., 2001b) without developing CO_2-induced injury (Amarante and Banks, 2002) or the induction of anaerobic respiration (Aamarante *et al.,* 2001a). It was suggested that high concentrations of coating deposits would be greatly beneficial in decreasing moisture loss and delaying ripening in that cultivar (Amarante *et al*., 2001b).

Effect on water vapour permeability

The water permeability of wax and zein protein coatings also depend on the concentration of wax (Amarante *et al*., 2001b) and the zein protein (Bai *et al*., 2003). Increased carnauba-wax concentrations in the coating reduced moisture loss (Amarante *et al.*, 2001b) by decreasing the water vapour permeability of the wax coating (Bai *et al*., 2003). When tomatoes (also a climacteric fruit) were coated with zein coating, moisture loss during storage of the fruit at 21°C was reduced more efficiently as the zein concentration in the coating increased from 9% (w/w, 5 µm thick) to 16% (w/w, 15 µm thick). In contradiction, excessive weight loss and shrivelling in tomatoes coated with a 27% (w/w, 66 µm thick) zein coating was observed. This was attributed to accelerated senescence and anaerobic fermentation in the fruit which was brought about by the thick coating that was too impervious to gasses during respiration (Park *et al.*, 1994). Amarante *et al.* (2001c),

however, stated that treating 'Packham's' pears with low wax concentrations (resulting in small increases in the coating deposit on the skin) were sufficient to achieve considerable reductions in moisture loss.

2.4.2.4 Intrinsic fruit factors: Fruit maturity

Pear maturity affects the efficiency of the edible coating in reducing ripening optimally. Pears should be coated directly after harvest, prior to cold storage, to reduce moisture loss and delay ripening optimally because storage prior to coating will increase pear maturity (Amarante and Banks, 2002). Pears that were cold stored prior to wax coating were more mature at the time of coating because they were entering the respiration climacteric (rapid ripening) phase at the time of coating. This resulted in a shorter shelf-life and faster ripening than the freshly harvested pears, which were still in a pre-climacteric phase (Amarante *et al*., 2001b) at the time of coating. Pear tissue sensitivity to ethylene (Von Mollendorf, 1996), hypoxic conditions (O_2 levels below 1%) and anaerobic fermentation also increases with fruit maturity (Amarante *et al*., 2001a).

Drake *et al.* (1991) reported that waxing of 'd' Anjou' pears extended shelf-life and retarded ripening, regardless of whether pears were coated directly after harvest or after three months of storage at 1°C. In contrast, coating apples of advanced maturity with SPE coatings resulted in no benefit to the change in titratable acidity during ripening (Santerre, Leach and Cash, according to Sümnü and Bayindirli, 1993). The different results obtained in the two coating scenarios may relate to how far the climacteric phase progressed in the pears and apples respectively, before coating and the difference in gas permeability between the two different coatings.

2.4.2.5 Intrinsic fruit factors: Fruit temperature

Fruit temperature may affect the appearance of the coating. Regardless of the type of edible coating used or the time of coating (after harvest or cold storage), pears were transferred to room temperature (20°C) for 24 hours prior to coating (Drake *et al.*, 1991; Amarante *et al.*, 2001a b, c; Amarante and Banks, 2002). Drake *et al.* (1991) used a coating containing both shellac and carnauba wax, which is often used to coat apples and pears (Baldwin, 1994). The reason for warming pears up prior to coating may relate to moisture condensation that occurs on fruit when moved from

cold storage to a warmer environment. Moisture condensation on the fruit will affect the appearance of the coating and may prevent the wax from adhering to the moist pear surface due to the hydrophobicity of the wax coating.

2.4.2.6 Intrinsic fruit factors: Fruit skin surface

Different pear cultivars have different skin surfaces which may affect coating efficiency in delaying ripening or reducing moisture loss. 'Beurre Bosc' pears have a rough skin (irregular epidermal cells) and require large deposits of coating to block the pores, reduce the skin permeability to gasses and modify the fruit internal atmosphere (Amarante *et al*., 2001c; Amarante and Banks, 2002). 'Doyenne du Commice' pears have smooth skin but the cuticle layer has an interconnected network of cracks, which possibly have much higher permeance to water vapour and other gasses than areas of intact cuticle would have. Increasing the amount of coating deposits (through increased coating concentration) filled these cracks and blocked the pores. The result was a reduction in gas and moisture permeability (Amarante *et al.*, 2001c). Conversely 'Packham's Triumph' pears have a smooth skin (without cracks in the cuticle) and require small increases in coating concentration to modify the internal atmosphere of the fruit (Amarante *et al*., 2001a, c; Amarante and Banks, 2002).

2.5 Gaps in the knowledge

Often the reason for using a particular pear cultivar in coating applications is not given thus the relevancy of the chosen pear cultivar for countries other than South Africa is unclear. There appears to be little information on the typical physiological behaviour (moisture loss, respiratory and ethylene production patterns) and corresponding changes in quality attributes of 'Packham's Triumph' pears during storage under different temperatures. Considering that export pears should be able to ripen normally once they reach the export destination and that pears may be stored for varying periods of time before they are ripened, little information is available on the typical ripening behaviour of 'Packham's' pears. No data on the typical quality attributes for eat- ripe 'Packham's' could be found although the pears are considered to be eat-ripe when the climacteric maximum is reached (Eksteen and Ginsburg, 1977).

Considering the similarity between zein and kafirin protein and although zein is already available commercially as a coating, no data is available on the use of zein protein coatings on any pear cultivars. Although zein coatings were used with great success on apples, the coating concentrations were high (4 to10%, w/w). Fruit coating data using a range of zein coating concentrations, particularly coating concentrations of 5% (w/w) or less, is limited. Although research on kafirin films and their properties exist, no research on kafirin as a coating could be found. As a coating could effectively be a thin film, no information on how zein or kafirin films of different thicknesses differ in functionality, could be found either. As a coating must work in conjunction with the natural mechanisms of a fruit to protect itself against excessive transpiration and respiration, the inherent functionality of films and coatings based on the same material may be quite different.

3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

The rate of physiological and biochemical changes, and hence the rate of ripening, senescence and ultimately quality deterioration of 'Packham's Triumph' pears will increase as the storage temperature in RA at 95% RH increases. This hypothesis is based on the fact that ripening rate increases as storage temperature increases.

It is hypothesized that 'Packham's Triumph' pears stored at 10°C, prior to ripening, will be physiologically more mature and ripen faster at 20°C than pears stored at -0.5°C, because storage at 10°C is not sufficient to retard the onset of pear ripening during storage.

It is hypothesized that a kafirin-based fruit coating will delay ripening and extend the shelf-life of 'Packham's Triumph' pears. This hypothesis is based on the fact that zein protein coatings exhibited good O_2 barrier properties by blocking the pores of the fruit and as a result delayed ripening and extended the shelf–life of apples (Bai *et al*, 2003) and tomatoes (Park *et al.*, 1994). Kafirin coatings have potential to produce similar results because zein and kafirin are both prolamin proteins and similar in solubility, structure (Shull *et al.*, 1991) and amino acid composition (DeRose, Ma, Kwon, Hasnain, Klassy, Hall, 1989). Kafirin may therefore be equally capable of blocking the pores of the fruit.

3.2 Objectives

To determine the physiological and biochemical behaviour of 'Packham's Triumph' pears exposed to ideal temperature (-0.5°C), temperature-abused (10°C) and normal ripening temperature (20°C) conditions used during export and retailing.

To determine the effect of storage duration at ideal temperature (-0.5°C) and temperature-abused (10°C) conditions, on subsequent ripening of 'Packham's Triumph' pears.

To determine the effect of a kafirin coating on the physiological and biochemical behaviour, ultimately shelf-life of ‘Packham’s Triumph’ pears, during ripening at 20°C for 24 days.

4. RESEARCH

The following two research chapters were written according to the format required by the journal, *Postharvest Biology and Technology*. The references used are listed in Chapter 7. A two-phased approach was used in the research. The objective of the first phase was to determine the effect of storage temperature and storage duration on export grade ‘Packham’s Triumph’ pear quality during typical refrigerated storage (-0.5°C), inadequate (temperature-abused) storage conditions at the export destination (10°C) and ripening (20°C) under regular atmosphere (RA) conditions. Two experiments, which ran concurrently, were carried out during Phase 1 and subsequently form research chapter 4.1. In Experiment 1, the effect of storage temperature and storage duration on pear quality during refrigerated storage (-0.5 and 10°C) and ripening (20°C) was determined. The effect of refrigerated storage time and temperature (-0.5 and 10°C) on the subsequent ripening rate (at 20°C) and the quality of the ripened pears was studied in Experiment 2. The results from these two experiments provided the baseline information that was required to plan Phase 2 of the research.

In Phase 2, ‘Packham’s Triumph’ pears were coated with a kafirin protein coating. Coated and uncoated pears were ripened at 20°C during which time the quality and shelf-life of the two pear groups were monitored to determine the effect of the coating on pear physiology and shelf-life. Phase 2 forms research chapter 4.2.

4.1 The effect of storage temperature and duration of storage on the physiological behaviour and quality of 'Packham's Triumph' pears

Abstract

In order to reduce postharvest losses of 'Packham's Triumph' pears during export, more information was required on the physiological and biochemical behaviour of this cultivar under ideal refrigerated (-0.5°C), inappropriate or temperature-abused (10°C) and typical ripening (20°C) conditions. In Experiment 1, pears were stored at –0.5°C, 10°C and 20°C and 95 to 98% RH for 42, 42 and 21d respectively. No ripening or significant (P> 0.05) ripening-related quality changes (i.e. colour, flesh firmness, titratable acidity, soluble solids content) occurred in pears stored at -0.5°C. Pear respiration rate at 20°C was significantly higher (P < 0.001) and quality changes occurred faster than that of pears at 10°C. For pears at 10°C, quality changes indicated that physiological ripeness was probably reached between 21 and 35d, while senescence set in around day 35. Pears stored at 20°C reached physiological ripeness within six days, after which senescence set in. Due to their accelerated ripening rate, only pears stored at 20°C lost moisture significantly (P < 0.001) and exhibited shrivelling.

In Experiment 2, 'Packham's Triumph' pears were stored at ideal, refrigerated (-0.5°C) and temperature-abused (10°C) conditions and 95 to 98% RH for 42 and 35 days, respectively. Subsequently, pears were placed at 20°C for 7 days to determine if storage prior to ripening affected the ripening rate and final quality of the ripe fruit. During storage at 10°C, pears exhibited an increased metabolic activity over pears stored at -0.5°C and consequently reached maximum softness and yellowness faster than pears stored at -0.5°C, when transferred to 20°C. Storage duration prior to ripening caused reduced flesh firmness and increased de-greening and yellowing of the ripe pears. The final texture and colour of ripe pears, stored at -0.5 and 10°C prior to ripening, were not significantly different after 7 days of ripening. Extended storage of pears at either -0.5 or 10°C did not significantly affect the acidity or sweetness of pears during ripening. Storage at temperature-abused (10°C) conditions accelerated ripening and the attainment of the required quality attributes.

Extended storage at 10°C resulted in softer, more yellow pears that reached senescence faster than pears stored at -0.5°C.

4.1.1 Introduction

The Southern African deciduous fruit industry is worth several billions of Rands in earnings, mainly from export. However, postharvest decay is a major limiting factor in successful fruit marketing and export. Foreign exchange earnings would increase if postharvest decay was reduced (Anon., 2003). Pears are climacteric fruit and harvested unripe but physiologically mature. 'Packham's Triumph', one of South Africa's favourite pear varieties, is exported in the largest quantities (Anon, 2003; Anon, 2005b).

Shelf-life was defined by Bester (1973) as the retention of original product quality for the period required to obtain marketing goals. The shelf-life is considered to be related to fruit respiration rate which, in turn, increases as temperature increases (Kader and Barrett, 1996). The cold chain (i.e. storage of pears at -0.5 or even 0°C) may be maintained relatively well during export of pears from South Africa to Europe. However, during the subsequent distribution and sale of pears at fruit and vegetable markets or small retailers abroad, storage conditions may be inappropriate (10°C or higher and under RA conditions) and thus not able to maintain pear shelf-life, effectively. Following the storage of pears at such inappropriate (temperature-abused) conditions (10°C), pears would be purchased by consumers and exposed to ambient temperature until the fruit are considered fit for consumption. This begs the question of how long inadequately stored pears will take to ripen and whether the physiological behaviour and quality of these pears will be different to ripe pears that were stored at ideal
(-0.5°C) conditions before ripening.

Apart from accelerated ripening, another undesirable occurrence during export and retailing of 'Packham's Triumph' pears is moisture loss, which results in stem-end shrivelling. 'Packham's Triumph' pears tend to shrivel during extended periods of cold storage (Coetzee, Logistics manager, Kromco Limited, personal communication, 2002). Shrivelling is the result of excessive weight loss through loss of water in fruit

and manifests as wrinkles on the fruit skin (Hatfield and Knee, 1988). Although the amount of water loss, and consequently weight loss that results in a shrivelled appearance in 'Packham's Triumph' pears is not known, shrivelling in pears starts at the proximal (stem-end) and gradually expands towards the distal (calyx end) during storage under conditions of low humidity (Asakura *et al*., 2001). When warm pears are cooled with cold air, the vapour pressure difference between the fruit and the environment results in water movement out of the fruit. During storage, the movement of cold air is considered the main reason for weight loss and consequently shrivelling (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication).

Although it is known that pear metabolism is slow at -0.5°C, typical respiration and moisture loss data for 'Packham's' Triumph' pears during storage is not available in literature. In addition, it is known that 'Packham's Triumph' pears reach physiological ripeness (the climacteric maximum) after seven days of ripening at 20°C (Amarante *et al*., 2001a; Amarante and Banks, 2002) but typical ripening behaviour information for 'Packham's' is not available in literature either. In order to design methods that would reduce shrivelling during storage and extend pear shelf-life, even under inappropriate storage conditions, information regarding the typical physiological behaviour of 'Packham's' under storage and ripening conditions are required.

The research reported here investigated the effect of storage temperature on physiological behaviour and quality of 'Packham's Triumph' pears (including moisture loss) during ideal refrigerated storage (-0.5°C), inappropriate (temperature-abused) storage at the export destination (10°C) and ripening (20°C), in air at 95 to 98% RH. Additionally, extended storage at -0.5 and 10°C, its effect on subsequent pear ripening at 20°C and the quality attributes of the ripe pears, were investigated.

4.1.2 Materials and methods

4.1.2.1 Plant material and storage conditions

In March of 2003, pears (*Pyrus communis* L.) of the export variety 'Packham's Triumph' were procured from Tru Cape (Grabouw, Western Cape). Pears of similar size (70 pears per 12.5 kg box; average pear surface area 0.0147 m^2) were used in

all experiments. No information was available from Tru Cape on the harvest and packing maturity of the fruit and fruit history prior to the start of Experiments 1 and 2, was therefore unknown. Pears were stored at -0.5°C for approximately 48 h prior to the start of the experiments.

For each storage condition (–0.5, 10 and 20°C) pears were randomly divided into six sealable plastic containers (80l in volume, 60 cm in length, 45 cm in width, 34 cm in height) by placing 38 pears per container in a mono-layer onto a shelf. Each shelf was suspended 12 cm above a layer of water (10l / container) to create a relative humidity of approximately 95 to 98% in each container. Three air holes (15 mm in diameter) were drilled in two sides of each container just below the lid, to prevent anaerobic conditions from developing. Inside each container fruit were placed loosely and six fruit were stored in an open glass bowl (25 cm in length, 20 cm in width, 8.5 cm in height) for respiration and moisture loss analyses.

4.1.2.2 Experimental design

Experiment 1

The effect of storage temperature on the physiological behaviour and quality of 'Packham's Triumph' pears during storage at -0.5°C, 10°C and 20°C (95 to 98% RH) was investigated. The physiological behaviour (respiration rate and moisture loss) during storage was measured on three groups of pears that were stored at -0.5, 10 and 20°C, respectively. Each group consisted of 36 pears (six glass containers of six pears each), which were used repeatedly at each time interval for the analyses. Pears stored at -0.5 and 10°C were analysed up to 42 days at two weekly and weekly intervals, respectively. Pears at 20°C were analysed daily up to day 12 and subsequently day 14 and 16.

Quality evaluations were conducted as follows. At each time interval, six pears from each storage temperature were evaluated at 20°C in terms of colour change, flesh firmness, titratable acidity and total soluble solids immediately after removal from storage. Pears stored at -0.5 and 10°C were analysed up to day 42 at two weekly and weekly intervals, respectively. Pears stored at 20°C were analysed every two days up to day 20. Day zero of the storage trial refers to the start of the trial.

Physiological behaviour and quality attributes of the fruit were measured on day zero after the fruit had been stored at the respective temperatures for four hours.

Experiment 2

Experiment 2 ran concurrently with Experiment 1. Experiment 2 determined the effect of refrigerated storage time at -0.5 and 10°C (95 to 98% RH) on the subsequent ripening rate at 20°C and the quality of pears after ripening. In Experiment 2 pears were refrigerated at -0.5°C and 10°C (95 to 98% RH) for 42 and 35 days respectively. Pears that required storage at -0.5°C were removed for analysis on day zero and subsequently every two weeks up to day 42. Pears that required storage at 10°C were stored at 10°C for four hours on day zero, prior to removal for analysis. Subsequently pears at 10°C were removed weekly up to day 35. Six pears from each storage temperature (one from each 80l container) were removed at each analysis interval and ripened at 20°C (95 to 98% RH) for 7 days in storage containers similar to those used for storing pears at -0.5 and 10°C. On days 0 and 28 (for pears from -0.5°C) and days 0, 14 and 28 (for pears from 10°C) respiration rate was measured daily during ripening at 20°C (95 to 98% RH). On day 7, quality analyses were performed on the ripened pears.

4.1.2.3 Analyses

Respiration rate was measured over a period of 10 min using an Infra Red Gas Analyser (LI-COR gas analyser, model LI-6262, CS Africa, Somerset West, South Africa) in a closed system. Preliminary trials indicated that monitoring respiration rate for 10 min was sufficient time for the fruit to respire normally. Nitrogen gas (99.9% pure) served as reference gas to the IRGA. Throughout the storage period, respiration rate was determined once per measurement interval on the same replicates (six groups of six pears each) for each storage temperature. Six pears were sealed in a gas tight glass container (3.5 l in volume) with ports in the lid for incoming and outgoing gas streams. For Experiment 2 respiration rate was determined on six replicates (six individual pears), one pear from each storage container at the respective storage temperatures. One pear was sealed in a gas tight glass container (3.5 l in volume) along with two plastic, sealed bottles with a volume of 1l each. The bottles were used to reduce the space in the glass container.

In both experiments the gas (air and CO_2 produced by the pears) in the system was circulated between the glass container and the IRGA via a small pump enclosed in a desiccator Preliminary tests indicated that gas circulation through the system for two minutes was required before respiration rate could be measured. Subsequently the linear increase in CO_2 concentration of the six pears was recorded for five minutes. On completion of the measurements the pears were removed, after which the glass container and the desiccator were opened and air was circulated through the system for one min prior to the analysis of the next group of pears. The respiration rate was expressed as mg CO_2 kg^{-1} h^{-1}. For Experiment 2 the daily respiration rate at 20°C was calculated as an average of the respiration rates of the six replicates and expressed as mg CO_2 kg^{-1} h^{-1}.

Weight loss, as an indication of moisture loss, was determined throughout the storage period using the same groups of pears used for the respiration rate analyses. Results were expressed as percentage moisture loss on a fresh mass basis, calculated by weight difference from the fruit weight on day zero, the start of the experiment.

Quality measurements (background colour, flesh firmness, titratable acidity and soluble solids content) were conducted at 20°C. For Experiment 1, one pear was removed from each of the storage containers and these six pears were used in all the quality measurements. For Experiment 2 quality measurements were also conducted at 20°C, but on the same six pears used for the respiration rate measurements.

Colour was measured in triplicate on the cheeks of the sun-side of six pears per storage treatment using a Unifruco colour chart and a ColourQuest Hunter colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, Va., U.S.A.). Only Hunter a* and b* values were measured, where a* and b* values represent the chromaticity dimensions as follows: a* measures redness when positive and greenness when negative; b* measures yellowness when positive and blueness when negative (Anon., 2006a).

Flesh firmness on the sun-side of six pears per analysis was measured with a Stable Micro Systems Texture Analyser (Model TA-XT2*i*, Wirsam Scientific Ltd., Johannesburg, South Africa) using a 2 mm stainless steel probe. The analysis method followed was based on the Stable Micro Systems Applications Study on the firmness measurement of St. William pears by probing (REF: PEAR 1/P2). Pears were cut open longitudinally. A strip of peel (10 mm wide, 30 mm long) was removed with a potato peeler from the pear cheek along the equatorial plane. The probe was inserted, at three points (10 mm apart) at a rate of 5 mm/s up to a distance of 10 mm and the compression force was recorded in Newton (N). Means were calculated from the three readings per side of the fruit. The firmness of the flesh directly below the peel (external flesh firmness) and at 10 mm deep (internal flesh firmness) was recorded.

Titratable acidity (g malic acid/ 100 g juice) and the Soluble Solids Content (SSC) were determined at 20°C on the clear pear juice of six individual pears per analysis interval. Each pear was liquidised into a pulp in a Kenwood kitchen liquidiser. The pulp was filtered through Whatman no. 4 filter paper and the filtrate (clear juice) was used for subsequent analyses. For determination of titratable acidity, pear juice (6 g) was combined with distilled water (50 ml) and titrated with NaOH (0.1 N) to an end point of pH 8.1 (measured with a pH meter). Juice mass and the volume of NaOH titrated were recorded.
The following formula was used to calculate the grams malic acid/ 100 grams of juice:

malic acid (g)/ 100g juice = $V \cdot N \cdot (67) \cdot (100) / M \cdot 1000$

where: V, volume of NaOH used in titration (ml); N, normality of NaOH (mEq/ml); 67, molecular mass of malic acid divided by 2 (g/mol); M, mass of juice (g); 1000, factor relating mg to grams (mg/g, 100/1000) (Garner, Crisosto, Wiley and Crisosto, 2005). Soluble Solids Content (SSC) was measured by a refractometer (ATAGO, Japan) and expressed as °Brix. Ethylene content could not be analysed consistently due to technical problems experienced with the equipment and the analysis was subsequently omitted from the study.

For Experiment 1 statistical analysis of data was conducted using the SAS for Windows programme (SAS 9.1.3, SAS Institute Inc. Cary, NC, USA, 2002-2003). Mean values, standard deviations, analysis of variance (ANOVA) followed by Tukey's multiple comparison tests, were performed at a 95% confidence limit ($P < 0.05$). It was combined with analysis of data using STATISTICA (version 7.1, StatSoft, Inc., Tulsa, USA). Statistical analyses of data in Experiment 2 were conducted by STATISTICA (version 7.1, StatSoft, Inc., Tulsa, USA). Least square mean values and standard errors, analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) multiple comparison tests, were performed at a 95% confidence limit ($P < 0.05$). In Experiment 2, Fisher's LSD test was chosen over Tukey's multiple comparison test, because it was found that Tukey's procedure can produce inconsistent results if the study contains only a few experimental replicates. Fisher's LSD procedure was found to be less inconsistent (Saville, 1990).

4.1.3 Results and discussion

4.1.3.1 Effect of storage temperatures on physiological behaviour and pear quality during ideal, refrigerated storage (0.5°C), temperature-abused storage (10°C) and ripening (20°C)

Physiological activity and ripening processes were accelerated as the storage temperature increased. This is evident by comparison of the respiration rates, moisture/ weight loss and quality attributes of the pears from the different storage temperatures.

Respiration rate

The respiration rates of the 'Packham's' pears measured in this study compared favourably with those reported in literature, for pears in general. Findlay and Combrink (1996) reported respiration rates ranging between 30 to 70, 8 to 21, and 3 to 7 mg CO_2 $kg^{-1}h^{-1}$ for pears stored at 20, 10 and 0°C, respectively. As expected, the respiration rate of 'Packham's' stored at 20°C was significantly higher ($P < 0.001$) than that of pears stored at 10 (Fig. 4.1.1) and more than 10 times higher than that of pears stored at approximately -0.5°C (Fig. 4.1.1). No climacteric peak was observed

throughout the storage period for pears at -0.5°C, which indicated that the metabolic activity of the refrigerated pears was low.

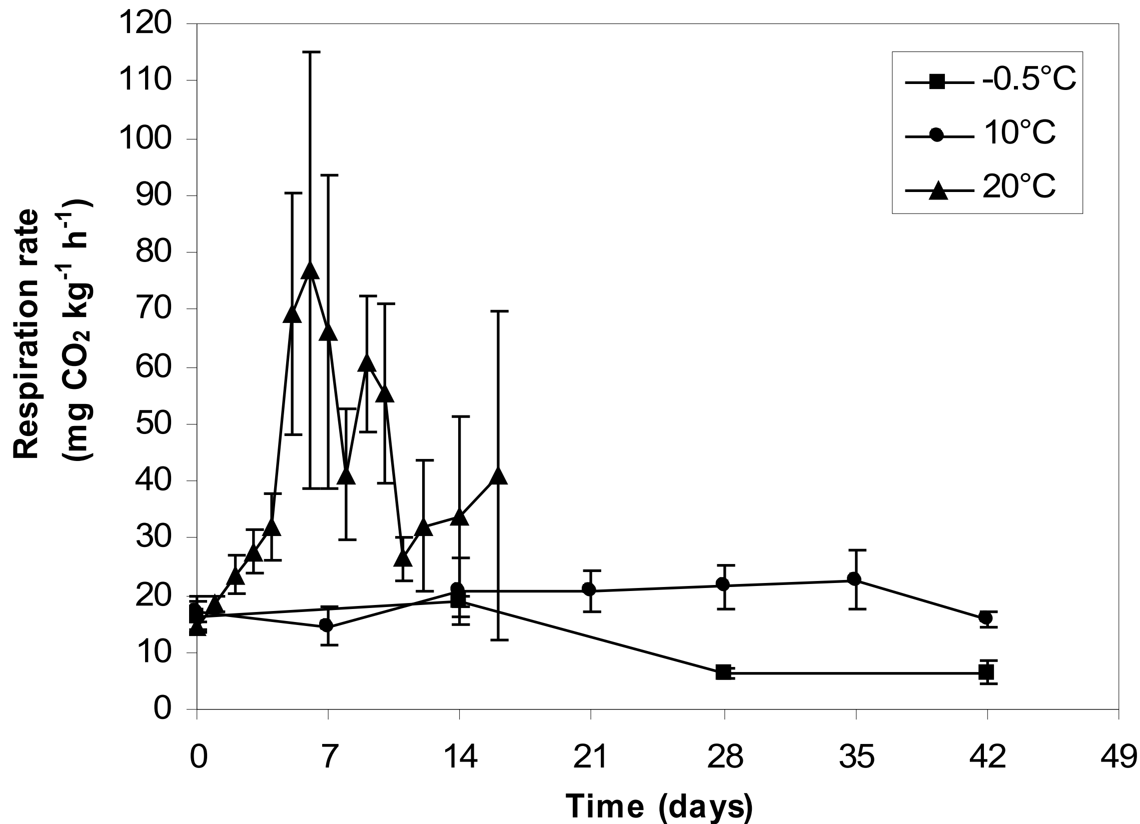


Fig. 4.1.1. Effect of storage temperature (-0.5, 10 and 20°C) on the respiration rate of 'Packham's Triumph' pears during storage at 95 to 98% RH, expressed as mg CO_2 kg^{-1} h^{-1}. Data are means of 6 samples of 6 pears each (n=36). Bars represent standard deviations of the means

The respiration rate of pears stored at 10°C (Fig. 4.1.1) increased significantly ($P < 0.05$) from day seven to 14 (from 14.6 to 20.6 mg CO_2 kg^{-1} h^{-1}, respectively), but subsequently showed no significant ($P > 0.05$) change up to day 35. However, the respiration rate declined significantly ($P < 0.01$) from day 35 to 42 (22.6 to 15.8 mg CO_2 kg^{-1} h^{-1}, respectively). Such a drastic decline in respiration rate is usually an indication of the progression of senescence in the pear when the fruit requires increasingly less energy to maintain its biochemical and physiological functions as it nears the end of its postharvest life. Thus, senescence may have progressed between days 35 and 42 (Fig 4.1.1). However, as testing intervals were not

sufficiently frequent, one can only speculate about the actual occurrence of the climacteric peak. Testing intervals should, at most, have been two days apart. Interestingly, the respiratory response in pears at 10°C up to day 14 appeared to be erratic and was not characterised by any quality changes. Furthermore, although the pears ripened, no sharp increase in respiration rate was observed.

According to Romani (1984), an increased respiration rate is a compensatory or homeostatic response to the stress of developing senescence. Thus, the climacteric represents the ultimate homeostatic response by the fruit to senescence and the postclimacteric phase is a result of unsuccessful homeostasis. Furthermore the magnitude of the homeostatic response decreases with senescence. Therefore the respiratory response of pears (at 10°C) up to day 14 was probably a result of successful homeostasis. However, pears may have matured during storage, which would have rendered them more prone to senescence. As a result the magnitude of the climacteric was low or had already passed.

For pears stored at 20°C (Fig. 4.1.1) there was no significant difference ($P > 0.05$) between the respiration rates recorded on days five to seven although respiration rates on days five and seven were significantly ($P < 0.05$) higher than that recorded on days 4 and 8, respectively. Thus, it would appear that the climacteric peak may have been reached between days five and seven (maximum rate recorded was 76.7 mg CO_2 kg^{-1} h^{-1} at day six). The respiration rates measured beyond day ten were significantly ($P < 0.05$) lower than the maximum rate of 76.7 mg CO_2 kg^{-1} h^{-1} on day six.

Weight loss and shrivelling

Pears stored at 20°C lost significantly ($P < 0.001$) more weight and thus more moisture than pears stored at either 10 or –0.5°C (Fig. 4.1.2). The first sign of shrivelling in pears stored at 20°C was observed after only four days of storage at 95 to 98% RH at which point the pears had lost approximately 1.2% moisture.

The fruit skin, and in particular the cuticle, acts as a barrier to moisture during transpiration (Maguire *et al.,* 2001). Many factors influence water vapour permeability of the cuticle, which in turn affects weight loss through transpiration and

ultimately causes shrivelling. The high moisture loss at 20°C may be attributed to the higher metabolic activity, brought about by the high storage temperature. An increase in temperature affected fruit respiration rate and possibly the permeance of the cuticle. Schonherr (according to Maguire *et al.,* 2001) found that increased storage temperatures increased the moisture permeability of the cuticle of apples (Maguire *et al.,* 2001). Similarly, Henze (1995) found that that fresh weight loss, and thus moisture loss, of 'Conference' pears at 1°C, 4°C and 16°C respectively, increased when storage temperature increased. From the decrease in respiration rate beyond day seven (Fig. 4.1.2), it may be assumed that the pears entered the post climacteric (senescent) phase after day seven.

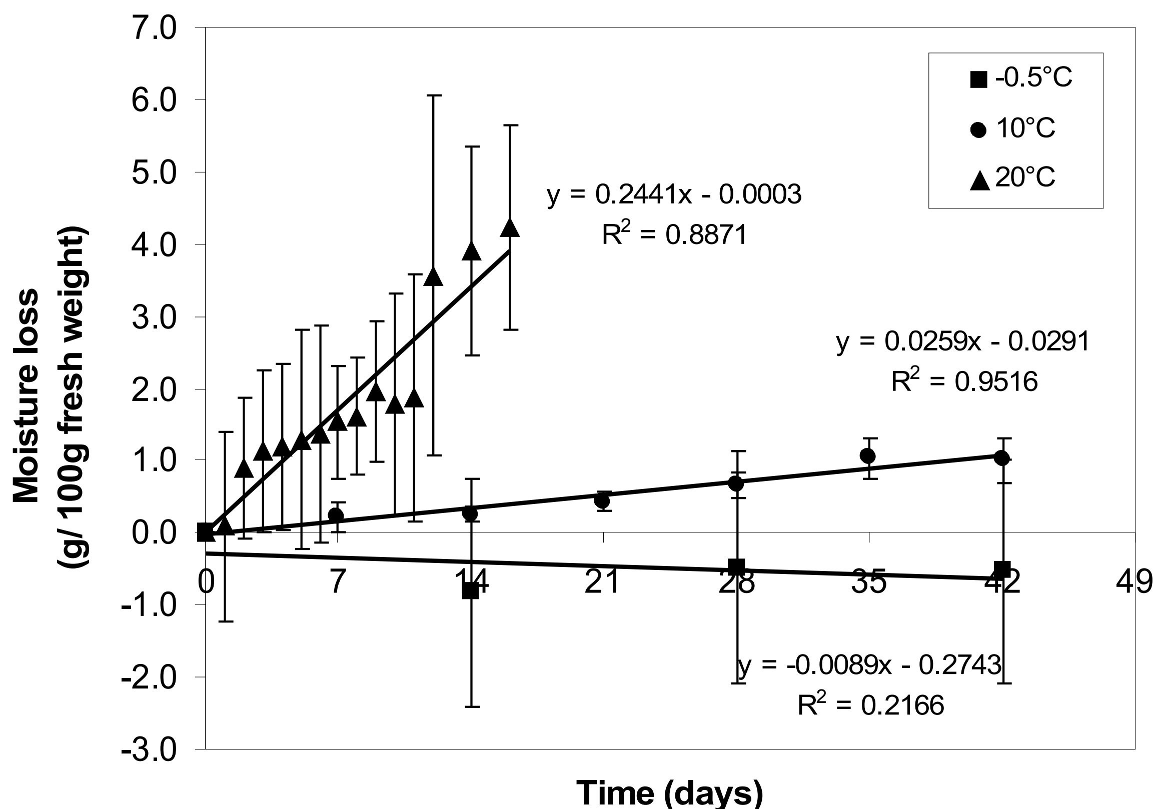


Fig. 4.1.2. Effect of storage temperature (-0.5, 10 and 20°C) on the cumulative moisture loss of 'Packham's Triumph' pears during storage at 95 to 98% RH. Lines represent the best fit for the moisture loss at the different storage temperatures. Bars represent standard deviations of the means (n=36)

Moisture loss and shrivelling was not only a function of temperature because 'Packham's' pears at 10°C did not loose significantly ($P > 0.05$) more moisture than

pears at -0.5°C and stem-end shrivelling was not observed on pears at -0.5 or 10°C either. This occurrence may be a result of the combination of sufficiently low storage temperature (10°C) and sufficiently high humidity to reduce vapour pressure difference between the fruit and the surrounding atmosphere. According to Kader and Barrett (1996) transpiration rate is dependant on the vapour pressure difference between the fruit and the surrounding atmosphere. The vapour pressure difference is influenced by temperature and relative humidity. Asakura *et al*. (2001) found that Japanese pears, stored at 10°C and 100% RH, exhibited a fresh appearance with minimal weight loss.

Colour and flesh firmness

According to the Unifruco colour chart (Fig. 4.1.3) pears at –0.5°C de-greened very slightly over the 42-day storage period, from a value between 2.0 and 2.5 (day 0, beginning of storage trial) to between 3.0 and 3.5.

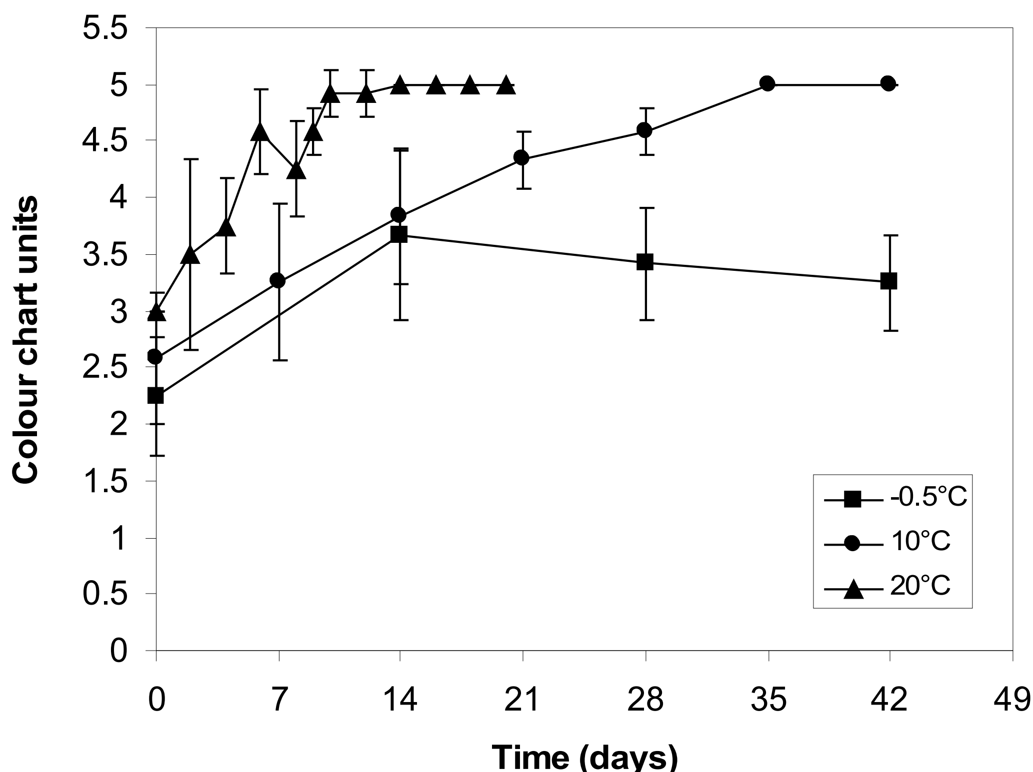


Fig. 4.1.3. Effect of storage temperature (-0.5, 10 and 20°C) and duration on the Unifruco colour chart values of ‘Packham’s Triumph’ pears during storage at 95 to 98% RH. A colour chart value increase of 0.5 indicates a marked colour change. Bars represent standard deviations of the means (n=6)

Preliminary tests on ripening ‘Packham’s’ indicated that when the climacteric maximum was reached, pear colour had a colour chart value of approximately 4. This colour value was achieved after 21 and six days of storage for pears at 10 and 20°C, respectively (Fig. 4.1.3).

The Hunter a* and b* values (Fig. 4.1.4a and 4.1.4b) supported the findings of the Unifruco colour chart. The increase in a* values, particularly its transition from negative to positive, as well as the increase in b* values generally indicates the loss of green colour and the revelation of yellow colour, respectively, in the pears. In apples, the colour changes occurring during fruit ripening can be attributed to chlorophyll degradation (loss of green colour), which reveals the underlying yellow carotenoids that are already present (Lurie, 1998). This may also be the case for pears as both fruits fall under the pome fruit sub-family (Jackson, 2003). Despite the apparent trend of increasing a* and b* values for pears stored at –0.5°C, no significant ($P > 0.05$) de-greening (a* values) or yellowing (b* values), occurred throughout the storage period. Although contradictory to the Unifruco colour chart results for pears at -0.5°C, the latter is less sensitive than the HunterLab for colour changes.

For pears stored at 20°C a maximum a* value (maximum de-greening) of 4.99 ± 1.05 was reached by day nine of storage. Pears stored at 10°C reached a similar maximum a* value (5.61 ± 1.58) after 28 days (Fig. 4.1.4a). Pears stored at 10 and 20°C de-greened significantly ($P < 0.05$) more and increased in yellowness significantly ($P < 0.05$) more (increasing b* values) than pears stored at –0.5 °C (Figs 4.1.4a and 4.1.4b). Similarly, Henze (1995) found that an increased storage temperature accelerated the colour change in pears from green to yellow. The argument that the climacteric peak for pears at 10°C was reached between day 21 and 35 is supported by the “ripened colour” (Fig. 4.1.3) that was achieved after 21 days and the maximum a* value reached at day 28 (Fig. 4.1.4a). With this in mind, the reduction in b* values between days 35 to 42 may suggest that the pears were becoming senescent after day 35.

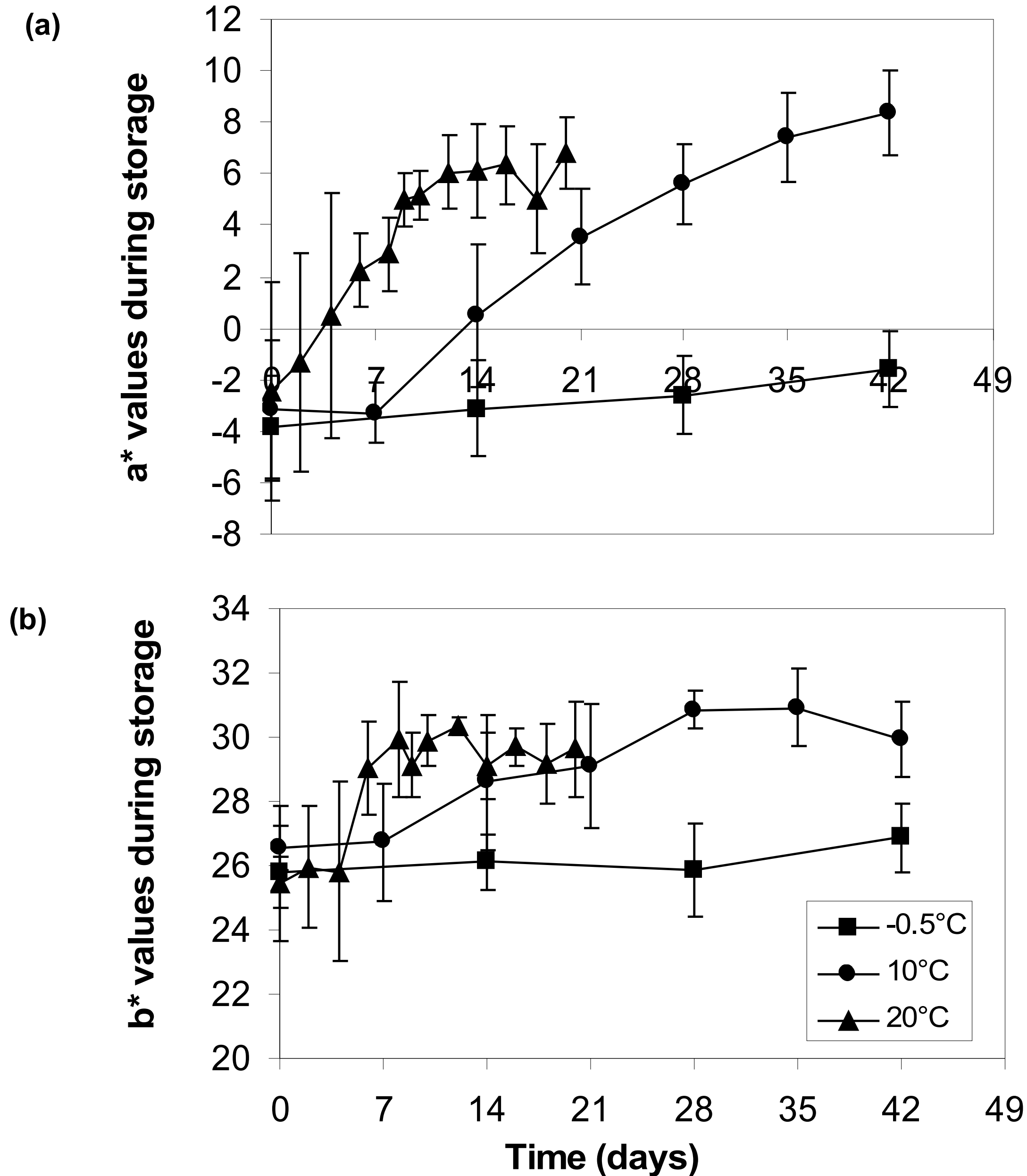


Fig. 4.1.4. Effect of storage temperature (-0.5, 10 and 20°C) and duration on (a) Hunter a* and (b) b* colour values during storage of 'Packham's Triumph' pears at 95 to 98% RH. Where negative a* values are green and positive a* values are red, negative b* values are blue and positive b* values are yellow. Bars represent standard deviations of the means (n=6)

Storage temperature had a significant ($P < 0.001$) effect on the external flesh firmness (immediately below the pear skin) and flesh firmness internal to the pear (10 mm deep) (Fig. 4.1.5). Interestingly, during storage at -0.5°C (Fig. 4.1.5) pears softened significantly ($P < 0.05$) during storage to ultimately reach an average

(between internal and external texture) value of 1.53 N on day 42. The low metabolic activity, indicated by the low respiration rate and lack of de-greening during storage at -0.5°C, rules out ripening as the primary reason for the softening that occurred during storage, especially between days 28 and 42.

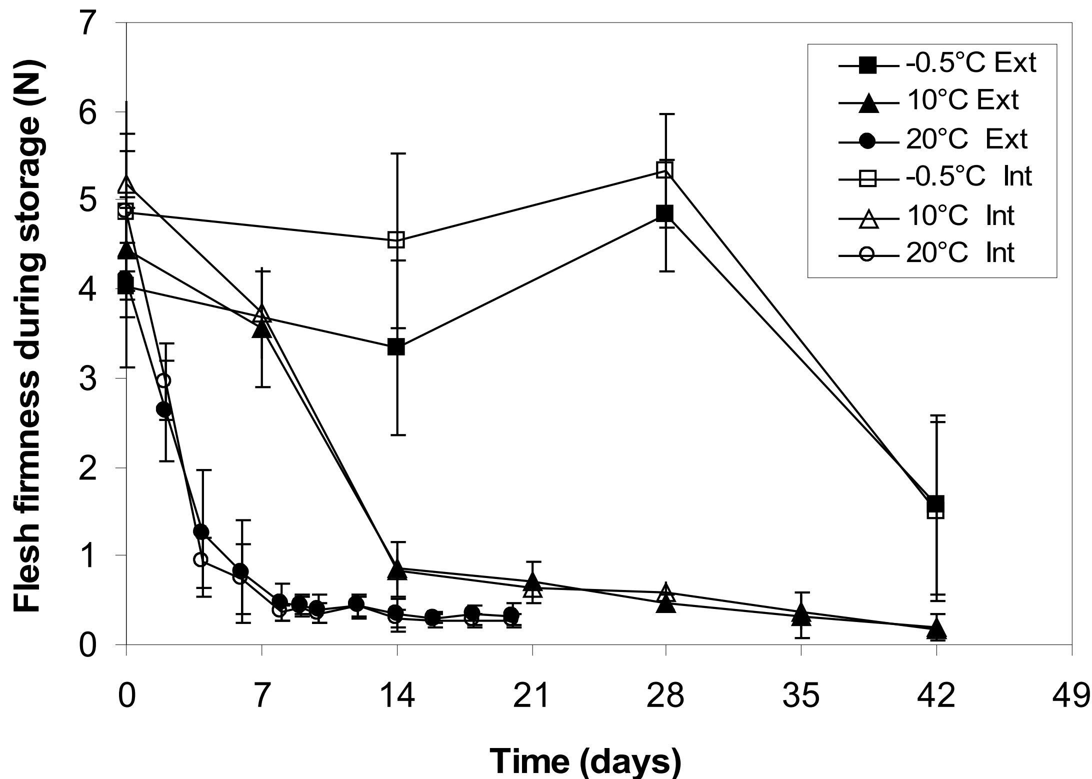


Fig. 4.1.5. Effect of storage temperature (-0.5, 10 and 20°C) and duration on flesh firmness immediately below the pear skin (external) and internal to the pear (10 mm deep), in 'Packham's Triumph' pears at 95 to 98% RH. Bars represent standard deviations of the means (n=6)

Some softening during storage is not uncommon. According to Retamales, Campos and Castro (1998) the storage of 'Packham's' pears at about -0.5°C for 120 days in air, resulted in a decline in flesh firmness during cold storage. Softening at -0.5°C (Fig. 4.1.5) may have been a result of a break in the cold chain prior to the start of the experiment. Alternatively, softening may have been due to poor temperature management in the refrigerator, which resulted in the formation of ice crystals in some of the pears, leading to damaged cells and a loss of firmness upon thawing. This theory is supported by Findlay and Combrink (1996) who found that in general,

storage of pears below –0.4°C resulted in the formation of expanding ice crystals within the cells that ruptured the cell walls and caused physiological injury to the pears. This leads to increased respiration and ethylene production rates once the pears are removed from storage (Fonesca *et al.*, 2002). In this study, however, an increase in respiration rate was not noted because respiration rate on the pears was not conducted after removal from -0.5°C.

'Packham's' pears ripened during storage at 10°C over the 42 day period (Fig. 4.1.5). Except for the significant ($P < 0.01$) difference between internal (± 4.1 N) and external (± 5.0 N) flesh firmness on day 0, internal and external texture did not vary significantly ($P > 0.05$) during storage. Similarly for pears at 20°C internal and external flesh firmness only differed significantly ($P < 0.001$) on day four (Fig. 4.1.5). This may imply that internal and external flesh firmness changes simultaneously during ripening. Overall flesh firmness at 20°C decreased significantly ($P < 0.001$) up to day eight, which coincides with the high metabolic activity depicted by the respiration rate data up to day eight (Fig. 4.1.1). The insignificant ($P > 0.05$) changes in flesh firmness after eight days may imply that pears were completely ripe by day eight and would not be able to soften further during senescence (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication).

Overall, the rate of softening increased as the storage temperature increased. In fact, a two or three-fold increase in the rate of biological reactions can be expected with every 10°C increase in temperature (Anon., 2006b). This may explain why pears at 10°C softened twice as fast in half the time of pears at -0.5°C. Similarly, it explains why pears at 20°C softened twice as fast as pears at 10°C in the same amount of time. Interestingly, irrespective of the temperature of storage (10 or 20°C) the pears softened to a similar extent by the end of the respective storage periods probably because pears were completely ripe (attained maximum ripeness).

Increases in colour and textural changes are related to increased respiration rates as a result of an increased storage temperature and are also linked to increased internal ethylene concentrations during storage. Although neither internal ethylene concentrations nor ethylene production rates were measured in this study, its action in pear ripening should not be ignored. Johnston, Hewett, Hertog and Harker (2002)

found that yellowing, softening and respiration rate in ‘Granny Smith’ and ‘Pacific Rose’ apples were most likely mediated by ethylene. A minimum ethylene (less than 1 µL kg^{-1}. h^{-1}) production rate is required to trigger a rise in respiration rate (the climacteric) and subsequent ripening (Macrae *et al*., 1993). Cold treatment is known to promote the synthesis of internal ethylene in pears (Murayama *et al*., 1995). Due to the autocatalytic nature of ethylene, continued synthesis during cold storage would mean that ethylene levels persisted to increase throughout the 42 day storage period of pears at 10°C, thereby mediating and hastening the ripening of pears at 10°C. This theory is supported by Romani (1984) who found that endogenous ethylene may hasten senescence of fruit.

Titratable acidity and soluble solids content

Generally, during fruit ripening, the levels of organic acids decline because they are converted to sugars during aerobic respiration. As a result, fruit acidity declines as fruit ripen (Wills *et al*., 1998). The low metabolic activity and thus the low energy requirement of the pears at -0.5°C may explain why the titratable acidity (TA) (Fig. 4.1.6a) and the soluble solids content (SSC) (Fig 4.1.6b) of pears did not differ significantly ($P > 0.05$) over the 42 days of storage at –0.5°C.

Although there was no significant ($P > 0.05$) change in TA within the first 28 days of storage at 10°C, TA decreased significantly ($P < 0.05$) from 28 to 35 days (Fig. 4.1.6a). The latter may suggest that fruit were entering the climacteric phase and required the conversion of acids to sugars for energy. In fact, this may suggest that the climacteric peak may have been reached on approximately day 35.

The steady decline in SSC of pears at 10°C during days 0 to 35 (Fig. 4.1.6b) may have been related to the relatively low energy requirements (lower at 10°C than at 20°C) of the pears during the pre-climacteric phase and the availability of organic acids for conversion into energy. The significant increase in the SSC content from day 35 may be the result of starch breakdown (not measured in this study) to provide the necessary energy for the biochemical changes occurring during the senescent phase.

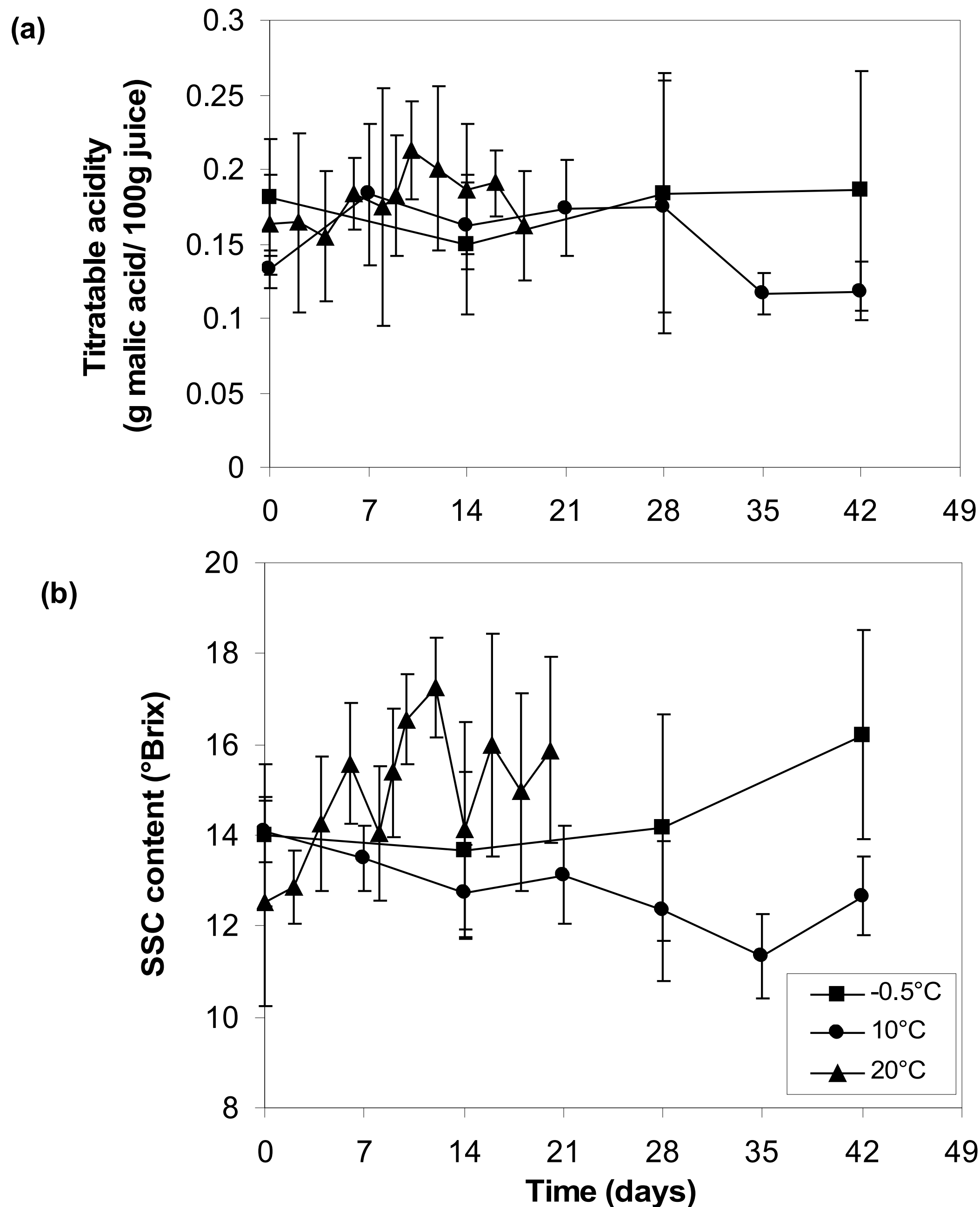


Fig. 4.1.6. Effect of storage temperature (-0.5, 10 and 20°C) and duration on the (a) titratable acidity and (b) soluble solids content of 'Packham's Triumph' pears stored at 95 to 98% RH. Bars represent standard deviations of the means (n=6)

The insignificant (P > 0.05) change in TA of pears stored at 20°C (Fig. 4.1.6a) may suggest that sugars were predominantly used as the preferred substrate for respiration as sucrose is the main respiratory substrate used for energy requirements in most ripening fruits (Macrae *et al.,* 1993). The increased levels of SSC in the pears during ripening at 20°C (Fig. 4.1.6b) supports this suggestion and can be

attributed to the enzymatic hydrolysis of starch to sugars that is typical of pear ripening (Macrae *et al.,* 1993).

4.1.3.2 Effect of storage duration at -0.5 and 10°C prior to ripening, on physiology and pear quality during and after ripening (20°C)

Respiration rate

Even though pears from Experiments 1 and 2 were from the same batch and harvested at the same time, pears from -0.5°C that were ripened on day zero of Experiment 2 (Figs 4.1.7a) did not reach physiological ripeness on the same day as pears at 20°C from Experiment 1 (day 6). No clear climacteric peak was observed during ripening for pears (from -0.5 and 10°C) that were ripened on day zero of Experiment 2 (Figs 4.1.7a and 4.1.7b). In addition, the maximum respiration rates of pears from -0.5 and 10°C in Experiment 2, was 60.2 mg CO_2 kg^{-1} h^{-1} on day 7 (Fig. 4.1.7a) and 82.8 mg CO_2 kg^{-1} h^{-1} on day 2 (Fig. 4.1.7b), respectively.

The rate for pears from -0.5°C was different to that of pears from Experiment 1 (76.7 mg CO_2 kg^{-1} h^{-1}). The variation is most likely the result of analysing six pears individually in Experiment 2. In fact, variability in the respiratory data of Experiment 2 (Figs 4.1.7a and 4.1.7b) emphasises the importance of measuring respiration rate on groups of pears (instead of just one pear) and the inclusion of more than six replicates or groups for each analysis.

When mean respiration rates of pears ripened on day zero and after 28 days of storage at -0.5°C were compared (Figs 4.1.7a and 4.1.7b), there was no significant difference ($P > 0.05$) between the respiration rates of pears on corresponding days of ripening. Data variation may account for the lack of difference between the two groups of pears. The individual maximum respiration rates for the six pears ripened on day zero were compared and large standard deviations were observed (raw data not shown). It appeared that the variation stemmed from the different pears reaching a climacteric peak on different days, rather than from variation in the magnitude of the maximum respiration rates (raw data not shown). The variation in time to peak may be related to the different levels of maturity of the individual pears. According to Van der Merwe (1996), pear maturity may vary as a result of fruit size and the

location of the fruit on the tree hence fruit from the bottom of the tree tend to be more mature than fruit from the top.

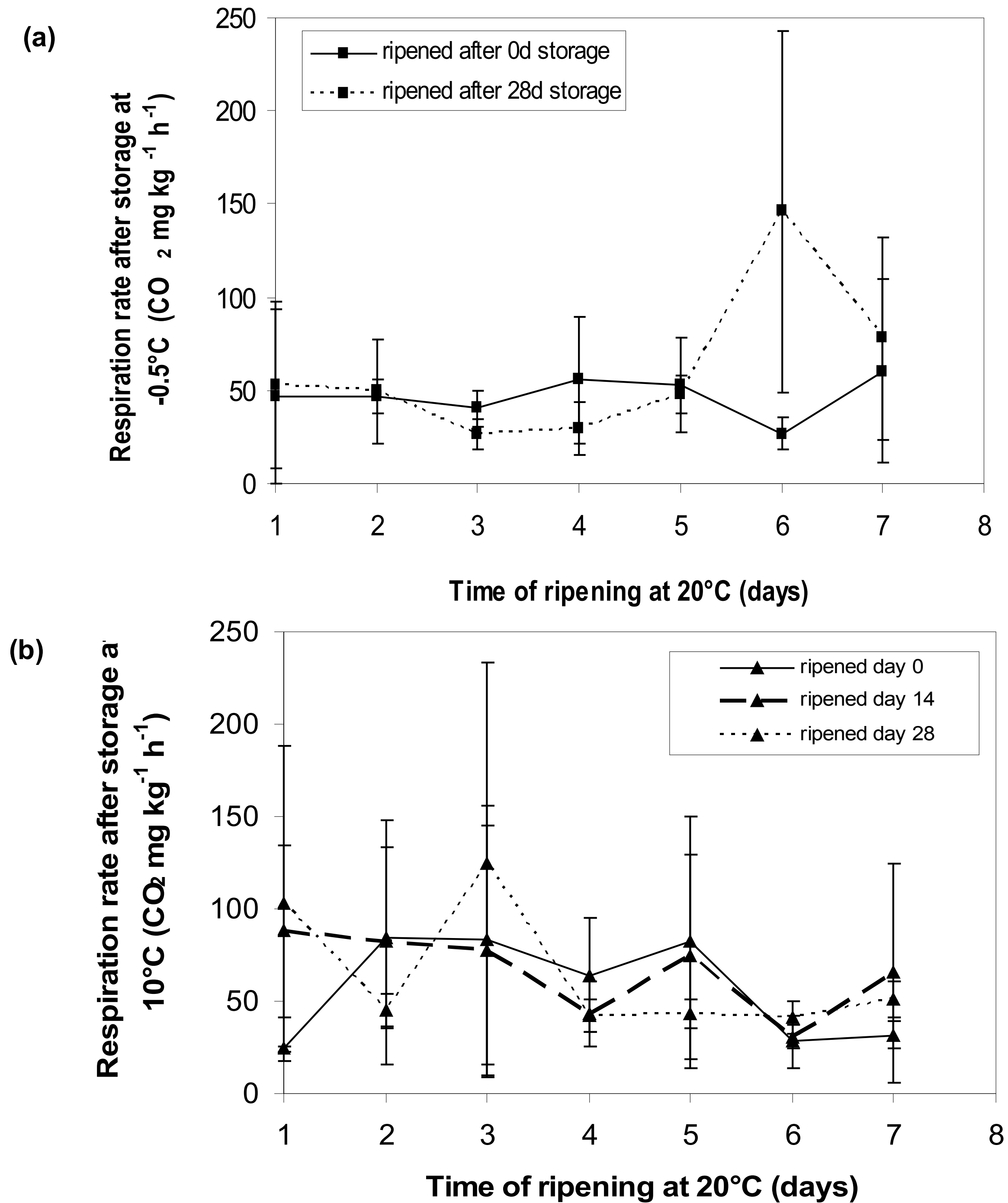


Fig. 4.1.7. Effect of storage duration at (a) -0.5°C and (b) 10°C prior to ripening, on the average daily respiration rate of 'Packham's Triumph' pears during ripening at 20°C over seven days. Bars represent standard deviations of the means (n=6)

During transport of the 'Packham's" pears for Experiments 1 and 2 and storage prior to the start of the experiments, some pears may have already entered the climacteric phase and thus ripened faster to optimum ripeness than the other fruit, when placed at 20°C.

Interestingly, pears stored for 28 days at -0.5°C exhibited a significant ($P< 0.001$) increase in the respiration rate and a clear climacteric peak (146.23 mg CO_2 kg^{-1} h^{-1}) on day six of ripening at 20°C (Fig. 4.1.7a). Individual respiratory data of the six ripening pears revealed that four of the six pears reached the climacteric peak on day six. Thus the variation between pears stored longer at -0.5°C (Fig. 4.1.7a) may stem from variation in the magnitude of the respiration rates at the climacteric peak, rather than in the time to reach the peak. The clear climacteric peak and the magnitude thereof may be the result of ethylene production during extended cold storage. 'Packham's' pears normally require four weeks (28 days) of refrigeration before sufficient autocatalytic ethylene is synthesised to initiate normal ripening at 20°C (Richardson and Gerasopoulos, 1993; Maage and Richardson, 1998). Although the duration of refrigeration, prior to procurement of 'Packhams' used in Experiments 1 and 2, was unknown pears were refrigerated after harvesting, during transport and before the start of the experiments.

It is possible that pears stored for 28 days were more mature and that their ethylene levels increased during the refrigerated storage. Ethylene is the trigger for ripening in climacteric fruit (Lelievre, according to Alexander and Grierson, 2002). It coordinates and accelerates ripening (Alexander and Grierson, 2002) and is known to increase the respiration rate of plant tissue (Solomos, according to Brady and Romani, 1988). Hence, it is proposed that increased ethylene levels in the 'Packham's' after extended storage may have been responsible for the increased respiration rate and the magnitude of the climacteric peak during ripening. Pears were probably also more sensitive to ethylene action after extended storage, which could have contributed to reaching of the climacteric peak on day 6. It is well known that more mature fruit are more sensitive to ethylene, although the factors that control the sensitivity are not well known (Wills *et al*., 1998). Respiratory results could not support findings by Maxie and Ginsburg (1974) regarding 'Bon Chretien' pears stored

at -1.1°C for 4 weeks that ripened faster at 21.1°C in air, because the climacteric peaks of pears ripened on day zero of the experiment were not clearly visible.

Pears stored at 10°C for 14 days (Fig. 4.1.7b) exhibited no significant difference (P > 0.05) in mean respiration rates during storage at 20°C and no clear climacteric peak was observed. Experiment 1 indicated that pears were able to reach the climacteric peak between 21 and 35 days of ripening. Thus, it is likely that pears started to ripen during storage at 10°C for 14 days. However, the fluctuating ripening respiratory pattern of pears after 14 days of storage at 10°C showed no clear indication of a climacteric peak but rather an attempt of the fruit at homeostasis. It appears unlikely that the elevated respiration values observed on days one and two during storage at 20°C represented a climacteric peak because another peak was observed on day 5 and respiration rate increased again on day seven (Fig. 4.1.7b). As explained before (Romani, 1984) the increased respiration rates may have been part of the homeostatic response of the pears to the approaching senescence. The respiration rates on days one and two may simply have been a response to the increased temperature, because (according to Findlay and Combrink, 1996) the respiration rate increases as storage temperature increases. It seems unlikely that pears from 10°C exhibited such an unusual ripening pattern at 20°C as a result of insufficient cold storage and ethylene production. Agar, Biasi and Mitcham (2000b) found that 'Bartlett' pears required two weeks of storage at -1°C before ripening could be induced whereas storage at 10°C for 3 to 4 days was sufficient to induce and hasten ripening at 20°C.

Interestingly, pears stored for 28 days at 10°C exhibited a normal ripening pattern with a distinctive climacteric peak that occurred on day three of storage at 20°C, with a respiration rate (124.43 mg CO_2 kg^{-1} h^{-1}) significantly ($P < 0.05$) higher than that recorded from the same pears on any of the other days during storage at 20°C. After 28 days of storage at 10°C pears may have been close to reaching the climacteric peak because ripening would have been initiated and the respiration rate accelerated as a result of the increased temperature. The high respiration rate would probably have been able to produce sufficient energy to drive normal ripening processes, as was hypothesised by Solomos (according to Romani, 1984).

The extended storage at 10°C would have enabled ethylene production and the pears would have been more sensitive to ethylene action as storage duration at 10°C and pear maturity increased (Wills *et al*., 1998). Thus, additional storage at 20°C was shorter and the respiration rate at the climacteric peak higher than that of pears after 14 days of storage. Thus, when comparing different storage durations within the respective temperatures, pears ripened on day zero (from both -0.5°C and 10°C storage) appeared to have had greater variability in their time to peak while variability in pears ripened after 28 days of storage stemmed more from the magnitude of the respiratory peak than from the time to peak.

Storage of pears at different temperatures for corresponding durations revealed the following. Data variability and the large standard deviations (Figs 4.1.8a) of pears stored at -0.5 and 10°C before ripening (day zero), may have accounted for the lack of significant differences ($P > 0.05$) that occurred between the respiration rates of the ripening pears. However, the trends in respiratory data indicated that pears (ripened after 4 hours of storage at 10°C) respired at a slightly higher rate for most of the ripening duration, than pears from -0.5°C (Fig. 4.1.8a). This is to be expected as it is known that respiration rate increases when the temperature increases (Findlay and Combrink, 1996). For pears stored for 28 days prior to ripening (Fig. 4.1.8b), there was no significant difference ($P > 0.05$) between the magnitudes of the peak rates during storage at 20°C, for pears removed from -0.5°C (146.22 mg CO_2 kg^{-1} h^{-1}) and 10°C (124.43 mg CO_2 kg^{-1} h^{-1}) storage.

However, the mechanisms that drove the manifestation of the climacteric peaks (Fig. 4.1.8b) may have been different for pears stored at -0.5 and 10°C, respectively. As cold storage for 28 days is required to stimulate sufficient ethylene production in 'Packham's' for immediate ripening at 20°C (Richardson and Gerasopoulos, 1993; Maage and Richardson, 1998), it is proposed that ethylene may have initiated ripening and subsequently increased the respiration rate of the pears during ripening at 20°C. This theory is supported by Miró, Graell, Larrigaudiere and López (2001) who found that quality changes in 'Comice' pears during ripening at 20°C are related to increased ethylene production after storage at 1°C for two weeks. In contrast, the storage of 'Packham's' pears at 10°C accelerated the respiration rate and ripening processes and ultimately the occurrence of optimum ripeness (climacteric peak).

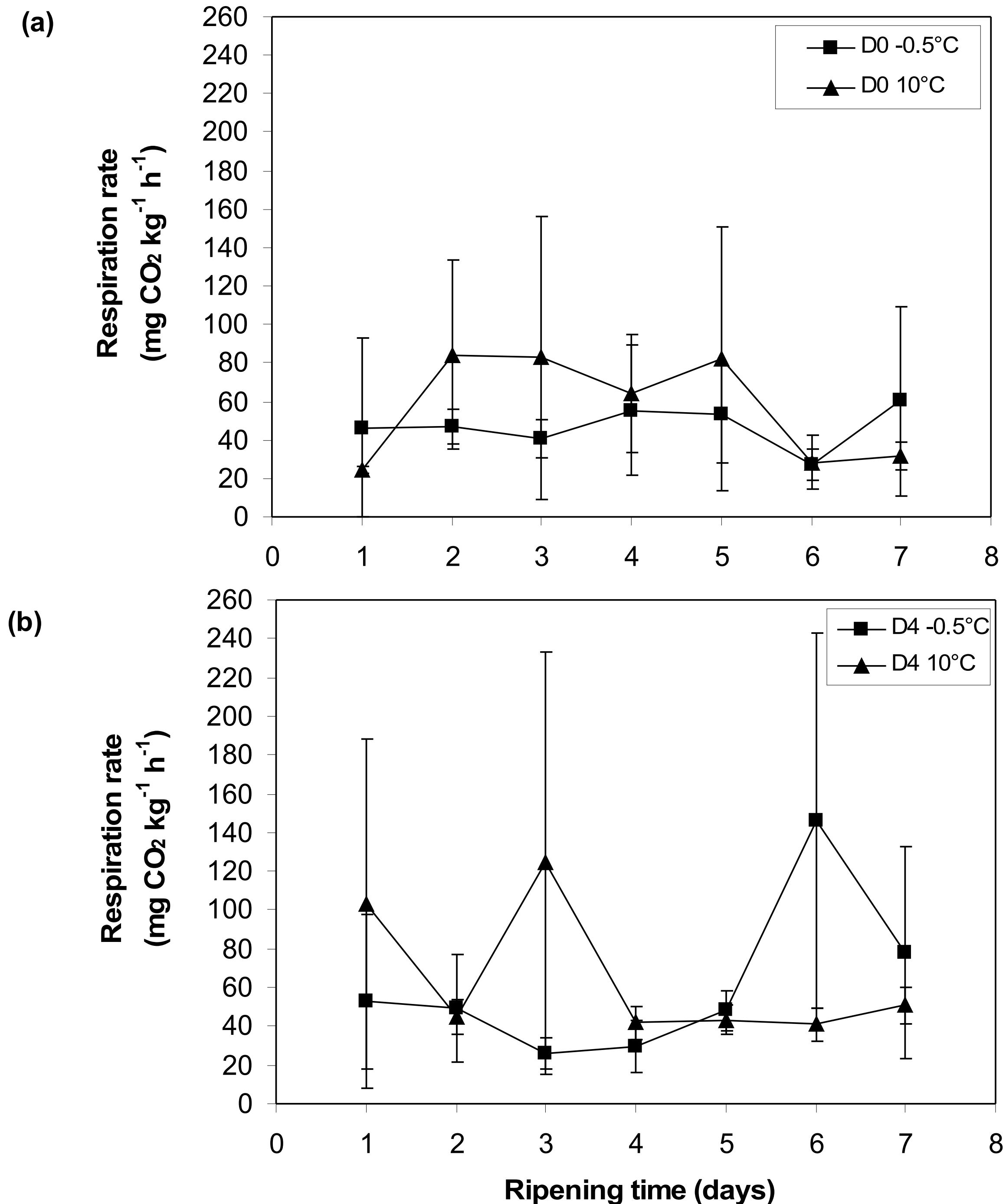


Fig. 4.1.8. Effect of storage temperature prior to ripening, on the average daily respiration rates of 'Packham's Triumph' pears during ripening, when analysed (a) on day zero and (b) after 28 days of storage prior to ripening. Bars represent standard deviations of the means (n=6)

Flesh firmness

Flesh firmness has long been considered as one of the most reliable measures of pear maturity and ripeness (Maxie and Ginsburg, 1974). In Experiment 2, no

significant (P > 0.05) differences were found between internal and external textures of the pears that were removed from storage at -0.5 and 10°C and subsequently stored for seven days at 20°C (Fig. 4.1.9). According to the trend in flesh softening (Fig. 4.1.9), pears stored at 10°C for up to 28 days were softer after 7 days at 20°C, than pears stored for the same duration of time at -0.5°C prior to ripening.

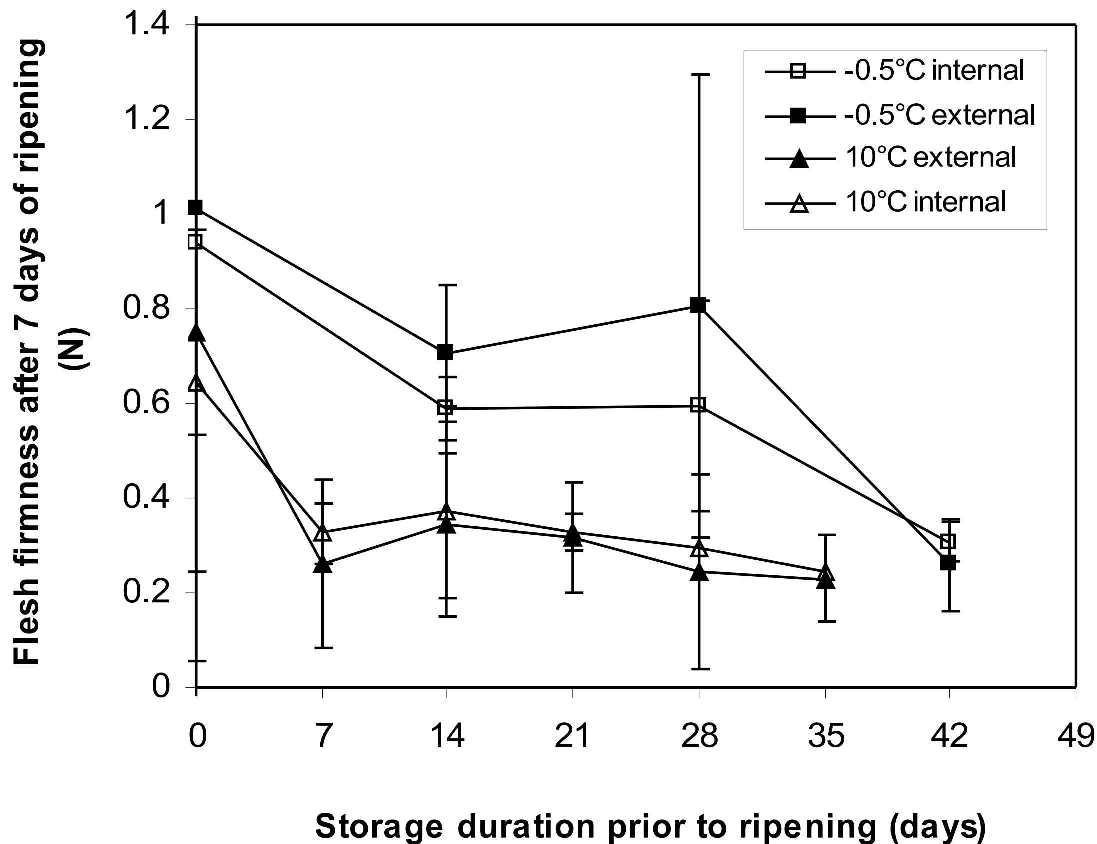


Fig. 4.1.9. Effect of storage duration at -0.5°C and 10°C, prior to ripening, on the average external and internal flesh firmness of 'Packham's Triumph' pears after ripening at 20°C for 7 days. Bars represent standard deviations of the means (n=6)

Interestingly, storage duration at 10°C only had a significant (P < 0.05) effect on the final flesh firmness of the ripe pears, on day 14 of storage. The lack of difference between pears removed from -0.5 and 10°C after zero and 28 days, respectively, may again be a consequence of sample variation. As previously described, pear respiration at 10°C may have accelerated quality changes, thereby initiating the onset and progression of ripening and ripening-related reactions such as flesh softening. Agar *et al.* (2000b) found that storage of 'Bartlett' pears at 10°C in air for up to four days induced ripening activity. Ripening was probably not the result of

higher ethylene production but rather of higher enzyme activity. 'Packham's' pears from 10°C in Experiment 2 were probably also more mature (as a result of progressing ripening) than pears at -0.5°C prior to removal from storage. Hence, it was to be expected that pears from 10°C would reach maximum softness at an earlier date (day 35) than pears from -0.5°C (day 42).

In this study, storage duration prior to ripening at 20°C (Fig. 4.1.9) had the following effects. Refrigerated storage of pears at -0.5°C for 14 and 42 days prior to ripening significantly ($P < 0.05$) reduced flesh firmness after ripening. Agar *et al*. (2000a) found that 'Bartlett' pears produced increased levels of ethylene as storage duration at -1°C increased and as a result ripening rates increased as storage duration increased. Similarly, Retamales *et al.* (1998) found that storage of 'Packham's' pears at -0.5°C in air for 30 days prior to ripening, resulted in rapid softening during ripening as a result of increased ethylene production during refrigerated storage. In fact, flesh firmness after ripening reduced as cold storage duration prior to ripening increased, regardless of whether ethylene during storage was controlled or not. These findings may support the theory that the final firmness of the ripe 'Packham's Triumph' pears in Experiment 2 decreased as ethylene production levels increased, as a result of increased storage time at –0.5°C.

Storage of pears at 10°C for up to 7 days significantly ($P < 0.001$) reduced flesh firmness after ripening, but additional storage had no significant effect on softening. Insignificant ($P > 0.05$) changes in firmness after seven days of storage at 10°C was first observed by Maxie and Ginsburg (1974) who found that the rate of softening of 'Bon Chretien' pears decreased drastically as fruit approached full ripeness. 'Packham's' pears in Experiment 2, stored at 10°C would have been more mature and reached the senescent phase and full ripeness faster as storage time was extended. This is supported by Amarante *et al*. (2001) who found that pears that were cold stored for several months prior to ripening were more mature and entering the respiration climacteric on removal from cold storage. This resulted in a shorter shelf-life and faster ripening than the freshly harvested pears, which were still in pre-climacteric phase. For pears stored at 10°C analysis was abandoned after 35 days of storage because fruit were rotting and considered unmarketable. Pears at the end of their storage duration, after 42 and 35 days at -0.5 and 10°C respectively,

exhibited similar flesh firmness, which is probably a result of all pears attaining full ripeness.

Colour

Storage duration affected the final colour of the ripe pears. Storage at -0.5°C for zero to 28 days prior to ripening had no significant effect on pear colour after ripening (Fig. 4.1.10). This is supported by the Unifruco colour chart values (Fig. 4.1.10) of the ripe pears that did not increase more than 0.5 units, which are considered by van der Merwe (1996a) as the indication of significant colour change.

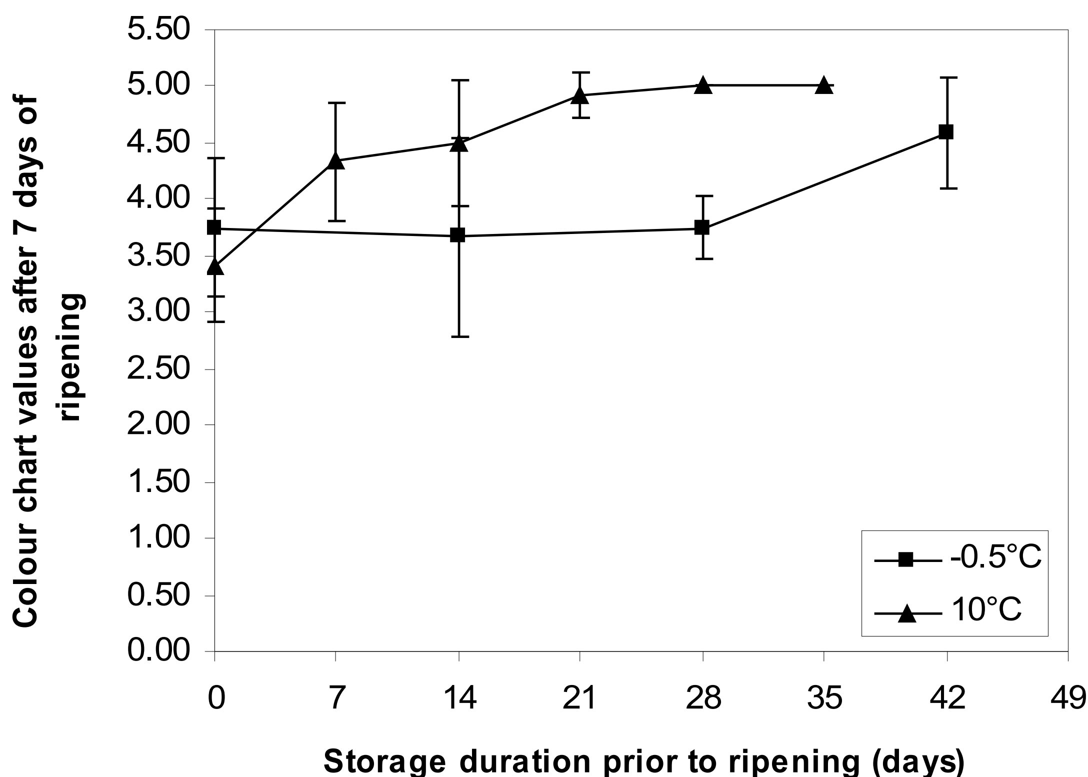


Fig. 4.1.10. Effect of storage duration at -0.5°C and 10°C prior to ripening, on the Unifruco colour chart values of 'Packham's Triumph' pears after subsequent ripening at 20°C for 7 days. A colour chart value increase of 0.5 indicates significant colour change. Bars represent standard deviations of the means (n=6)

Colour chart values are supported by Hunter a* and b* values (Figs 4.1.11a and 4.1.11b). Pears stored at -0.5°C prior to ripening only exhibited a ripe colour (Unifruco chart value of ≥ 4.0) after 42 days of storage. Similarly, after 42 days of storage pears de-greened significantly ($P < 0.05$) during ripening to an a* value of

4.97± 2.81 (Fig. 4.1.11a) and yellowed significantly ($P < 0.05$) to a b* value of 28.57± 0.61 (Fig. 4.1.11b). Although not dramatic, colour change in 'Packham's' pears, stored for up to 28 days at -0.5°C is possible during storage at 20°C.

If the colour values of pears after six days at 20°C in Experiment 1, are used as a guide for physiological ripeness of the pears in this study, then the Unifurco chart, Hunter a* and b* values should be 4.5 (Fig. 4.1.3), + 2.25 (Fig. 4.1.4a) and 29 (Fig. 4.1.4b), respectively. However, the colour values of ripened pears on day zero of Experiment 2 were slightly lower than their green counterparts, as indicated by 3.5 (Fig. 4.1.10), -1.9 (Fig. 4.1.11a) and 27.2 (Fig. 4.1.11b) for the Unifruco chart and Hunter a* and Hunter b* values, respectively. Thus, colour values of pears ripened on day zero at 20°C (Experiments 1 and 2) is different to that of pears ripened after 42 days at -0.5°C prior to ripening, as a result of extended cold storage of the latter group of pears. The ability of pears to change colour and firmness prior to sufficient cold storage is supported by Gerasopoulos and Richardson (1997).

The fact that pears from Experiment 1 reached an average climacteric maximum, accompanied by a green-yellow colour, within seven days of ripening at 20°C (the required time as indicated by Amarante *et al.*, 2001) must indicate that refrigeration time at -0.5°C was sufficient for pears in this study to start ripening immediately after removal from cold storage. The ripened, yellow colour of pears in Experiment 2 after 42 days of storage at -0.5°C (Fig. 4.1.10) followed by 7 days at 20°C, indicated that the pears were fully ripe.

'Bartlett' pears showed increasing ethylene production rates and subsequently faster ripening rates as storage duration at -1°C increased but ripening attributes were influenced to different degrees by extended storage duration (Agar *et al.*, 2000b). The different degrees to which extended storage affected different ripening attributes may explain why pears significantly ($P < 0.05$) softened after 14 days storage at -0.5°C and subsequent ripening at 20°C, but colour change during ripening remained relatively similar to pears ripened on day zero of the experiment.

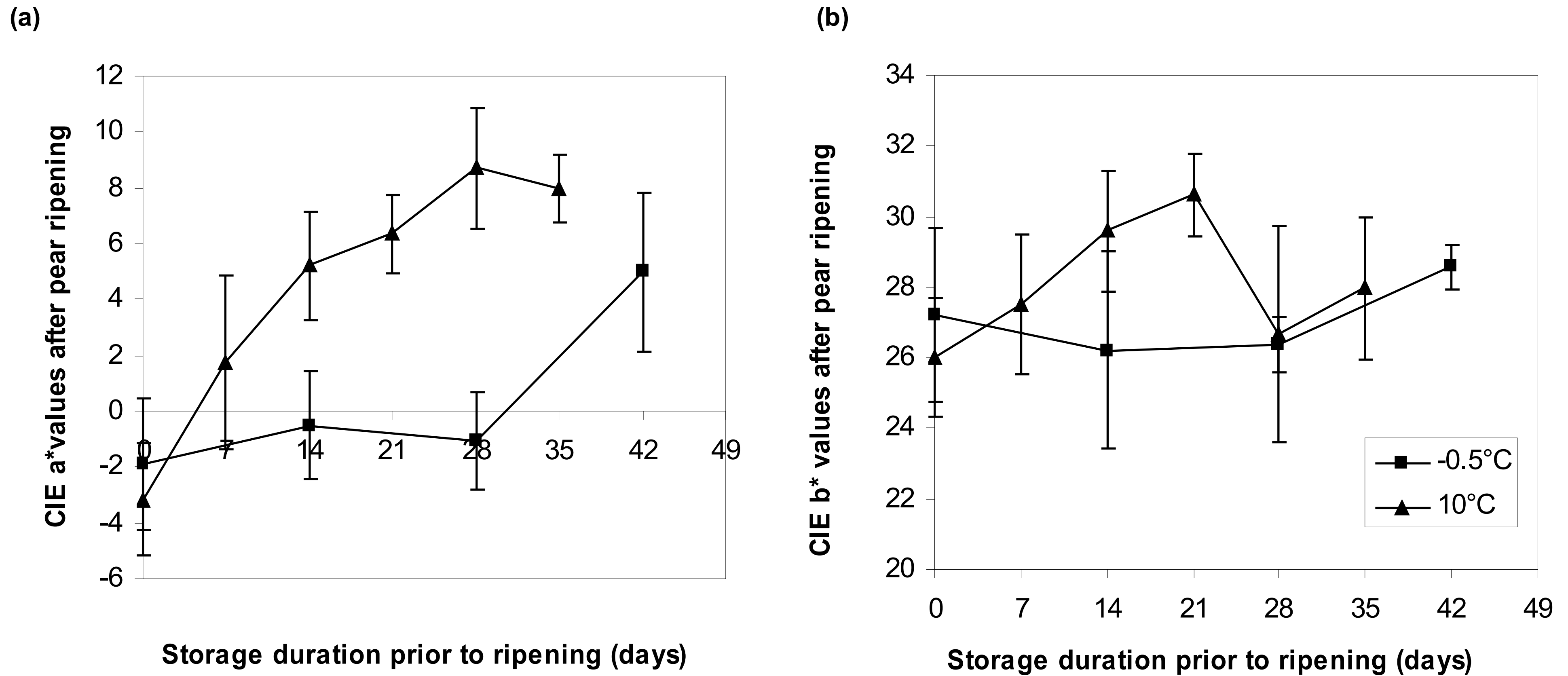


Fig. 4.1.11. Effect of storage duration at -0.5°C and 10°C prior to ripening, on the colour (a) a* values and (b) b* values of 'Packham's Triumph' pears after subsequent ripening at 20°C for 7 days. Negative a* values are green and positive a* values are red, negative b* values are blue and positive b* values are yellow. Bars represent standard deviations of the means (n=6)

As refrigerated storage duration of pears at 10°C increased, Unifruco (Fig. 4.1.10), Hunter a* (Fig. 4.1.11a) and Hunter b* (Fig. 4.1.11b) colour values of ripe pears after storage at 20°C continued to increase. When these colour values were compared to those of pears at 20°C on day six, colour values similar or exceeding that of Experiment 1 were reached by pears after 14 days of storage at 10°C and subsequent placement at 20°C for seven days. As seen in Experiment 1, pears stored at 10°C started ripening during storage but ripening was slower than at 20°C. A delay in ripening was to be expected for pears stored at 10°C. A review by Knee (1990) indicated that ethylene concentration in air at high temperatures was responsible for accelerated chlorophyll loss in apples and other fruit. The respiration rate of pears at 20°C, after 28 days at 10°C, reached a maximum on day 3 and pears were well into senescence by day 7 (Figs 4.1.7b and 4.1.8b), This is supported by the colour values that indicated a yellow colour (Figs 4.1.10 and 4.1.11b) and near maximum de-greening (Fig. 4.1.11a).

Comparison of ripe colour values for pears from 10°C at corresponding testing intervals to pears from -0.5°C (Figs 4.1.10 and 4.1.11a and b) revealed higher values for pears stored at 10°C prior to ripening. Maximum colour values were not only higher than those of pears stored at -0.5°C, but maximum values were also reached earlier in pears stored at 10°C than in pears stored at -0.5°C prior to ripening. This supported respiration rate data (Fig. 4.1.8) where an increase in storage temperature accelerated the manifestation of the climacteric peak. The insignificant ($P>0.05$) differences between the a* and b* values for ripe pears after 42 days at 0°C, compared to the a* and b* values of pears stored at 10°C for 14 to 35 days prior to ripening indicated that pears stored at -0.5°C for 42 days prior to ripening de-greened (a* values) and yellowed (b* values) to a similar extent as pears stored at 10°C for 14 to 35 days prior to ripening (Figs 4.1.11a and 4.1.11b). Thus, these storage durations at the respective temperatures prior to storage at 20°C may possibly be an indication of how long pears should be stored at -0.5 and 10°C for sufficient ethylene to be produced in order for pears to reach maximum colour change after ripening. Henze (1995) also found that optimum eating quality of 'Conference' pears and colour change from green to yellow was accelerated when storage temperature prior to ripening was increased (stored at 1, 4 or 16°C). The intensity of the change in colour and texture during ripening increased as storage duration prior to ripening was

increased. Ultimately, regardless of storage time, ripe 'Conference' pears exhibited similar colour and softness values.

Titratable acidity and soluble solids content

The titratable acidity and total soluble solids of pears after storage at -0.5 or 10°C and subsequent ripening (results not shown) did not differ significantly (P > 0.05) as a result of extended refrigerated storage at the respective temperatures. This is to be expected as the decrease in acidity and conversion of starch to sugars (increase in TSS) is optimal during the climacteric phase (Wills *et al*., 1998). By the time fruit passed the climacteric peak, changes in acidity or TSS were not as dramatic (Von Mollendorf, 1996). After seven days of storage at 20°C most of the pears would probably have been in the senescent phase where biological processes require little energy to maintain. The clear and dramatic changes observed in texture and colour and the minimal changes observed in acidity and total soluble solids of pears refrigerated prior to ripening, correlated well with trends observed by Miró *et al.* (2001) with 'Doyenne du Comice' pears stored at -0.5°C for 2 weeks and ripened for 4 days at 20°C. Extended storage had the greatest effect on colour and textural changes whereas only slight changes in titratable acidity were observed.

4.1.4. Conclusions

To extend the storage life of 'Packham's Triumph' pears, temperature control is of the utmost importance. In Experiment 1, the physiological behaviour and quality of 'Packham's Triumph' pears does not change significantly during storage at -0.5°C. However, pears can ripen during inappropriate (temperature-abused) storage conditions (10°C), although ripening progresses at a slower rate and the degree of physico-chemical changes associated with ripening is less pronounced than for pears stored at 20°C. Pears stored at 10°C eventually ripen to similar skin colour and flesh softness values as pears stored at 20°C. When the metabolic activity of pears is slow (during storage at -0.5 and 10°C) and the RH around the pears is 95 to 98%, pears lose insignificant amounts of moisture and do not shrivel. Conversely, despite the presence of a high RH, at a high storage temperature (20°C), the metabolic activity of 'Packham's' pears and hence their rate of moisture loss and shrivelling, is accelerated.

In Experiment 2 the variability in fruit, stored at -0.5 and 10°C and ripened on day zero, stems from the differences in pear maturity and thus time to reach the climacteric peak. Conversely, pears from extended storage at the above mentioned temperatures vary less in their time to peak and more in the magnitude of the peak value. Measurement of ethylene production is of utmost importance to accurately explain changes in pears during storage and ripening. For pears stored at -0.5°C, the respiration and ripening rate upon transfer to 20°C is increased as the storage duration at -0.5°C is extended. Storage of pears at temperature-abused conditions (10°C) will result in faster ripening fruit that reach the end of their storage life faster than fruit refrigerated prior to ripening. Colour and texture are more affected by cold storage prior to ripening at 20°C, than SSC and titratable acidity.

4.2 Extending the quality and shelf-life of 'Packham's Triumph' pears with a kafirin protein coating

Abstract

The effect of selected kafirin coatings on the postharvest physiology and shelf-life of 'Packham's Triumph' pears was investigated by studying changes in physico-chemical and sensory properties as well as microbiological quality over a storage period of 24 days. A 2% (w/w) kafirin coating was able to extend the shelf-life of the pears significantly by decreasing the ripening rate of the coated fruit. The kafirin coating was not able to retard the progression of the climacteric phase as the latter probably set in prior to fruit coating. However, the coating was able to decrease the respiration rate and retard the progression of the senescence phase. The coating inhibited de-greening most, probably because it reduced the amount of oxygen available for chlorophyll degradation. Moisture loss was exacerbated in the kafirin-coated fruit during ripening at 20°C, probably as a result of the dehydrating effect of the ethanol in the coating solution. The 2% (w/w) kafirin coating seemed to be more effective at retarding ripening than in preventing moisture loss and shrivelling, because the shelf-life of the coated pears was extended but they shrivelled more than the uncoated pears. The microbiological counts on the coated and uncoated pears during storage at 20°C, were low and there were no significant ($P > 0.05$) differences in the total aerobic mesophile or yeast and mould counts between the coated and uncoated pears. The respiration rate, ethylene production rate and quality attribute data supports the finding that the kafirin coating acted as a gas barrier and that the shelf-life of the coated pears were extended by the kafirin coating.

4.2.1 Introduction

Pome fruit exports earn approximately R 2.6 Billion in revenue for the South African fruit industry (Anon., 2006c). From 2001 to 2005, the pear cultivar that was exported in the largest quantities (approximately 5 Million cartons per annum) (Anon., 2006c), is 'Packham's Triumph' (Anon., 2006c; Anon., 2005b). With this information in mind, it appears imperative that changes in fruit quality during export are minimised. In South Africa, fruit quality during export is primarily maintained by refrigerated storage of 'Packham's' pears at –0.5°C (Anon., 2005a) after they have been packed in boxes lined with polyethylene bags (Bester, 1973). The latter is a form of passive modified atmosphere packaging to retard respiration, increase the shelf-life, reduce mass loss (primarily through moisture loss) and prevent shrivelling (Bester, 1973). The prevention of shrivelling is important because 'Packham's Triumph' pears are particularly prone to skin shrivelling as a result of moisture loss, during extended periods of cold storage (Coetzee, Logistics Manager, Kromco Limited, 2002 - personal communication). However, the plastic bags have some disadvantages. Without refrigeration, the temperature inside the plastic bags will rise and moisture may condense on the surface of the pears. Under the warm, moist conditions mould growth may lead to increased postharvest losses. As pears respire faster CO_2 levels within the packaging will increase which may induce CO_2 injury in some pear cultivars (Bester, 1973), although 'Packham's Triumph' is not prone to CO_2 injury (Amarante and Banks, 2002). The polyethylene bags may also contribute to packaging waste. Ultimately, once pears are removed from the packaging at fresh fruit markets, pear respiration and ripening continues at ambient temperatures.

Edible coatings for fruits have long been investigated to extend fruit quality and shelf-life. To retard ripening and moisture loss a coating would have to act as both a gas and moisture barrier. Carnauba-based wax coatings, used on pears (Amarante *et al*., 2001a; 2001b), and the hydrophobic maize protein (zein), used on tomatoes (Park, 1999) and apples (Bai *et al.*, 2003), have proven able to fulfil such requirements under ripening conditions (20°C). The proposed mechanism of the edible coating as a gas barrier is through modification of the fruit's internal atmosphere by increasing CO_2 and decreasing O_2 concentrations, typical of Modified Atmosphere Packaging (MAP) (Park, 1999). Amarante *et al*. (2001a; 2001b) found

that waxes delayed ripening in coated pears by blocking the pores. Although carnauba-based wax coatings were found to be effective because they modified the internal O_2 and CO_2 concentration of pears like 'Packhams' Triumph' (Amarante *et al*., 2001a), consumers tend to be weary of wax coated fruit due to the waxy taste on the peel. Thus the development of an edible coating that does not impart a waxy taste is advantageous (Park, 1999).

One such alternative is zein, the prolamin protein from maize, which has proven to be an effective gas and moisture barrier on tomatoes (Park *et al*, 1994) and apples (Bai *et al.*, 2003). Sorghum grain is indigenous to Africa and the prolamin protein from sorghum, called kafirin, is (according to Shull *et al*., 1991) similar to zein in amino acid composition, structure and molecular weight. In addition kafirin protein is known to be hydrophobic and non-allergenic in nature. In fact of all the cereal prolamin proteins kafirin is more hydrophobic than other prolamins such as wheat gliadin and maize zein (Duodu *et al*., 2003). Hence, kafirin should be a better moisture barrier than zein while providing the necessary gas barrier properties exhibited by zein. This theory is supported by Gao *et al.* (2005) who found that kafirin films of similar sensory qualities and water vapour transmission rates to films from commercial zein, may be produced. Buffo *et al*. (1997) not only had similar findings on the similarity in barrier properties of kafirin and zein films but they were the first to suggest that kafirin had potential as an edible coating. Thus by coating pears with kafirin protein, the coating may provide a barrier against moisture loss and gas exchange, which may extend the quality and shelf-life of export grade 'Packham's Triumph' pears.

In this study 'Packham's Triumph' pears (first stored under CA conditions for 18 weeks) were left uncoated or coated with a kafirin protein coating and stored for one week under RA conditions at –0.5°C. The effect of the coating on the physiological behaviour and shelf-life of the pears was investigated while pears were exposed to ambient (typical ripening) conditions (20°C, 35 to 45% RH).

4.2.2 Materials and methods

4.2.2.1 Raw materials

In May of 2004 'Packham's Triumph' pears were procured from Colours Fruit in Paarl, South Africa. Pears of similar size (70 pears per 12.5 kg box) were used during shelf-life testing. Pears were stored in controlled atmosphere (CA) stores at -0.5°C, O_2 and CO_2 concentrations of 1.5%, respectively, and 95% relative humidity (RH) for eighteen weeks prior to procurement.

4.2.2.2 Coating of pears

Sorghum kafirin (83.6% protein on dry basis) was extracted on large-scale at the Centre for Scientific and Industrial Research (CSIR), Modderfontein. The kafirin was defatted and ball-milled for 16 h before incorporation into the coating solution. Propylene glycol or 1,2-propanediol (code 123638, from Sigma Aldrich Chemicals, Johannesburg, South Africa) and glucono-delta-lactone (from CC Immelman, Southdale Johannesburg, South Africa) were used as plasticisers. Ethanol (96%, AR, from Labchem, Edenvale, South Africa) was diluted with distilled water to 70% (v/v). The aqueous ethanol served as the solvent in the coating solution.

Following preliminary coating experiments to determine the appropriate protein concentration that would allow normal respiration of coated 'Packham's Triumph' pears, a 2% (w/w) kafirin coating was selected for use in the shelf-life study of the coated 'Packhams Triumph' pears. The formulation for the 2% (w/w) kafirin coating solution is given in Table 4.2.1.

Table 4.2.1

The formulation for a 2% (w/w) kafirin coating solution

Ingredients	Amount (g / 100g coating solution)
Kafirin protein (83.6% pure)	2.39
Aqueous ethanol (AR) (70%, v/v)	96.53
Propylene glycol (1,2-propanediol)	0.72
Glucono Delta Lactone (GDL)	0.36

Coating solutions were prepared by weighing the kafirin protein into an Erlenmeyer flask. Warm (70°C) aqueous ethanol was added. The weight of the container and its contents was noted. The mixture was heated in a 70°C water bath while it was stirred rapidly (using overhead stirrer) for 20 min. The flask and its contents were reweighed and 70% (v/v) aqueous ethanol added until the original weight of the mixture was obtained (to replace ethanol lost during evaporation). The mixture was left overnight (16 h), at room temperature.

Prior to pear coating the plasticiser mixture was weighed into the ethanol/protein mixture. The container and solution weight was noted. The solution was heated in a 70°C water bath while being stirred continuously until it reached 70°C. Aqueous ethanol (70%, v/v) was added to replace amount lost during evaporation, after which the container was covered and left to cool to 20 to 25°C.

Sixteen hours prior to coating, all the pears (still unripe) were removed from refrigerated storage and from the carton boxes, and left at 20°C to equilibrate overnight. The pears were divided into two groups of equal size. One group of pears were dipped into ± 300 ml coating solution, for five seconds and hung up by the stem to dry for four hours at 20°C. The other group of pears (uncoated control) remained untreated. After application of the kafirin coating, coated and uncoated pears were packed back into the cardboard boxes (with plastic liners) that they were procured in and refrigerated at 0 ± 1°C for seven days prior to commencement of Experiment 1 of the shelf-life study.

4.2.2.3 Experimental design

During shelf-life testing the coated and uncoated pears were stored separately from each other at 20°C and ambient RH (35 to 45%) although the methods for pear selection and analyses were the same for both groups. Storage of pears at 20°C is generally considered as ripening and storage during this shelf-life study will hence forth be referred to as ripening.

Fruit were selected for the shelf-life study (Experiment 1) as follows. Coated and uncoated pears were removed from their original packaging (cardboard boxes and pallets) and randomly divided into six plastic containers (60 cm in length, 45 cm in

width, 34 cm in height) to yield 36 pears per container. Pears were placed in mono-layers at the bottom of each container. The containers remained open for the duration of the shelf-life study. Inside each of the six containers a group of six fruit were stored in an open glass bowl (25 cm in length, 20 cm in width, 8.5 cm in height) for respiration and moisture loss analyses. The remaining 30 fruit from each container were used for the assessment of quality changes during the course of the shelf-life study. During Experiment 1 of the the shelf-life study, coated and uncoated pears were ripened at 20°C (35 to 45% RH) for 24 days. Coated and uncoated pears were removed form their respective containers for analyses as follows. The amount of pears given for the respective analyses represents the amount removed per group (either coated or uncoated). To measure respiration rate and moisture loss, 36 pears (one group of six pears from each container) were removed from storage on days 0, 3, 4, 5, 6, 7, 9, 10, 11, 14, 17, 21 and 24. To measure quality attributes (colour, firmness, soluble solids content and titratable acidity) six pears (one from each container) were removed on days 0, 3, 7, 10, 14, 17, 21 and 24. Microbiological quality was conducted on the same days as that of the quality attributes but six different coated and uncoated pears were used for microbial analyses.

During Experiment 1, problems were experienced with the analysis of ethylene production during the shelf-life study. Although the quantity of pears available was limiting, the measurement of ethylene production was considered crucial to the study. Subsequently, measurements of respiration rate and ethylene production of coated and uncoated pears were repeated during a second experiment (Experiment 2). Pears used in Experiment 2 were from the same batch of pears used in Experiment 1. Pears for Experiment 2 were stored in the original packaging at 0 ± 1°C for 24 days (the duration of Experiment 1) prior to coating. After application of the kafirin coating, the coated and uncoated ‘Packham’s Triumph’ pears were respectively divided into only five plastic containers (60 cm in length, 45 cm in width, 34 cm in height) (12 pears per container). Pears were placed in mono-layers at the bottom of each container, which remained open for the duration of the shelf-life trial. Inside each container two groups of six fruit each were stored in open glass bowls (25 cm in length, 20 cm in width, 8.5 cm in height) for analysis of respiration rate and ethylene production.

Experiment 2 commenced as Experiment 1 concluded. The shelf-life study of Experiment 2 was conducted on coated and uncoated pears, ripened at 20°C (35 to 45% RH) for up to 17 days. For respiration rate determination five groups of six pears each were removed from the storage containers for conduction the analyses on days 0, 1, 3-12, 14, 17. For determination of ethylene production five groups of six pears each were removed on days 0, 3, 5, 7, 12, 14, 17.

4.2.2.4 Respiration rate and moisture loss determination

Analyses of respiration rate, ethylene production and moisture loss were performed at 20°C. Respiration rate was measured using an Infra Red Gas Analyser (LI-COR gas analyser, model LI-6262, CS Africa, Somerset West, South Africa) in a closed system. Nitrogen gas (99.9% pure) served as reference gas to the IRGA.

Throughout the storage period in both Experiments 1 and 2, respiration rate was determined once on the same six pears from each plastic container for coated and uncoated pears. Six pears were sealed in a gas tight glass container (3.5 l in volume) with ports in the lid for incoming and outgoing gas streams. Respiration rate was measured as described in section 4.1.

Weight loss was measured throughout the storage period using the same pears used for the respiration analyses. Moisture loss was calculated from the weight loss data and expressed as percentage moisture loss (on fresh weight basis).

4.2.2.5 Ethylene production determination

In Experiment 2 ethylene production was measured by Gas Chromatography (GC) headspace analysis. The equipment consisted of the following components: GC (Varian 3700 plus FID); column (Fused silica PLOT; 30 m); injector (splitless mode; temperature 250°C); Flame Ionisation Detector (FID) (temperature 290°C); column temperature (isothermal; 75°C); carrier gas (N_2; inlet pressure 30 psi). A standard of 2.0% ethylene in N_2 was used for calibration (Lowest Lethal Dose, LLD = 7 ppm, according to material safety data sheet for gas standard). Measurements were performed on five groups of coated and five groups uncoated fruit at 20°C on days 0, 3, 5, 7, 12, 14, 17 during Experiment 2. A group of fruit (six fruits each) were placed in a plastic vacuum bag. Each plastic bag was sealed using a layer of vacuum

grease and clips, 24 h prior to analysis. The bags of pears remained at 20°C up to the point of analysis. Care was taken to ensure that all the bags contained approximately the same volume of air. During analysis, 1.00 ml of gas was withdrawn from the plastic bag using a 1.00 ml gas syringe with a Poly- Tetra-Fluoro- Ethylene (PTFE) plunger (Precision Sampling Corp., USA). The 1.00 ml gas sample was injected directly into the GC through the septum of the injector. Each sample was analysed in triplicate and results were expressed as averages of the three values, in μl^{-1}.

4.2.2.6 Quality attribute evaluation

Skin colour change, flesh firmness, titratable acidity and soluble solids content were measured as previously described in section 4.1.

4.2.2.7 Microbiological quality evaluation

Pears were sampled over the 24-day shelf-life study of Experiment 1. Six uncoated and six coated pears were sampled at each measurement interval. Measurement intervals were on days 0 (baseline), 3, 7, 10, 14, 17, 21 and 24. The pear skin was sampled by peeling with a standard all-metal, sterilisible fruit and vegetable peeler. The same peeler was used throughout the 24-day period. The 10 g (± 0.5 g) sample removed consisted primarily of peel. For unripe pears, the 10 g sample was found to correspond with a surface area of approximately 55 cm^2. As the pears ripened, the surface to mass ratio decreased so that after 24 days the 10 g sample corresponded to a surface area of around 40 cm^2.

The peel (10 g) was added to 90ml of sterile Maximum Recovery Dilutent (MRD), consisting of 8.5 g/l sodium chloride and bacteriological peptone in water. The peel and MRD was homogenised for 2 min in a Colworth 400 stomacher (AJ Seward, London, UK). Serial dilutions with the same dilutent were carried out. Four microbiological analyses were performed after each sampling interval over the 24-day storage period according to standard methods (Pouch Downes and Ito, 2001). For the aerobic mesophile count, tryptone soya agar (CM 0131, Oxoid Ltd., England) was used as medium. The pour-plate method was used and plates were incubated at 30 ± 2°C and examined after 48 ± 4 h and again after 72 ± 4 h. For determining the yeasts and moulds counts acidified potato dextrose agar was used as medium.

After autoclaving of the prepared potato dextrose agar (Code 01-483, Scharlau Chemie S.A., Barcelona, Spain) a 10% (w/v) sterile tartaric acid solution was added to the agar to obtain a pH of between 3.5 and 4.0. Plates were incubated at 25 ± 2°C and examined after 3 and 5 days, respectively. Lactic acid bacteria counts were determined using MRS (de Man, Rogosa, Sharpe) agar (01-135, Scharlau Chemie, Barcelona, Spain). Plates were incubated aerobically at 30 ± 2°C for 72 ± 4 h. The Most Probable Number (MPN) method was used for the Coliform test. This method is more sensitive than plate methods with a lower detection limit. Lauryl sulphate tryptose broth (CM 451, Oxoid Ltd., England) was used for the first stage of growth detection. From the positive tubes where gas formation occurred, inoculations were made into tubes containing the more selective brilliant green bile (2%) broth (CM 31, Oxoid Ltd., England). All tubes were incubated at 37 ± 1°C for 24 ± 4 h before the coliform count was confirmed with the MPN table.

4.2.2.8 Statistical analyses

Mean values, standard deviations, analysis of variance (ANOVA) followed by the LSD multiple comparison tests were performed at 95% confidence limit ($P < 0.05$) using STATISTICA® (version 6, Stasoft, 2003) software.

4.2.3 Results and discussion

4.2.3.1 Respiration rate (Experiment 1)

During aerobic respiration, the respiration rate is dependant on the amount of O_2 consumed for the oxidation of carbohydrates and organic acids, which are subsequently metabolised to CO_2 (Wills *et al*., 1981). The respiration pattern of the uncoated pears (Fig. 4.2.1) exhibited a sharp incline (climacteric phase) that lead to a clear climacteric peak (optimum physiological ripeness) on day four, which was followed by a steady decline (start of senescent phase) in respiration rate. These characteristics are typical of aerobic respiration in climacteric fruit. The respiration rate of the coated pears (Fig. 4.2.1) also exhibited typical climacteric behaviour. From the significant difference ($P < 0.001$) between the respiration rates of the coated pears on day four (28.6 ± 2.8 mg CO_2 kg^{-1} h^{-1}) and that of all the respiration rates on the other days during ripening, the climacteric peak of the coated pears was

probably also reached on day four (Fig. 4.2.1). Thus, the 2% (w/w) kafirin coating allowed the fruit to respire normally.

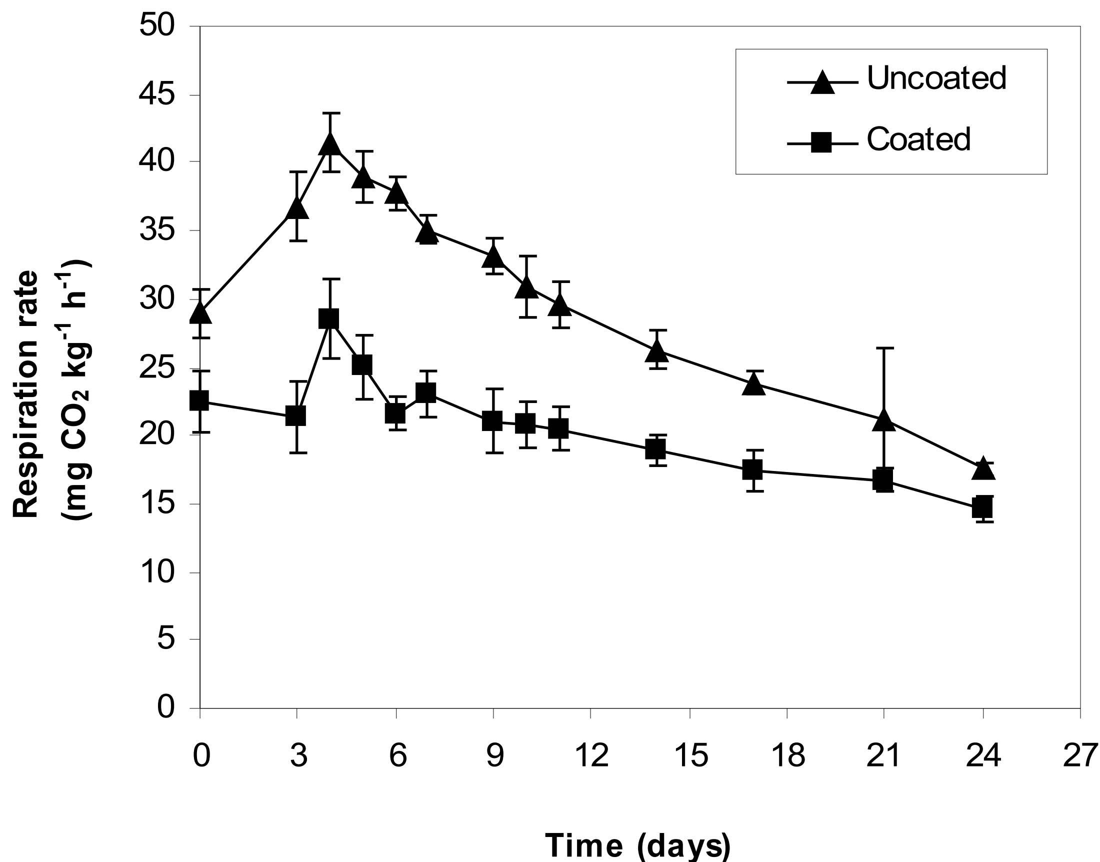


Fig. 4.2.1 Effect of a 2% (w/w) kafirin coating on the respiration rate of 'Packham's Triumph' pears ripened for 24 d at 20°C (35 to 45% RH), expressed as mg CO_2 kg^{-1} h^{-1}. Data are means of 6 groups of 6 pears each (n=36). Bars represent standard deviations of the means

Based on the climacteric peaks that were reached on day 4 (Fig. 4.2.1), the coated and uncoated pears appeared to ripen faster than expected during the shelf-life study, when compared to previous work (own results). The quality attributes would confirm whether day four was truly the point of optimum physiological ripeness for both groups of pears. Many factors may have contributed to the accelerated ripening rate of the pears. The quality of the pears used in the shelf-life study may not have been ideal because pears stored in CA chambers at -0.5°C (95% RH) for 18 weeks prior to procurement, were used. Crouch (Manager, Pome Fruit, ExperiCo, June 2004 – personal communication) commented that the biological processes of fruit from CA storage exhibited an initial lag phase prior to the commencement of ripening

after which ripening commenced faster than it would in fruit stored only under RA conditions. Thus, the accelerated respiration rate and attainment of the climacteric peak on day four may have been a result of the CA storage of the pears prior to the start of the shelf-life study. Storage at 20°C prior to coating may also have induced ripening in the pears and may have contributed to the accelerated ripening rate. In addition, Maree (Colours Fruit exporters) and Crouch (Manager, Pome Fruit, Experico) confirmed that pears from the 2004 season had a shorter storage life and ripened faster than pears used in the 2003 season (personal communication, April 2004).

Most importantly, the initial storage of the coated and uncoated pears (18 weeks under CA conditions prior to coating plus one week at 0°C, RA) prior to the start of the shelf-life study may have contributed to pear maturity. Amarante *et al.* (2001a) found that cold storage of pears prior to coating increased pear maturity because pears were entering the respiration climacteric (rapid ripening) phase at the time of coating. This resulted in a shorter shelf-life and faster ripening than the freshly harvested pears, which were still in the pre-climacteric phase at the time of wax-coating. Considering that pears in this study may have been prone to ripen fast, the pears may have already entered the climacteric (rapid ripening) phase prior to coating and subsequent refrigeration at 0°C for one week under RA.

This may explain why it appeared that the kafirin coating was not able to prevent the onset and commencing of the climacteric phase (ripening) in the coated pears and thus was unable to retard ripening. However, the question remains whether the coating had any effect on the rate of senescence of the pears. Overall, the average respiration rate of the kafirin-coated pears (20.9 mg CO_2 kg^{-1} h^{-1}) (Fig. 4.2.1) was significantly ($P < 0.001$) lower than that of the uncoated pears (30.9 mg CO_2 kg^{-1} h^{-1}) over the 24-day period. In addition, the respiration rate of the uncoated pears (41.4 ± 2.2 mg CO_2 kg^{-1} h^{-1}) at the climacteric peak was significantly ($P < 0.05$) higher than that of the coated pears (28.6 ± 2.8 mg CO_2 kg^{-1} h^{-1}) pears on day four. It was found by Solomos (according to Kader, 1986) that the respiration rates of fruits decrease in response to reduced levels of O_2. In addition, according to Sfakiotakis (as reviewed by Mir and Beaudry, 2001), a reduction in O_2 uptake is followed by reduced respiration rate as a primary metabolic response to low O_2 concentrations. Thus, the

2% (w/w) coating was probably able to limit the O_2 availability to the pears in this study (Fig. 4.2.1).

Eksteen and Ginsburg (1977) stated that the climacteric peak was the point when 'Bon Cretien' pears were considered eat-ripe and that senescence followed the eat-ripe phase. Thus it may be said that eat-ripe pears are at the beginning of senescence. From the trends in the respiration rates of Experiment 1 (Fig. 4.2.1), the slope of the chart for the coated pears after day four (start of senescent phase) appeared less steep when compared to that of the uncoated pears. Senescence and deterioration may have progressed slower in the coated pears, but can only be confirmed after evaluation of the quality attribute results. This may support the theory that although the coating may not have retarded the climacteric phase or delayed the reaching of the climacteric peak, it may have retarded the senescence phase.

4.2.3.2 Respiration and ethylene production rate (Experiment 2)

In Experiment 2, both coated and uncoated pears exhibited atypical climacteric respiratory patterns and no clear climacteric peaks were observed (Fig. 4.2.2). For the uncoated pears (Fig. 4.2.2) the atypical curve may be a result of variations in the respiration rates (standard deviations) and in the time required by the different pear groups, to reach the climacteric peak. In the coated pears the climacteric phase, respiratory peak and senescence phase are not clear (Fig. 4.2.2). Two very similar but insignificant ($P > 0.05$) respiratory peaks manifested on days four (20.42 ± 3.1 mg CO_2 kg^{-1} h^{-1}) and ten (20.45 ± 2.9 mg CO_2 kg^{-1} h^{-1}), respectively.

The respiration rate of the coated pears was not indicative of anaerobic respiration because during anaerobic respiration (in the absence of O_2) CO_2 is produced without the consumption of equal amounts of O_2 (Salveit, s.a.). When O_2 is limiting, carbohydrates are converted to pyruvate, which is metabolised to either lactic acid or acetaldehyde, and the latter is converted to ethanol during fermentation (Wills *et al*., 1981). However, CO_2 did not increase much during ripening of the coated pears (Fig. 4.2.2). The atypical respiratory behaviour may have been a result of over-storage or over- maturity because pears for Experiment 2 (from the same batch used

in Experiment 1) were stored for effectively 31 d (at 0 ± 1°C, RA) prior to pear coating and the start of Experiment 2.

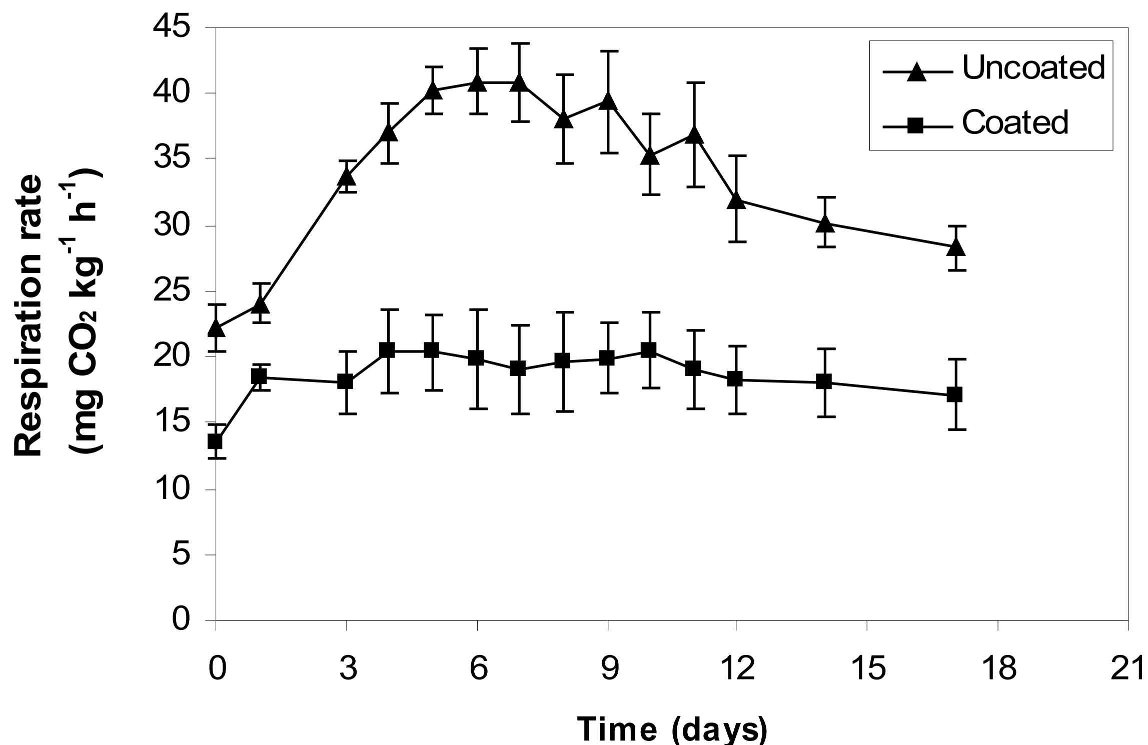


Fig. 4.2.2. Effect of a 2% (w/w) kafirin coating on the respiration rate of ‘Packham’s Triumph’ pears after 24 d storage at 0°C and subsequent ripening over 18 d at 20°C (35 to 45% RH), expressed as mg CO_2 kg^{-1} h^{-1}. Data are means of 5 groups of 6 pears each (n=30). Bars represent standard deviations of the means

Over storage of fruit often results in delayed and abnormal ripening (Blanpied, 1990). It is proposed that the absence of a clear climacteric phase and climacteric peak in pears from Experiment 2 may have been the result of fruit maturity due to the extended storage. According to Romani (1984) the climacteric (increased respiration) is an indication of homeostasis. During fruit ripening, anabolic and catabolic processes counteract each other as the fruit constantly tries to prevent the onset of senescence (post climacteric peak phase). The small peaks prior to the climacteric peak are indicative of successful homeostasis but the climacteric peak manifests when the fruit can longer prevent senescence. The intensity of homeostasis, and thus the intensity of the climacteric, decreases in more senescent fruit. The variation between the ripening patterns in fruit from the two experiments

may in part also be ascribed to the extended storage of the fruit used in Experiment 2.

The respiratory climacteric peak of the uncoated pears (40.9 ± 2.4 mg CO_2 kg^{-1} h^{-1}) may have occurred on day six of ripening at 20°C (Fig. 4.2.2), which correlates better with findings by Amarante *et al.* (2001a) of seven days to reach the climacteric peak, than the respiratory climacteric data of the uncoated fruits from Experiment 1 (requiring four days to peak) (Fig. 4.2.1). The level of CO_2 evolution for the uncoated fruits at the respective climacteric peaks from Experiments 1 and 2 were similar at 41.4 ± 2.2 mg CO_2 kg^{-1} h^{-1} (day 4, Fig. 4.2.1) and 40.9 ± 2.4 mg CO_2 kg^{-1} h^{-1} (day 6, Fig. 4.2.2), respectively.

The ethylene concentration of the coated pears reached a maximum on day ten (Fig. 4.2.3). When it is added to the respiration rate data (Fig. 4.2.2), the climacteric peak in the coated pears may have occurred around day ten. Contrary to expectations, ethylene production in the coated pears (Fig. 4.2.3) was not delayed by the kafirin coating because the maximum concentration was reached on day 10. As previously proposed, ripening may have commenced in all the pears prior to coating (Fig. 4.2.1). Cold treatment is known to promote the synthesis of ethylene in pears (Murayama *et al*., 1995) hence ethylene production may also have started in the fruit during storage prior to coating. This may explain the occurrence of the ethylene peaks on the same day (Fig. 4.2.3) although the ethylene concentration for the uncoated fruit was significantly ($P < 0.05$) higher than that of the coated fruit. In fact the maximum ethylene concentration of the coated pears was more than three times lower, and significantly ($P < 0.05$) so, than that of the uncoated pears. Reason being that the ethylene biosynthetic pathway, especially the conversion of the ethylene precursor to ethylene, is O_2 dependant.

Thus a reduction in O_2 reduces the rate at which the fruit can synthesise ethylene. Wang (1990), noted that when apples were stored in 3% O_2 they produced two to three times less ethylene than those stored in air (Wang, 1990). Similarly, Xu *et al*. (2001) found that a soy protein isolate coating delayed ethylene production of kiwi fruit and that the ethylene concentration was 3 to 4 times less than that of the

uncoated fruit. Decreased O_2 levels, brought about by the kafirin coating, appear to be effective in reducing ethylene production (Kader, 1989).

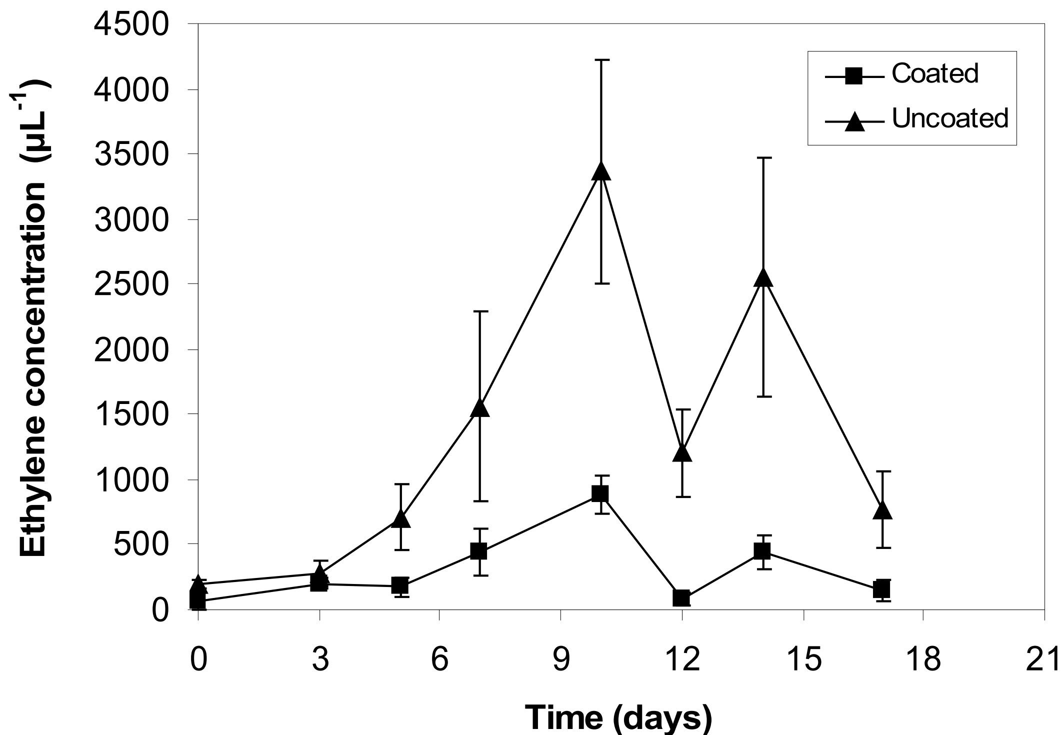


Fig. 4.2.3. Effect of a 2% (w/w) kafirin coating on ethylene production in 'Packham's Triumph' pears after 24 d ripening at 0°C and subsequent ripening over 18 d at 20°C (35 to 45% RH). Data are means of 5 groups of 6 pears each (n=30). Bars represent standard deviations of the means

The reduced respiration and ethylene production rates of coated pears in Experiment 2 support the finding from Experiment 1 that the kafirin coating acts as a gas barrier by restricting the penetration of O_2 from the atmosphere through the coating to the fruit for respiration. Thereby fruit respiration rate, as a reflection of fruit metabolism (Mir and Beaudry, 2001), was reduced. Similarly, Eksteen and Ginsburg (1977) found that a decrease in O_2 levels retarded respiration and reduced metabolism in 'Bon Cretien' pears and retarded the time taken to reach the climacteric peak and the associated processes of senescence.

4.2.3.3 Moisture loss

The coated and uncoated pears lost a significant (P < 0.05) amount of moisture during ripening (Fig. 4.2.4). This was expected as the metabolic activity of fruits at the typical ripening temperature (20°C) would be high and the ambient relative humidity (35 to 45%) very low. Surprisingly, however, there was no significant (P > 0.05) difference in moisture loss between the coated and uncoated pears. Although not significant, the trend was for coated fruit to lose less water.

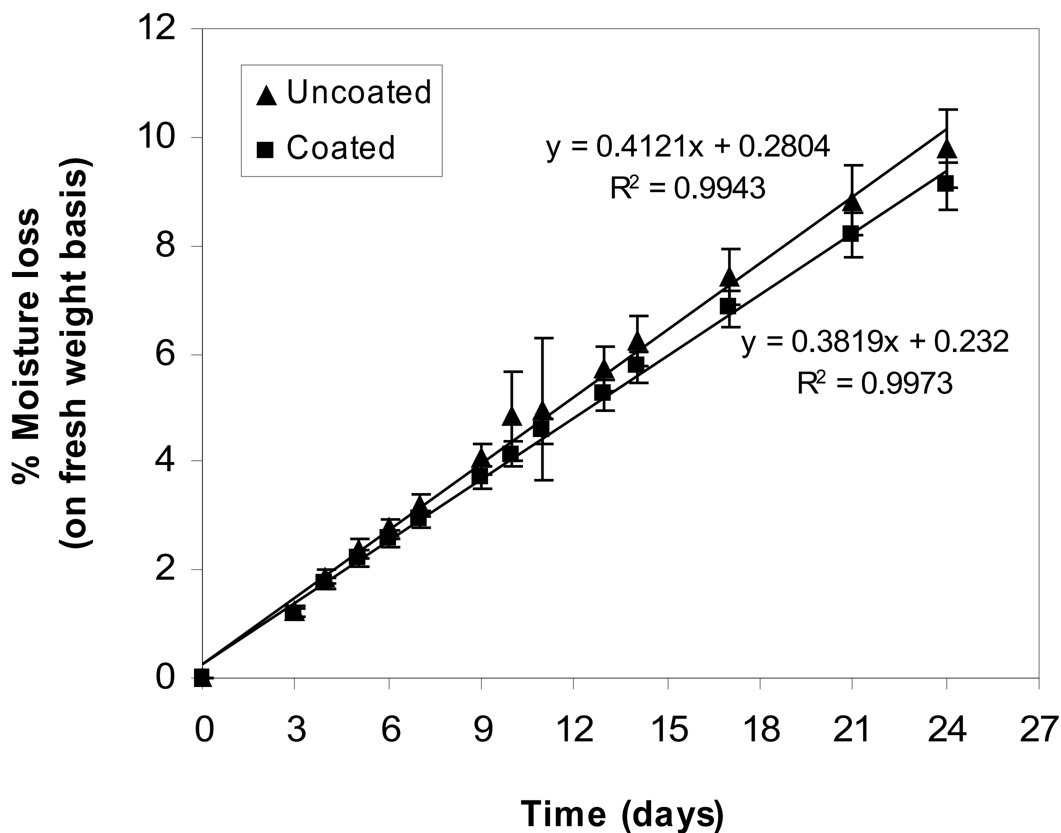


Fig. 4.2.4. Effect of a 2% (w/w) kafirin coating on the cumulative moisture loss of ‘Packham’s Triumph’ pears ripened at 20°C (35 to 45% RH). Lines represent the best fit for % moisture loss on a fresh weight basis for coated and uncoated pears. Data are means of 6 groups of 6 pears each. Bars represent standard deviations of the means

It is proposed that moisture loss in the coated pears may have been aggravated by the age of the fruit and the ethanol in the coating solution may have damaged the soluble cuticular lipids (SCL). SCL affect the barrier properties of cuticle membranes

to water and are considered to be the main barrier against moisture transport through plant cuticles (Baker, according to Maguire *et al.*, 2001). Changes in the quantity and structure of SCL as fruit approach physiological ripeness have been thought to decrease the barrier properties of fruit to water vapour (Maguire *et al.*, 2001). In addition the wax crystals of the SCL degrade as fruit tissues senesce as a result of mechanical abrasion and termination of wax production. Subsequently, the water vapour permeance of over-mature fruit increases (Jenks and Ashworth, according to Maguire *et al*., 2001).

On arrival in Pretoria from Cape Town, the procured pears all exhibited slight stem-end shrivelling. However, the coated pears appeared to shrivel faster (over the entire pear surface) than the uncoated pears after 10 or more days of ripening at 20°C (35 to 45% RH) (Fig. 4.2.5).

Fig. 4.2.5. Shrivelling on the surface of the 2% (w/w) coated 'Packham's Triumph' pears (right) and uncoated pears (left) after 22 d of ripening at 20°C (35 to 45% RH)

The lack of difference in moisture loss between the coated and uncoated pears and the shrivelling on the coated pears after 10 d of ripening indicated that the 2% kafirin coating was not an effective moisture barrier to reduce moisture loss and shrivelling.

As alcohol is known to be a dehydrating agent, there may be a relationship between the alcohol content of this formulation and the shrivelling observed on the coated fruits (Fig. 4.2.5). Saltveit (according to Podd and Van Staden, 1998) proposed that the treatment of tomatoes with high levels (4.7 mg/g) of external ethanol, increased cell membrane permeability. The effect of the ethanol was more pronounced on the mature tomatoes than on younger fruit. Thus, the surface shrivelling on the kafirin coated pears may have been the combined effect of mature fruit (due to the CA storage for 18 weeks prior to coating) that was dipped in a solution that contained high levels of ethanol, which weakened the cell walls. Subsequently, at the low RH (35 to 45%) it was probably easier for moisture in the coated fruit to escape through the weakened cell walls and the, possibly, damaged cuticle. The effect of the ethanol may have been less damaging to the fruit cell and cuticle structures if the fruit were not as mature i.e. if fruit were only stored at RA for a few weeks prior to coating.

It should, however, also be considered that there may be an optimum protein concentration to prevent moisture loss and shrivelling, which may be different from the protein concentration required to retard ripening (gas barrier properties of the coating).

4.2.3.4 Quality attributes

Colour

According to Van Der Merwe (1996), a skin colour difference of 0.5 units on the Unifruco colour chart indicates a visible colour change. The uncoated pears de-greened markedly within the first ten days (Fig. 4.2.6). Physiologically ripe (eating-ripe) 'Packham's Triumph' pears typically have a green or green- yellow skin colour. A yellow 'Packham's' pear is considered over-ripe and past its best eating quality (Crouch, Manager: Pome Fruit, Experico, 2006 – personal communication). Although it was expected that a "physiologically ripe colour" (a value of 4) would have manifested between days three and seven to coincide with the respiration climacteric peak on day four, it developed between days seven and 10.

The coated pears only reached a "ripe colour" (green - yellow) on day 24, which does not coincide with the respiration peak at day four (Fig. 4.2.1). The respiration rate is

usually an indication of the ripening pattern of the fruit and it was expected that some colour change would occur around day four. The delay in colour change in the coated pears did not coincide with normal, climacteric ripening pattern of pear at all.

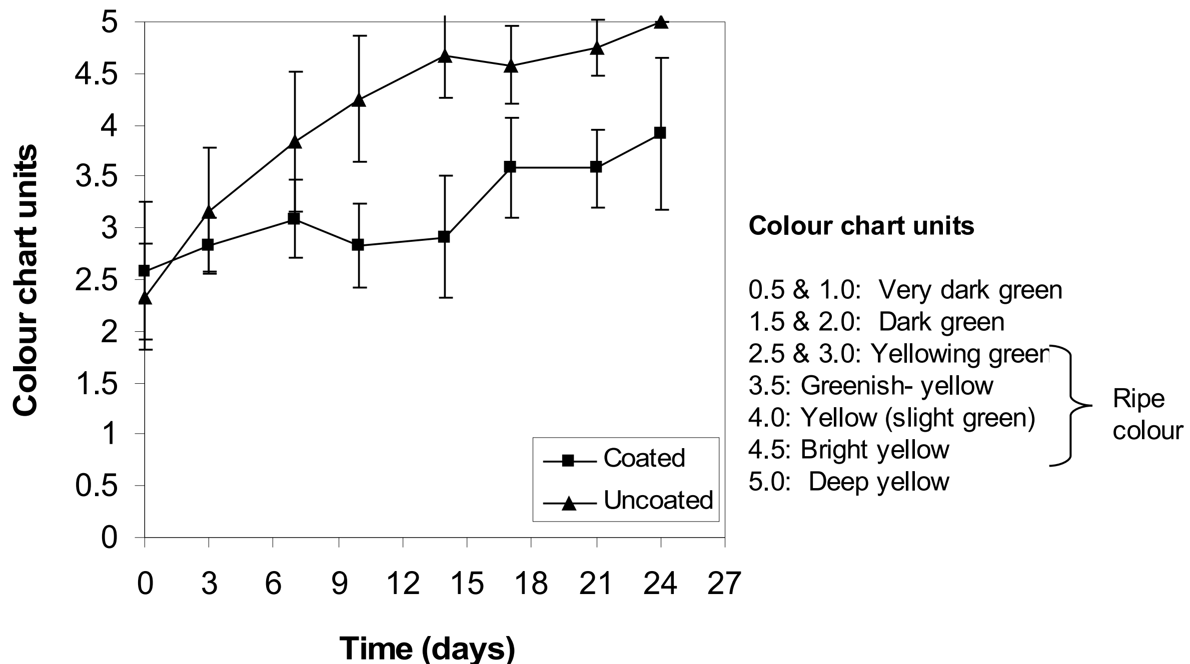


Fig. 4.2.6. Effect of a 2% (w/w) kafirin coating on the colour of 'Packham's Triumph' pears during 24 d of ripening at 20°C (35 to 45% RH), measured with a Unifruco Colour chart. Data are means for 6 pears analysed in triplicate on the sun side (n=6). Bars represent standard deviations of the means

The a* values (Fig. 4.2.7a) showed that significant ($P < 0.05$) de-greening in the uncoated pears occurred between days 3 and 14 and that the predominantly green colour (negative a* values) was replaced by yellowing (positive a* values on the red side) between days 7 and 10. This correlates well with the colour chart values where the "ripe colour" developed between days 7 and 10 (Fig. 4.2.6). The significant ($P < 0.05$) increase in b* values of the uncoated pears (Fig. 4.2.7b) between days 0 to7 suggests that yellowing increased, thus supporting the a* values.

The Hunter a* and b* values for the coated pears (Figs 4.2.7a and 4.2.7b) supported the findings of the Unifruco colour chart (Fig. 4.2.6), indicating that the colour remained predominantly green for most of the storage period. According to the trend in the a* values the colour of the coated pears shifted away from predominantly

green after day 17 although there was no significant difference (P > 0.05) between the values for days 17 to 24.

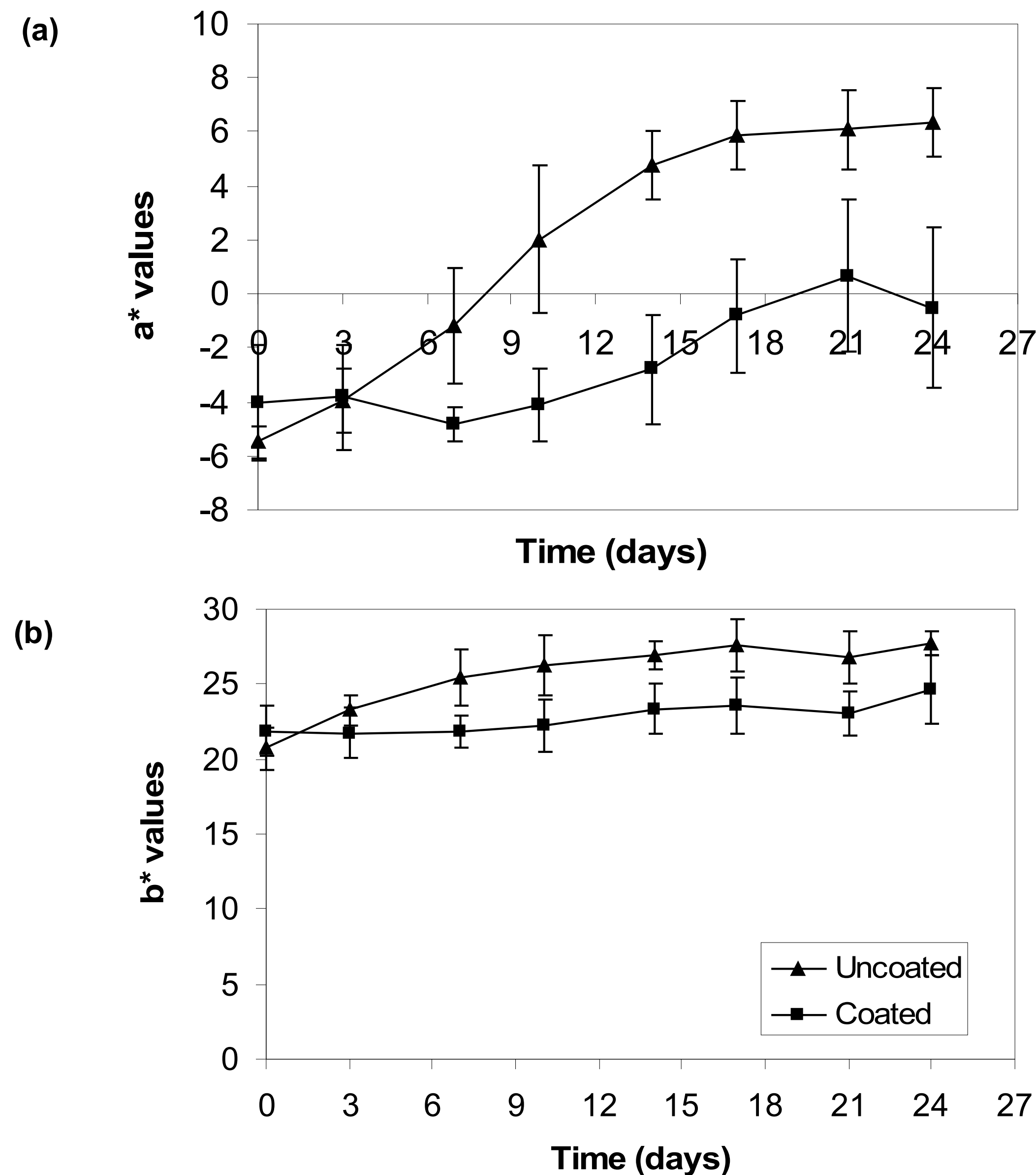


Fig. 4.2.7. Effect of a 2 % (w/w) kafirin coating on the Hunter (a) a* and (b) b* colour values during 24 d of ripening of 'Packham's Triumph' pears for 24 d at 20°C (35 to 45% RH). Data are means of 6 pears analysed in triplicate on the sun side. Bars represent standard deviations of the means

The delay in skin colour change of the coated pears may be attributable to the kafirin protein as well as the initial exposure to ethanol in the coating solution. According to a review by Podd and Van Staden (1998), it is well documented that ethanol can

disrupt the ethylene synthesis pathway through the inhibition or reduction of ACC oxidase that converts ACC to ethylene. As a result, the respiration rate is decreased and ripening is inhibited. Beaulieu and Saltveit (according to Podd and Van Staden, 1998) the alcohol concentration required for ripening inhibition depends on the maturity of the fruit.

Amarante and Banks (2002) found that colour change in wax-coated pears was sensitive to small changes in the internal O_2 concentration. The latter decreased when the coating thickness was increased, which caused a delay in colour change. The delay in skin colour change of the kafirin-coated pears along with the reduced respiration rate of the coated pears (Fig. 4.2.1), supports the theory that the kafirin coating was able to reduce the amount of O_2 available to the fruit from the atmosphere during respiration. Subsequently, the retarded colour change was probably a reaction to the low internal oxygen levels. The effect of low O_2 levels on de-greening retardation may possibly be explained by the “pheophorbide-a-oxygenase” (PaO) pathway of chlorophyll breakdown in senescent leaves, which requires oxygen for the step in the pathway where the first colourless compound (pFCC) is formed (Matile *et al*., 1999). Therefore, when O_2 was limiting in the coated pears during the shelf-life study, there may not have been sufficient O_2 for de-greening and the latter only occurred towards the end of ripening when less O_2 was required for respiration and other O_2 -requiring physico-chemical changes.

Flesh firmness

In the uncoated pears, flesh firmness immediately beneath the skin and internally (Figs 4.2.8a and 4.2.8b) decreased significantly ($P < 0.05$) between days zero and three. This coincided with the climacteric phase (days zero to four) of the respiration rate data (Fig. 4.2.1) as rapid textural changes typically occur during the climacteric phase (Wills *et al.*, 1981). The less dramatic flesh softening of the uncoated pears that occurred between days three and seven (Figs 4.2.8a and 4.2.8b) coincided with the onset of senescence according to the respiration rate data (Fig. 4.2.1). Flesh softening during senescence is typically less dramatic than during the climacteric phase (Wills *et al.*, 1981).

Significant ($P < 0.05$) flesh softening in the coated pears (Figs 4.2.8a and 4.2.8b) occurred between days 0 and 3, which supports the theory that the kafirin coating was unable to retard the climacteric phase because the latter probably set in prior to pear coating.

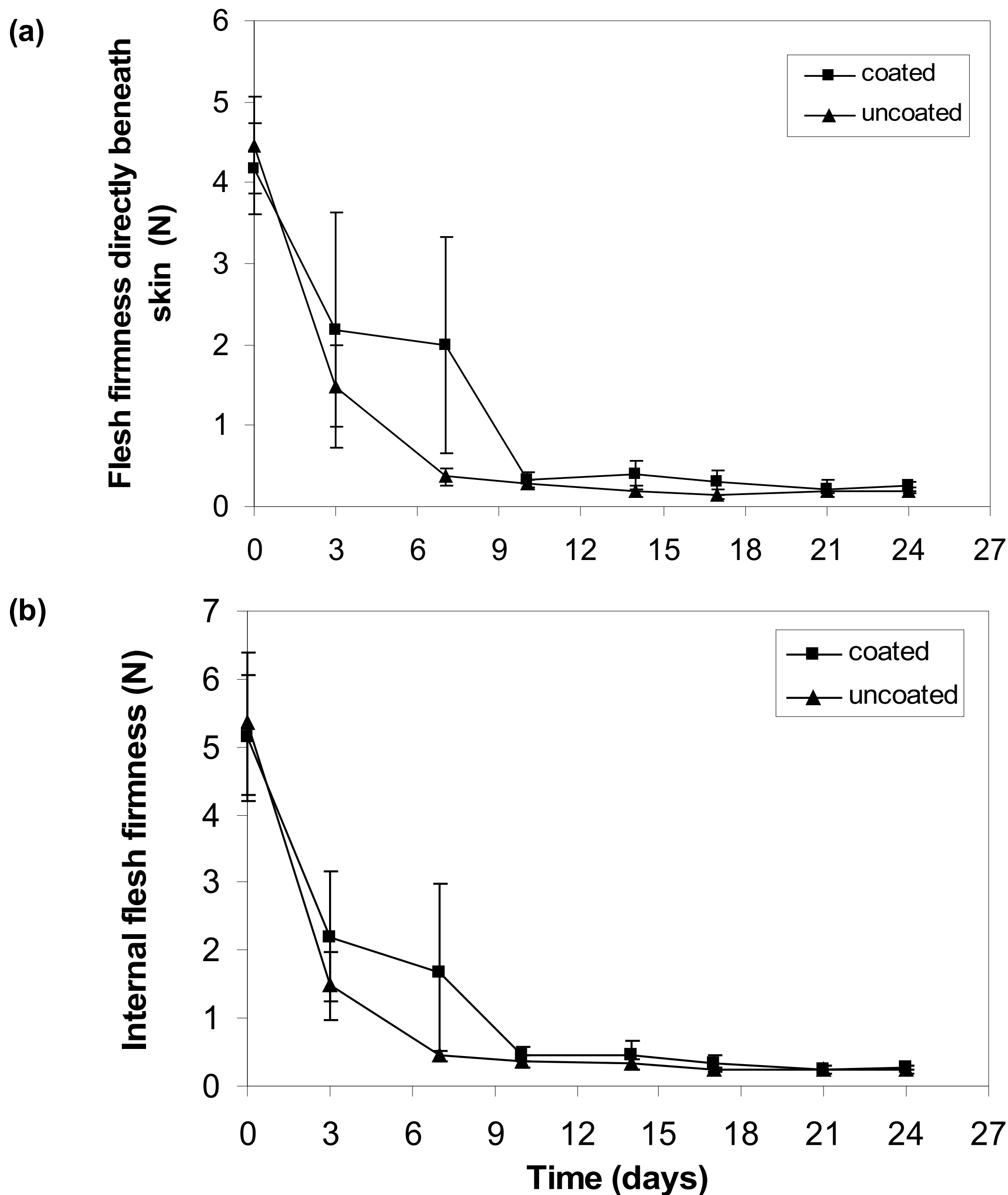


Fig. 4.2.8. Effect of a 2 % (w/w) kafirin coating on the fruit firmness immediately beneath the skin (a) and on the internal fruit firmness (b), in 'Packham's Triumph' pears ripened for 24 d at 20°C (35 to 45% RH). Data are means of 6 pears analysed in triplicate on the sun side (n=6). Bars represent standard deviations of the means

The insignificant ($P > 0.05$) textural changes between days 3 and 7, followed by the significant ($P < 0.05$) textural changes between days 7 and 10, and the insignificant ($P > 0.05$) textural changes after day 10, all support the theory that the coating delayed the onset of senescence to day ten. By day 10 (Fig. 4.2.8a and 4.2.8b) the coated pears were fully mature and could not ripen further (Crouch, Manager: Pome Fruit, Experico, 2006 – personal communication), which would explain the lack of change in flesh firmness after day 10 of ripening. The variation on days 3 and 6 was probably a result of the variation in flesh firmness between the six coated pears. The standard deviations are large because of too few replicates that were used.

Apart from the flesh firmness measured on day seven (Fig. 4.2.8a), flesh firmness directly beneath the skin of the coated and uncoated fruit did not differ significantly ($P > 0.05$) from each other. Only the internal texture of the coated and uncoated fruit (Fig. 4.2.8b) on days seven and 14 differed significantly ($P < 0.05$) from each other. This may further support the theory that even though the coating was not able to delay fruit reaching optimal physiological ripeness (climacteric peak) on day four, the coating may have been able to retard the onset of senescence in the coated fruit.

It is proposed that the dramatic change in texture and little change in skin colour of the coated and uncoated pears up to day three of ripening may have been the result of ethylene production. This is supported by Lelièvre (according to Agar *et al*., 2000a) who stated that softening is more sensitive to and thus initiated at lower levels of ethylene than colour change. In fact, in mature 'Anjou' pears with 2 μl^{-1} internal ethylene or low external ethylene concentrations of 0.05 to 0.2 μl^{-1}, it was found that softening was initiated before the respiratory climacteric (Wang, according to Watada, 1986). Although external ethylene treatment is widely used to initiate ripening (including de-greening) in pears, Mitcham, Agar, Biasi, Gross and Douglas (2000) found that treatment of 'Bartlett' pears with 100 p.p.m. ethylene significantly increased the rate of softening and yellow colour development, when pears were treated for 24 h at 20°C.

The kafirin coating appeared to have a less dramatic effect on flesh firmness than on skin colour. This may be related to the storage temperature of the kafirin coated pears. Amarante *et al.* (2001a) found that during storage of wax-coated pears at

20°C, softening and respiration rates were not delayed as dramatically as colour change, because the coating modified the internal O_2 concentrations more than the internal CO_2 concentrations during storage at 20°C. Conversely, the CO_2 concentration in the wax-coated pears was modified more during storage at 0°C, which resulted in CO_2 accumulation and greater firmness retention. Thus texture was more sensitive to changes in CO_2 concentration. Gas accumulation during storage related to the solubility of the gasses in the hydrophobic coating at the different temperatures.

Typically during fruit ripening, the total soluble solids content (SSC) increases as starch is broken down to sugars and the titratable acidity content decreases (Von Mollendorf, 1996) because organic acids are converted to sugars or respired (Wills *et al.*, 1998). Overall, there were no significant ($P > 0.05$) differences between the coated and uncoated pears with respect to titratable acidity (TA) (Fig. 4.2.9a) or the SSC content (Fig. 4.2.9b). However, the SSC (Fig. 4.2.9a) and TA (Fig. 2.2.9b) of the uncoated pears changed significantly ($P < 0.05$) over time during ripening at 20°C. In contrast, the SSC of coated pears (Fig. 4.2.9a) did not change significantly ($P > 0.05$) during ripening.

In the coated and uncoated pear, the declining trend in titratable acidity up to days 7 and 10, respectively (Fig. 4.2.9b), and the insignificant ($P > 0.05$) change in SSC content (Fig. 4.2.9a) may be a result of organic acids (malic acid), rather than carbohydrates, which provided energy for respiration. The significant ($P < 0.05$) increase in acidity of the uncoated fruit after day ten may be attributed to the loss of cell integrity during senescence, which resulted in mixing of the cell contents. The subsequent decline in organic acids indicates its continued use as fuel for respiration.

According to Mir and Beaudry (2001) the metabolic responses of fruits to low O_2 include a reduction in starch degradation and sugar consumption. Similarly, the insignificant ($P > 0.05$) change in SSC content during ripening of the coated pears may have been a response to the reduced internal O2 levels brought about by the gas barrier properties of the coating. Organic acids (malic acid), rather than carbohydrates probably declined because they provided energy to the coated pears for respiration. According to Wang (1990) the metabolysis of organic acids requires

less O_2 for the production of CO_2. The behaviour in the coated fruit is indicative of a low metabolic activity, manifested as a low respiration rate, which was brought about by the coating providing a barrier against O_2 consumption.

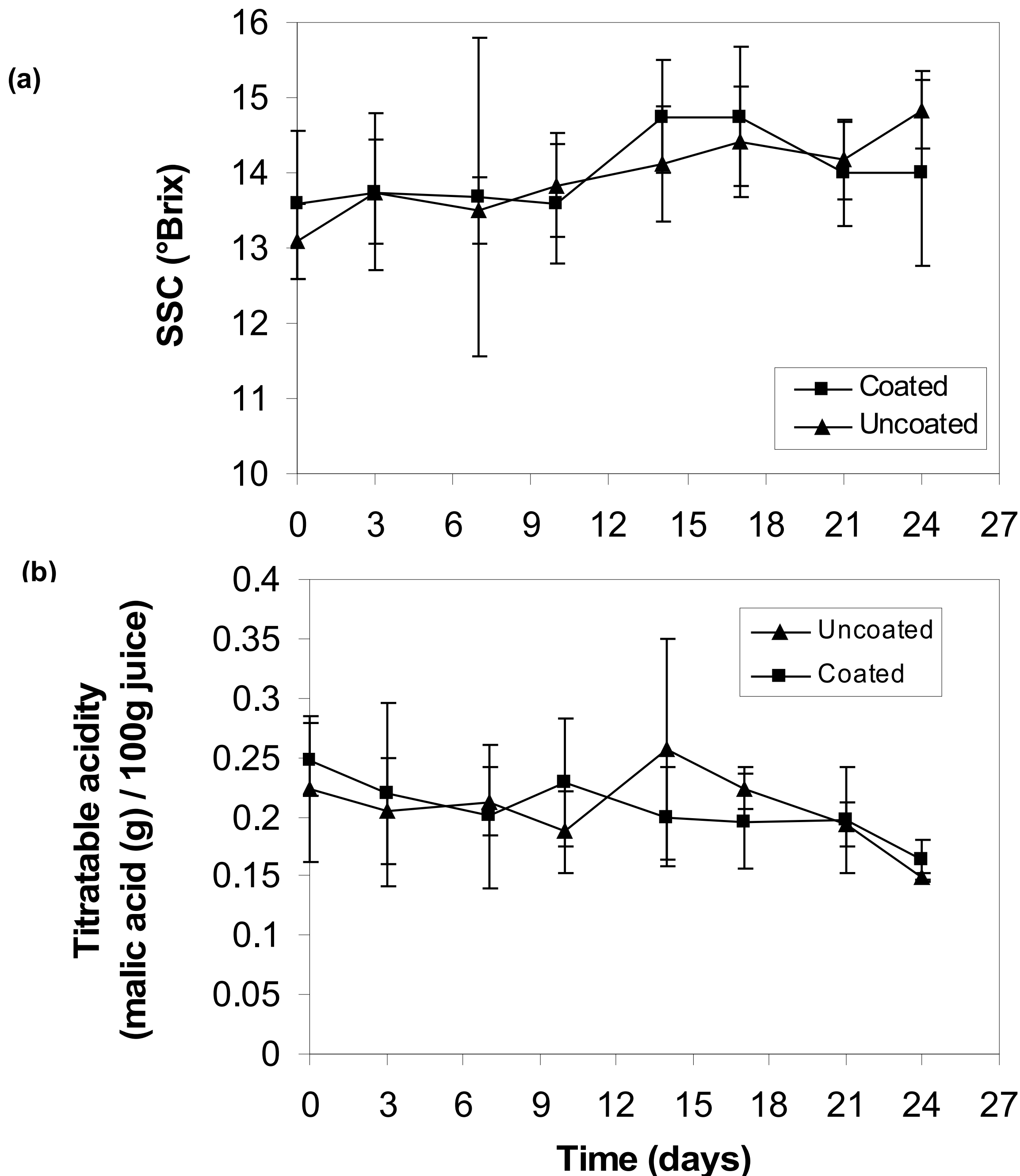


Fig. 4.2.9. Effect of a 2% (w/w) kafirin coating on the soluble solids content (SSC) (a) and titratable acidity (b) in 'Packham's Triumph' pears at 20°C (35 to 45% RH). Data are means of the juice of 6 individual pears (n=6). Bars represent standard deviations of the means

It appears that 18 weeks CA followed by 1 week of RA storage of the pears prior to the shelf-life study dramatically influenced the physiological behaviour of the fruit by increasing fruit maturity and inducing ripening. This has been confirmed by Amarante and Banks (2002) who found that pears should be wax coated directly after harvest and prior to cold storage for the optimal reduction of moisture loss and delay of ripening.

4.2.3.5 Microbiological quality

During ripening at 20°C, significantly ($P < 0.01$) higher aerobic mesophile counts (also known as total plate count) and yeast and mould counts ($P < 0.001$), were observed for the uncoated pears (Figs 4.2.10a and 4.2.10b). The large standard deviations are probably the consequence of analysing too few samples. Not only is biological material (such as pears) known to exhibit much variation, but relatively small variations that occurred in the microbiological population density on the skin of the six analysed pears (0 to 3 log_{10} cfu/ g) may have increased sample variation between such few replicates.

Coliforms did not increase significantly ($P > 0.05$) over the 24-day period. Counts were below 3 cfu/g (or 0.48 log_{10} cfu/g) and were thus not detectable in the peel samples for both the coated or uncoated pears. Lactic acid bacteria were also not detected in either the coated or the uncoated pears over the 24-day period. Coliforms and lactic acid bacteria were probably not detected on the pear peels because (according to Martin-Belloso and Soliva-Fortuny, 2006) yeasts and moulds mostly constitute the native microflora on fruits. The pH of pear flesh varies between pH 3.4 to pH 4.7 depending on the cultivars and the degree of ripeness (Roberts, Pitt, Farkas and Grau, 1998). As a relatively acidic fruit, the predominant spoilage organisms of pears are mostly moulds. The deterioration and spoilage of fruit is usually first detected on the surface (peel) as a result of surface damage or fruit being overripe and decaying. Consequently, soft rots due to mould growth form at the foci of the surface damage (Roberts *et al*., 1998).

The aerobic mesophile counts (Fig. 4.2.10a) of the coated and uncoated pears differed significantly ($P < 0.05$) from each other at the beginning of storage (days 0 to 3) and again on day 17.

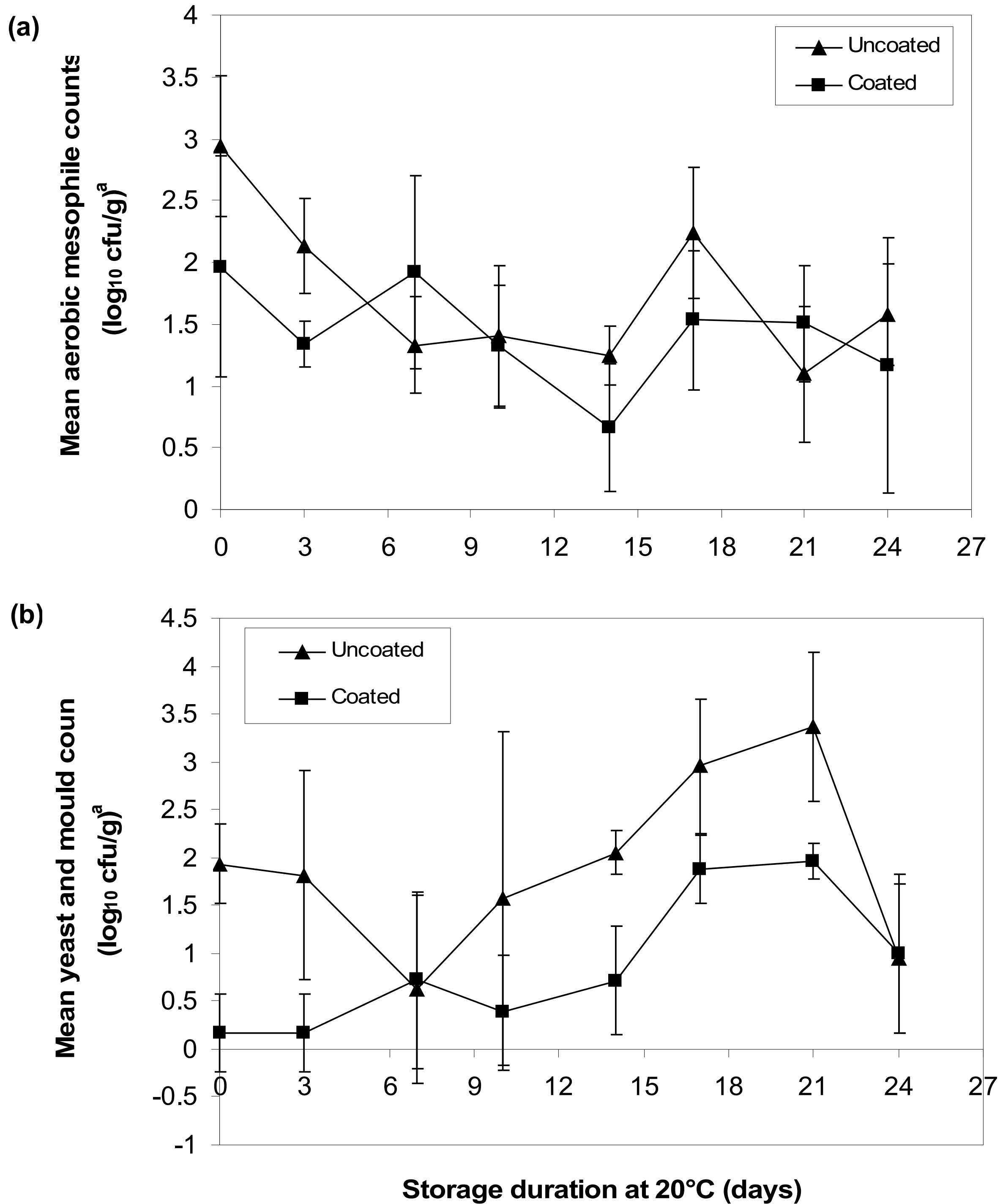


Fig. 4.2.10. Effect of a 2 % (w/w) kafirin coating on the (a) aerobic mesophile counts and (b) the yeast and mould counts in uncoated and coated ‘Packham’s Triumph’ pears during ripening for 24 d at 20°C (35 to 45% RH). Bars represent standard deviations of the means. [a]Minimum growth detection level: 1 $\log_{10}$ cfu/g

The lower levels of yeast and mould growth on the peel of the coated pears may be attributed to the ethanol in the coating solution, which may have reduced initial mould growth upon dipping of the fruit because ethanol is a known disinfecting agent. This theory is also supported by the low yeast and mould counts on the coated pears (Fig. 4.2.10b) measured on days 0 and 3. The anti-fungal action of ethanol on fruit has been confirmed by Karabulut, Gabler, Mansour and Smilanick (2004), who found that germination of *Botrytis cinerea* was inhibited completely after table grapes were dipped for 10 s in ethanol (at a concentration of 30% or more) at 24°C. Interestingly, the grapes were immersed in the solutions while contained within ventilated polyethylene bags. This is important for pears because *Botrytis cinerea* can cause calyx-end rot during pear storage or grey mould rot. Spores of this fungus are able to penetrate undamaged tissue and ultimately cause decay that can spread to surrounding fruit (de Kock and Combrink, 1996).

The significant ($P < 0.05$) increase in aerobic mesophile (Fig. 4.2.10a) and yeast and mould counts (Fig. 2.2.2b) on the coated and uncoated pears between days 14 to 17 and days 14 to 21, respectively, may be related to surface damage, bruising or decay of insect-penetration holes on the senescent, soft fruit. By day 10 of the shelf-life study, the uncoated pears were already in the senescent phase (since day 7) and had reached maximum softness (Fig. 4.2.8). Although the coated pears probably just entered senescence on day 10 (Fig. 4.2.8), flesh firmness was no different to that of the uncoated pears. Soft fruit would have been the most prone to bruising from day 10 onwards, which would have provided opportunity for more mould and yeast growth.

Bruising and wound decay on the coated and uncoated fruit by day 15 of ripening is illustrated in Fig 4.2.11. However, bruising on the uncoated pears appeared more pronounced than on the coated pears. The latter exhibited bruising or probably surface decay on two of the pears (Fig. 4.2.11b), which was probably the result of insect bites or stings while the fruit were on the tree.

On day 24 the aerobic mesophile counts of the coated and uncoated pears were not significantly ($P > 0.05$) different to that of day 21 yet the yeast and mould counts of the coated and uncoated pears were significantly ($P < 0.05$) lower. In addition, days

7 and 24 were the only measurement intervals where the yeast and mould counts (Fig. 4.2.10b) of the uncoated pears were not significantly (P > 0.05) higher than that of the coated pears.

Fig. 4.2.11. Surface bruising and wound decay on the skin of the coated (a, b) and uncoated (c, d) pears after 15 days of ripening at 20°C (35 to 45% RH)

This appears odd because, judging from the amount of bruising on the uncoated pears by day 24 (Fig. 4.2.12), there should have been ample opportunity for yeast infection and growth. In contrast, bruising on the uncoated pears appears less than that of the coated pears, thus the yeast and mould growth should be less than that of the uncoated pears (Fig. 4.2.12). It was observed that sampling of the peel became increasingly difficult as pears became softer. The yeast and mould counts (Fig. 4.2.10b) may have declined by day 24 as a result of the lower pH of the cell contents, (which contained organic acids) of the soft flesh that mixed with the peel during

sampling of the peel. Thus the pH of the sample (peel and flesh) would have been lower, which may have made circumstances less ideal for yeast and mould growth.

Fig. 4.2.12. Visible bruising, decay and mould growth on the surface of the uncoated (a, b) pears vs. less bruising and visible mould growth on the 2% (w/w) coated (c, d) 'Packham's Triumph' pears after 24 days of ripening at 20°C (35 to 45% RH)

Overall there was no obvious visible microbial spoilage or microbial spoilage detectable by analysis in the pears over the 24-day period. The only effect that the coating appears to have had is the reduction in yeast and mould growth on the coated pears. It is well documented that moulds require O_2 to grow (Richard-Molard, 1990) although yeasts are able to grow under anaerobic conditions as well (Roberts *et al*., 1998). The gas barrier action of the kafirin coating may have contributed to the reduced growth of moulds by reducing the amount of O_2 available for their growth. In

addition, ethanol is a well know bactericide that has been used in the past to reduce mould growth on bakery products (Seiler, 1989). Thus, the ethanol in the kafirin coating solution probably also had a disinfecting effect on the fruit peel when pears were dipped during coating. In general, the levels of microbiological growth on the pear skins of the coated and uncoated fruit were lower than those expected in overt spoilage, which is usually around 6 log_{10} cfu/g when spoilage is sensorically detectable (Roberts *et al*., 1998).

4.2.4. Conclusions

The 2% (w/w) kafirin coating is able to extend the shelf-life of CA-stored 'Packham's Triumph' pears during ripening at 20°C. Fruit maturity prior to coating has a marked effect on the efficiency of the coating to extend pear shelf-life and reduce transpiration and shrivelling. In pears coated after ripening has set in, the coating only decreases the respiration rate and retards the progression of senescence. The coating inhibits de-greening mostly because colour change is O_2 dependant and the coating reduces the amount of O_2 available. Conversely, moisture loss is exacerbated in the coated fruit during ripening at 20°C (35 to 45% RH). This is possibly a result of damage caused by the ethanol in the coating solution, to the cuticle and underlying cell walls of fruit that were physiologically over-mature and ripening by the time they were coated. In this study, the 2% (w/w) kafirin coating appeared more suitable in retarding ripening than in preventing moisture loss and shrivelling.

The microbiological data indicates that, in all instances, the levels of microbiological counts on the coated and uncoated pears are low. The bactericidal effect of the ethanol in the coating solution and the reduced O_2 levels brought about by the coating reduces the yeast or mould growth on the coated pears. The action of ethanol in reducing microbiological growth is supported by the significant differences in aerobic mesophile counts of the coated and uncoated fruit.

To optimally extend the quality and shelf-life of 'Packham's Triumph' pears with a kafirin coating, the pears should be coated directly after harvest or after minimal RA storage to ensure that pears are pre-climacteric, as extended or insufficient storage

conditions may increase pear maturity and reduce pear shelf-life. The kafirin concentration may also have to be increased to sufficiently reduce moisture loss and shrivelling during ripening.

5. GENERAL DISCUSSION

The main objective of this study was to assess the effect of a kafirin protein coating on the postharvest physiology and shelf-life of 'Packham's Triumph' pears (Phase 2). The research conducted in Phase 1 provided important baseline data on the physiological behaviour and ripening characteristics of 'Packham's Triumph' pears under different storage conditions, i.e. ideal refrigerated storage (-0.5°C), temperature-abused storage conditions at a market or the retail shelf (10°C) and typical ripening conditions (20°C). As most published research was done on cultivars other than 'Packham's Triumph', information from Phase 1 provided an understanding of the physiological and biochemical behaviour of 'Packham's Triumph' pears, stored at different temperatures under RA conditions. The information also aided the experimental design of Phase 2. However, in both Phases 1 and 2 several aspects of the research were not ideal and could have been improved upon.

5.1 General issues

5.1.1 The raw material

Its climacteric nature, propensity to shrivel, extensive availability during nine months of the year, and popularity as an export fruit and for local sale, rendered 'Packham's Triumph' pears the ideal model fruit for the development and evaluation of the kafirin protein coating. Its uniform green colour when unripe and its gradual, but clear, change from green to green-yellow (during ripening) and eventually to yellow (when over-ripe), simplified assessment of the ripening process. However, it was suggested by Van Niekerk (Director, Fruits Unlimited, 2005 – personal communication) and Crouch (Manager: Pome Fruit, Experico, 2005 – personal communication) that, considering the small profit margins made on exported fresh fruit and the increased retail cost brought about by the kafirin coating, choosing a pear cultivar prone to shrivel, and susceptible to large post harvest losses during export, may be more lucrative to the export industry for extending export earnings.

The cost and advantages of the kafirin coating would have to be compared to that of the polyethylene bags and taken into account for selection of suitable pear cultivars to coat. One such a suitable cultivar may be the summer pear, 'Bon Cretien'. It is exported in large quantities, but may yellow during export causing large losses (Van Niekerk, Director, Fruits Unlimited, 2005 – personal communication). The 'Forelle' cultivar may also qualify for coating because it is known to shrivel and experience ripening problems during shelf-life (Crouch, Manager: Pome Fruit, Experico, 2005 – personal communication). The current kafirin coating may be able to extend the shelf-life of these two pear cultivars.

Despite the availability of raw material, some problems were experienced during the procurement of pears. Pears were acquired through local channels. The distance between the South African pear growing regions and the city where the study was conducted (Pretoria) is more than 1000 km apart. It was not possible for the researcher to ensure the maintenance of the cold chain during transport to, and storage at the fresh produce market in Johannesburg from where pears were collected. When pears are exported, cooling regimes during transport to the harbour and during export, are strict (Anon, 2005a). However, during local transport and storage the cold chain may not be maintained so diligently. Therefore short breaks in the cold chain may have occurred and impacted on the maturity of the pears and their subsequent ripening behaviour. Information on the harvest date, harvest maturity and duration of storage prior to the transport of the different pear batches was also lacking in this study. Such information may have assisted in explaining ripening behaviour that appeared inconsistent with published data, such as that of pears in Phase 2, which required only four days to reach the climacteric peak during ripening at 20°C instead of seven days as was found in Phase 1 and by Amarante and Banks (2002).

As a consequence of the time lapse between conducting Phases 1 and 2 of the study, pears were not of the same season or necessarily of the same maturity at the start of each phase. Phase 1 was conducted on freshly harvested pears at the beginning of the season (March/ April of 2003) while Phase 2 was conducted on pears after storage under CA conditions for 18 weeks (2004 season). Except for the kafirin coating, the different storage regimes (under RA for a few weeks or CA for 18

weeks) of the pears prior to the start of each phase probably had the greatest effect on ripening behaviour of the pears, because it affected the pear maturity, which in turn affected the ripening rate.

5.1.2 Storage conditions

Storage of pears at -0.5°C for several weeks proved to be challenging. Regular refrigerators appeared unable to maintain temperatures at -0.5°C. Fluctuations in the temperature as a result of the cooling and defrosting cycle may have caused the pears to warm up and eventually enter the climacteric, or alternatively freeze the pears at temperatures as low as -2°C. In fact, data accumulated in Phase 1 after 56 days of storage at -0.5°C had to be discarded because pear flesh was damaged by possible freezing and thawing. After thawing the flesh texture was very soft and watery while the respiration rates appeared higher than that of fruit at 42 days. Increased respiration rates at low temperatures usually indicate that the fruit is under stress (Romani, 1984). The fruit did not appear bruised, which means that the increased respiration rates measured during storage at -0.5°C and the softening must have been caused by damage to the pear flesh during freezing.

The storage of pears at 10°C to simulate temperature-abused storage conditions at the export destination or at a market indicated that pears do not only ripen faster under such conditions, but that ripening is driven by a temperature-induced, increased metabolism rather than by ethylene action. However, the choice of lower temperatures for several days to depict: a break in the cold chain (2 to 3°C); or the temperature rise as a result of fruit distribution (2 to 5°C); or storage of fruit in a domestic refrigerator (5 to 8°C), may have been more relevant for understanding how pears may behave during export, distribution and prior to consumption (Crouch, Manager: Pome Fruit, Experico, 2005 - personal communication).

5.1.3 Experimental design

In Experiment 1 (Phase 1) respiration rate measurements were conducted on six groups of six pears each while quality analyses were conducted on only one pear per group (six pears in total per testing interval). In Experiment 2 (Phase 1) six pears

were analysed individually for respiration rate and quality analyses were also conducted on only six pears. However, variability in the results of Experiment 2 (Phase 1) indicated that, due to the intrinsic variability of fruit, six individual pears for quality or respiration rate analysis were not sufficient for optimal results with small standard deviations.

Published information appears contradictory in terms of the amounts of climacteric fruit used for gas and quality analyses. Respiration rate measurements ranged from using four (Cisneros-Zevallos and Krochta, 2003) to 10 individual fruit (Bai *et al.*, 2003) or between three and five groups of 3 to 12 fruit per group (Ju and Curry, 2000; Ju, Duan, Ju, 2000; Park *et al.*, 1994). Quality-attribute analyses ranged from using 4 to 5 individual fruit (Bai *et al.*, 2003) to 10 single fruit (Ju and Curry, 2000; Ju *et al*., 2000), and ultimately to using several groups containing 3 to 5 fruit each (Park *et al.*, 1994; Sümnü and Bayindirli, 1994). It appears that the use of at least three groups containing several fruit is preferred for gas production and quality analyses of climacteric fruit.

The necessity for sufficient replicates may imply that conducting Experiments 1 and 2 in Phase 1 concurrently, was not a good idea. The only advantage of the two experiments running concurrently was that the pears were from the same batch and physiologically the same age. Thus, results would not have been clouded by over-storage behaviour. Unfortunately, the two concurrent experiments required a lot of storage space and analyses capacity, which resulted in the use of fever replicates. However, storage space and analysis capacity may have been utilised best if the experiments were conducted separately with sufficient fruit as replicates. According to Crouch (Manager: Pome Fruit, Experico, 2006 - personal communication), five replicates of at least 10 fruit, but preferably a carton of fruit, per replicate are typically used for analyses in industry.

To present the side of the pear that may be most likely to reduce in quality first, colour change and flesh firmness was measured on the sun sides of the pears. Considering the intrinsic variation between pears and the firmness variation between the sun and shade sides, which may be difficult to distinguish when pears are still green, more than six pears are required for measuring colour and flesh firmness

changes. The microbiological growth measurements on the pear surface may also present less variability if more pears were used as replicates.

Measurement intervals for respiration rate and quality attributes were not frequent enough in this study. In Experiment 1 (Phase 1), pears stored at -0.5 and 10°C were analysed two-weekly and weekly, respectively. Consequently, physiological behaviour of pears from the different storage temperatures could not be sufficiently compared as only two measurement intervals corresponded. In addition, pears at 10°C ripened during storage but the progress of ripening and its time frame could only be speculated about due to the 7-day measurement intervals. As ethylene measurements were lacking during the storage trials of Phase 1 and Experiment 2 of Phase 2, it was even more unclear when ripening commenced and was completed. Respiration rate and quality attribute measurements should preferably be conducted every two days for pears at 10°C and if quality is to be compared to that of pears at -0.5 or 20°C, measurement intervals should also correspond. Even though the respiratory rate of the pears stored at 20°C was monitored daily in Experiments 1 (Phases 1 and 2) and at selected two-day and daily intervals in Experiment 2 (Phase 1) and Experiment 1 (Phase 2), quality attribute analysis may have been monitored best if analysed twice daily during ripening, as suggested by Mitcham and Thompson (1998).

The experimental design of Phase 2 could have been improved if respiration rate, ethylene production rate and quality measurements were conducted on the same measurement intervals. It would have made analyses much more labour intensive, but the combined information would have provided a complete picture of the physiological and biochemical behaviour of the pears at each interval. In addition, the control pears used in Phase 2 was not coated. However, the effect of a “coating” solution without kafirin protein should have been included to establish whether the behaviour of the coated pears was a result of the kafirin protein or of the exposure of the pears to ethanol during dipping into the coating solution. Research by Plotto and Baldwin (2004) on the treatment of whole mangoes with ethanol vapours has indicated that ethanol treatment resulted in a reduced respiration rate, maintained fruit firmness and surface colour.

5.1.4 Methodologies

In this study. a static or closed system was chosen for conducting respiration rate analysis due to the simplicity of the system set-up and the availability of equipment for analysis. As rightly pointed out by Saltveit (s.a.), leaks may affect the accuracy of the system and the accumulation of CO_2 may affect the respiration rate of the fruit. Thus, preliminary tests were conducted on pears during ripening at 20°C to determine the amount of time (in minutes) required for the CO_2 concentration to start increasing linearly and to deviate from linearity. The latter would indicate the point of CO_2 accumulation, which indicated the need for the system to be opened and pears exposed to air before the onset of anaerobic respiration due to exposure of pears to insufficiently low levels of O_2. Therefore, gas was circulated for 2 min prior to the start of each respiration rate measurement (for each replicate) and respiration rate was measured for no longer than an additional eight minutes. During processing of the results, graphs were drawn from the data collected at each measurement interval to ensure calculation of the respiration rate from the slope of a linear line, containing at least five data points.

The use of a closed or static system and an infra-red gas analyser is not uncommon. In fact, Agar et *al.* (2000a, b) sealed six 'Bon Chretien' pears in a 3.7 l glass container for 5 to 30 min and measured CO_2 production rates by analysing CO_2 in the headspace with an infra-red gas analyser. However, the measurement of the change in partial pressure of CO_2 in individual fruit over 30 min (Amarante *et al.*, 2001; Amarante and Banks, 2002) or sealing several pears in containers connected to a flow through system and measuring respiration rate over several hours or days (Baldwin *et al.*, 1999; *Ju et al*., 2000) appear to be the methods of choice for pears. According to a review by Fonesca *et al.* (2002) the greatest limitation of the flow through systems is its inaccuracy in determining low respiration rates i.e. respiration of produce at low temperatures or at low O_2 levels such as pears coated with an edible coating. The low respiration rate of wax-coated pears (including 'Packham's Triumph') in the research of Amarante *et al*. (2001), is probably the reason for their measurement of partial pressure of CO_2 in the wax-coated fruit, rather than measurement of respiration rate in a sealed container.

The measurement of ethylene production rate failed because the system available for ethylene concentration detection required modification to be able to measure low concentrations of ethylene. The measurement of internal ethylene concentrations was not considered as it is a destructive method, which required the storage of large amounts of additional pears for analysis at every measurement interval. The required storage capacity and analysis resources were not available to accomplish this. However, failure to determine ethylene production rates during the largest part of this study complicated storage and ripening data interpretation. It is difficult to interpret respiration rates and quality analyses without ethylene data (whether internal ethylene content or ethylene production rate), because ethylene data assists greatly in the explanation of respiratory behaviour. As respiration rate is closely linked to the volume of ethylene released (Xu *et al.*, 2001), ethylene production measurements would have been especially useful for explaining the ripening behaviour of pears during and after storage at 10°C.

Colour was measured with a HunterLab ColourQuest Colorimeter and a Unifrico colour chart because the South African fruit industry uses this chart. Unfortunately no published information for colour chart values on 'Packham's Triumph' pears could be found. However, it was pointed out by Crouch (Manager: Pome Fruit, Experico, 2006 - personal communication) that physiologically ripe 'Packham's Triumph' pears are typically still green or green/yellow in colour. On the Unifruco colour chart this green/yellow description corresponds to a value of 3.5. Additionally, a full yellow colour is an indication that the pears are overripe (Crouch, Manager: Pome Fruit, Experico, 2006 - personal communication). Our own preliminary trails revealed that the majority of the pears exhibited a yellow/ green colour (a little more yellow than green) at the climacteric peak. As a result a Unifruco colour chart value of 4 was used in the research as an indicator of optimum ripeness. Such discrepancies should be avoided if laboratory results are to be compared to results obtained by industry.

Although colour chart data supported the Hunter a* and b* values in this study, colour chart values may have been superfluous for colour evaluation. In addition, colour change during ripening (from green and yellow) is often indicated by changes in hue angle (h°) (Agar *et al.*, 2000a, b; Drake and Gix, 2000; Amarante *et al.*, 2001a, b, c);

Amarante and Banks, 2002). However, some research contained only a* values (Henze, 1995; Miró *et al*., 2001) or a combination of a* and b* values (Galvis-Sánchez *et al*., 2004; Ju *et al*., 2000). It can only be assumed that the reason for the differences in data presentation (i.e. hue angle, only a* values or a combination of a* and b* values) is a matter of personal choice. For the present study on colour change in 'Packham's Triumph' pears, presentation of colour data as a* and b* values appeared the easiest for observing colour change in the pears. However, for comparison with published research it may be best to present colour change data as the change in hue angle.

Comparison of textural changes that occurred in 'Packham's' pears in this study, to published research, was difficult. Not only are 'Packham's' pears seldom used in storage trials, but the fruit industry measures flesh firmness with a penetrometer that is fitted with an 8 mm diameter probe. If an 8mm diameter probe were used on the Texture Analyser in this study, instead of a two millimetre probe, it would have been possible to compare if fruit firmness was still within marketable standards. Unfortunately, Stable Micro Systems does not have an 8 mm diameter probe in its product range but probes such as the Magness-Taylor/ USDA fruit probe set (probes of 3, 6 and 11 mm diameters) are available and may have been more appropriate. According to Crouch (Manager: Pome Fruit, Experico, October, 2006 - personal communication) an adapter for a Texture Analyser that enables the attachment of an 8mm penetrometer tip, is used in industry. The Texture Analyser has the advantage over a penetrometer in that it excludes operator variability and can thus be set to penetrate the fruit at a constant speed and to a constant depth. Conversely, when using a penetrometer, the angle and speed at which the operator applies the force is critical to the accuracy of the measurement because the firmness reading will increase as the speed of pressure application increases (Bramlage, 1983).

In the present study the sun-side of pears was chosen because it would be the softest side of the 'Packham's' pear. It is to be expected that a probe with a larger diameter (8 mm) will damage larger areas of pear flesh. Thus, in order to evaluate undamaged pear flesh for true flesh firmness, more than six pears will be needed to take sufficient firmness measurements along the equatorial plane of the pear. It would be best to follow the method of Agar *et al*. (2000a, b) where skin on two sides

of the equatorial plane was removed and firmness measured on both sides. In addition, to ensure that measurements are not taken from incorrectly identified sides (sun or shade side), it may be best to measure firmness on opposite side of the pear (both the sun and shade sides) and average the readings.

5.2 Research findings

5.2.1 Relating results from Phases 1 and 2

Phase 1 revealed how storage temperature and storage duration affected export grade 'Packham's Triumph' pear quality and physiological behaviour during typical refrigerated storage (-0.5°C), inadequate (temperature-abused) storage (10°C) and ripening (20°C) under RA conditions. The findings from Phase 1 regarding the storage and ripening behaviour of 'Packham's Triumph' pears were not novel for pears in general, as similar findings for 'Bartlett' pears were shown by Agar *et al*. (2000a, b). However, Phase 1 provided indispensable information for Phase 2 of this study. Phase 2 revealed the effect of a kafirin coating on the shelf-life and physiological behaviour of coated pears, when ripened at 20°C and compared to uncoated, ripened pears. In Phase 1 the metabolic activity of 'Packham's Triumph' pears at -0.5°C did not change significantly during storage for 42 days. Conversely, pears at 20°C ripened faster than pears at 10°C or -0.5°C, because the climacteric peak was reached earlier at the higher temperature and the magnitude of physico-chemical changes associated with ripening was more pronounced. If a kafirin coating was to extend the storage and shelf-life of 'Packham's' pears it would ideally have to do so at any temperature, particularly when pears are not refrigerated.

Generally, to achieve a low respiration rate and ultimately a low metabolic activity in climacteric fruit, other than by refrigerated storage, one could subject the fruit to low O_2 concentrations (Mir and Beaudry, 2001). This is normally achieved by creating a modified atmosphere around the pears by the use of modified atmosphere packaging or creating it in the pears through the use of appropriate edible coatings. Bai *et al.* (2003) proved that a zein protein edible coating was able to create a modified atmosphere in apples, which resulted in an extended apple shelf-life at 20°C.

Therefore it was decided to store (and thus ripen) coated and uncoated 'Packham's Triumph' pears in Phase 2, at 20°C only, to determine the effect of the kafirin coating on pear metabolism.

Phase 1 indicated that the climacteric peak of 'Packham's' pears ripened at 20°C, directly after removal from storage at -0.5°C, may occur around days 5 to 7 with maximum respiration rates of up to 150 mg CO_2 kg^{-1} h^{-1}. Thus, the experimental design of Phase 2 was constructed to include daily respiration rate analysis between days 3 to 9 to enable more accurate assessment of the metabolic rate of the pears during ripening at 20°C. Unfortunately, Phase 2 could only be conducted towards the end of the season (August/ September) and pears from CA storage behaved differently to the freshly harvested pears used in Phase 1.

The results indicated that physiological behaviour and physico-chemical changes were accelerated by CA storage and total storage duration of pears, used in Phase 2. Uncoated pears at 20°C (Phase 2) respired at a lower rate (below 45 mg CO_2 kg^{-1} h^{-1}) than uncoated pears at 20°C (Phase 1) because the CA-stored pears were possibly more mature and were thus exhibiting a less intense homeostatic response (Romani, 1984) than the freshly harvested pears. The first signs of shrivelling in pears (Phase 1) stored at 20°C were observed after four days of storage at 95 to 98% RH, while pears from CA-storage exhibited signs of stem-end shrivelling on arrival. Interestingly, pears in both Phases 1 and 2 (uncoated pears), analysed on day zero, exhibited similar levels of de-greening and yellowing, as well as internal and external texture. After ripening for 20 (Phase 1) and 21 (Phase 2) days at 20°C, pears from Phases 1 and uncoated pears from Phase 2, respectively, de-greened and yellowed to similar extents and the coated and uncoated pears in Phase 2 softened to a similar extent to pears in Phase 1. The reason may be that the pears were fully ripe and were not able to ripen any further.

It is evident from the findings that, although storage temperature had an effect on the rate of quality deterioration, fully ripe 'Packham's' pears from different storage conditions exhibited similar final colour and firmness values. Storage duration (at -0.5 or 10°C) or storage at -0.5°C (under CA or RA conditions) prior to ripening of

pears at 20°C, may cause differences in the rate of pear ripening but ultimately ripe 'Packham's' pears ripen to similar final colour and firmness values.

Generally, statistical analysis of results is required to improve the accuracy of result interpretation. However, Crouch (Manager: Pome Fruit, Experico, October, 2006 - personal communication) commented that results which appear to be statistically insignificant, may have value in the export industry and make the difference between fruit being accepted or rejected at the export destination. Thus, the trend followed by a certain attribute over time (such as the change in flesh firmness or moisture loss) should also be considered in the interpretation of results.

5.2.2. Physiological vs. eating ripeness

It is well known that the point of physiological ripeness in climacteric fruit, including pears, is the climacteric maximum, which is followed by senescence when fruit quality rapidly deteriorates (Eksteen and Ginsburg, 1977). The eating ripeness of pears is very much a question of consumer preference because some consumers may prefer to eat pears when still crunchy and green while other may prefer a soft, juicy, yellow pear. It is understood that physiological ripeness is the point at which most of the physico-chemical changes have taken place. Although the respiratory climacteric peak of the coated and uncoated pears were reached on the same day (day 4 at 20°C) in Phase 2, coated pears were noticeably different to uncoated pears in all aspects except moisture loss, from day three of storage and onwards. Thus, physiological ripeness in the coated pears cannot be directly correlated with the eating ripeness or the manifestation of the majority of physico-chemical changes in this study. For kafirin coated pears, descriptive sensory and consumer sensory evaluations may best indicate what the correlation is between physiological ripeness and eating ripeness in the coated pears.

5.2.3 The kafirin protein coating

A model for the proposed effect of the kafirin coating on the shelf-life of coated and uncoated 'Packham's Triumph' pears is given in Figure 5.1. There is much to learn about the behaviour of the kafirin coating under different storage conditions.

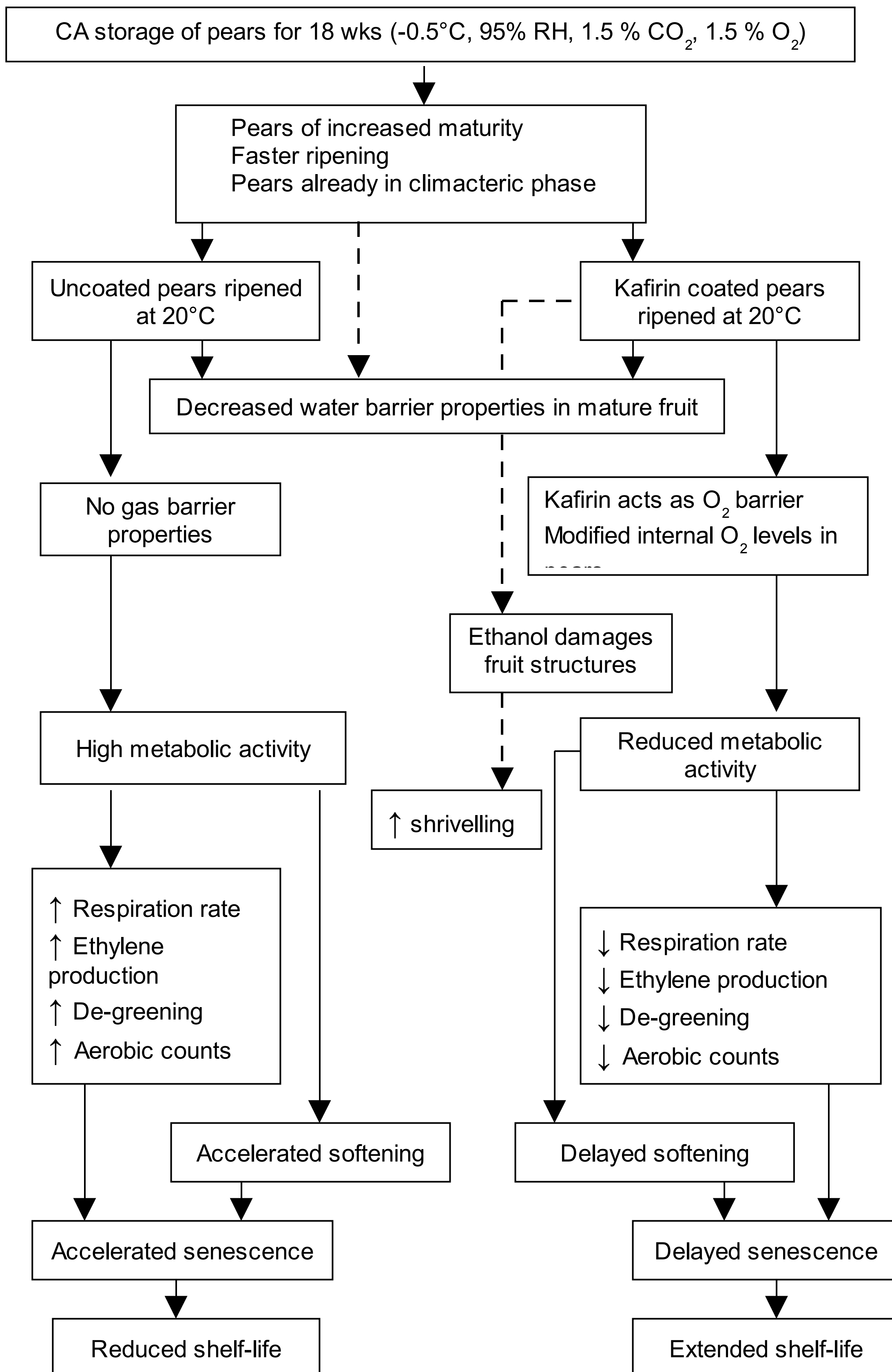


Fig. 5.1. Proposed model for the effect of the kafirin coating on the shelf-life of coated pears during ripening at 20°C, in comparison to uncoated pears

Nevertheless, based on what was learnt from the current study on 'Packham's Triumph' pears, the ability of the kafirin coating to extend pear shelf-life during storage at 20°C, may be improved by taking the following aspects into account.

5.2.3.1 The fruit

Although the study was meant to be conducted on export-grade pears, the quality of the pears used, were not considered export grade. Shelf-life was defined by Bester (1973) as the retention of original product quality for the period required to obtain marketing goals. Although the kafirin coating was able to extend the shelf-life of the pears by retarding senescence for up to 10 days compared to between 3 and 7 days in uncoated fruit, the kafirin coating did not retard ripening. This may be related to the pear quality. Pears used in this shelf-life study, could be considered as a "worst case scenario", because the 2004 pear season was known for its fast-ripening pears (Maree, Colours fruit, 2004 – personal communication). The pears were also from CA storage (for 18 weeks) and after such extended storage (according to Amarante *et al.*, 2001b) the pears were probably more mature than freshly harvested pears would have been (Fig. 5.1).

Although the coated and uncoated pears were stored under RA conditions at 0°C for a week, prior to the start of Phase 2, the coated and uncoated pears all reached the climacteric peak on day 4 of ripening at 20°C. Thus, ripening had to have set in prior to coating of the pears. Storage at 20°C prior to coating may have initiated ripening, which means that the pears were in the climacteric phase prior to coating (Fig. 5.1).

5.2.3.2 The kafirin coating

As good gas barrier properties are one of the prerequisites of a good fruit coating, the kafirin coating has exhibited potential as a good gas barrier and therefore a good fruit coating. Preliminary respiration rate monitoring of 'Packham's' pears coated with excessively thick kafirin coatings, indicated that the respiration rate readings of anaerobically respiring pears during a single measurement interval, do not increase linearly. The resulting respiration rate graph of the CO_2 concentrations emitted during storage at 20°C for several weeks, does not include a sharp increase, peak or decline in respiration rate (Buchner, Matsane, Kinnear and Minnaar, 2004). The graph is an increasing line, which (according to Saltveit, s.a.) is the result of an

increased use of the substrate (sugars, organic acids) and the subseqeunt production of higher amounts of CO_2, to compensate for the lower amounts of energy produced during anaerobic respiration. Through monitoring the CO_2 levels and the slope of the resulting graphs (as explained in section 5.1.4.), it was verified that the kafirin coating reduced the respiration rate of the coated pears, without inducing anaerobic respiration.

Colour change in the coated pears was dramatically retarded by the coating during ripening at 20°C (Fig. 5.1). The drawback of the delayed colour change in the kafirin coated pears is that the flesh will continue to soften while the colour portrays the pear to be not yet ripe. According to Crouch (Manager: Pome Fruit, Experico, October, 2006 - personal communication) such a delay in colour change could pose a problem for 'Bon Chretien' pears that need to yellow to ripen. Thus, by the time the consumer associates pear ripeness with its colour, the flesh may already have softened excessively. Similar findings were made by Amarante and Banks (2002) on the softening in wax coated pears (including 'Packham's' cultivar) while the colour remained unchanged. However, the coating technology may reduce incidences where fruit at the export destination are rejected as a result of early colour break during export (Crouch, Manager: Pome Fruit, Experico, October, 2006 - personal communication).

The ethanol solvent in the kafirin coating solution appears to have accelerated skin shriveling over the entire pear surface (Fig. 5.1), which renders the current coating formulation unsuitable for use on any quality pear. However, modification of the coating formulation to prevent skin shrivelling is not an easy problem to solve. Other than ethanol, there are no other food-grade solvents that kafirin protein can be dissolved in, that will not damage the skin of the pear. Kafirin protein is soluble in aqueous ethanol (when heated to 70°C) (Cuq *et al*., according to Taylor, Taylor, Dutton and de Kock, 2004). Taylor *et al*. (2004) also identified glacial acetic acid and lactic acid as good solvents for kafirin. Although these acids are known to be food grade, inclusion of these acids in the kafirin coating formulation during coating formulation development caused browning of the coated area. The lactic and glacial acetic acid probably damaged the cells and cell walls of the fruit, thereby causing the cell contents, particularly polyphenols and enzymes, to mix. As a result, the

phenolase enzyme probably oxidised the phenolic substances, which manifested as a browning reaction (Marshall, Kim and Wei, 2000).

To fully utilise the hydrophobic nature of the kafirin coating, the protein content of the coating may be increased to increase the thickness of the coating and hence reduce moisture loss through the cuticle. During development of the kafirin coating it was found that when the kafirin protein concentration in the coating solution was 3% (w/w) or above, no shrivelling was observed over the pear skin surface, even after storage at 20°C for 3 weeks. Unfortunately, these coatings caused anaerobic respiration in the pears (Buchner *et al*., 2004). However, this phenomenon may be counteracted by increasing the amount of plasticiser because (according to Miller and Krochta, 1997) increased levels of propylene glycol increased the O_2 permeability of the coating. This may explain why a zein coating formulation for apples (Bai *et al*., 2003) required 10% zein and 10% propylene glycol. The latter not only increased the glossiness of the coating (Bai *et al*., 2003), but may also have increased the O_2 permeability of the coating sufficiently to allow normal ripening of the apples. As the current kafirin coating served merely to investigate its ability to retard quality loss in pears, there is much room for improving the efficiency of the coating.

Although the kafirin protein was extracted with food-grade chemicals and the coating formulation contained food-grade chemicals, the coating has not yet been authorised by the South African Department of Health as being safe for consumption. Therefore, the kafirin coating cannot be claimed as edible at this stage. Sorghum, which includes kafirin protein, has not been found to be allergenic (Lopata, Principal Investigator, Allergy and Asthma Research Group, UCT Faculty of Health Sciences, 2005 – personal communication), which is an advantage for an edible coating.

5.2.3.3 Application of the coating in the pack house

Under commercial conditions, pears are usually blast-refrigerated (at -0.5°C) in bulk bins directly after harvest to get rid of the field heat and reduce the pear-core temperature, to preserve fruit quality. Subsequently, pears that are not destined for CA storage are stored at -0.5°C for 4 to 6 weeks before packing and export (Coetzee, Logistics Manager, Kromco, 2002 – personal communication). Prior to packing pears are washed in water flumes and will need to be dried before the kafirin

coating can be applied (Crouch, Manager: Pome Fruit, Experico, October, 2006 - personal communication).

Although pear shelf-life would benefit most from an edible coating if pears were coated directly after harvest, rather than after cold storage (Amarante *et al.*, 2001a), it was not feasible in this study. Export grade 'Packham's Triumph' pears are in great demand at the beginning of the season and if the pears are not procured before a certain time, the bulk of the 'Packham's' pears go into CA storage (at -0.5°C) for 18 weeks. After CA storage the shelf-life of the pears were found (in Phase 2) to be shorter. However, these CA-stored pears may especially benefit from the kafirin coating, applied prior to packing, as the coating may extend the time that the pears are still marketable.

An alternative to the model given in Fig. 5.1, is to coat pears after RA storage, just prior to packing, which should optimally extend the quality of kafirin coated pears and ensure that pears are coated when still in the pre-climacteric phase. As a consequence, the coating may be able to retard the onset and progression of ripening, instead of only retarding the progression of senescence as was the case in Phase 2. Transporting of pears to a different facility prior to coating, as conducted in this study, may increase the risk of the onset of ripening, which will further reduce pear quality prior to coating. Thus, pears should ideally be coated directly at the pack house, as soon as possible after harvesting.

Regardless of whether pears are coated after harvest or after storage under RA or CA conditions, pears may inevitably be at a temperature much lower than 20°C at the time of coating. Determination of whether pears can be coated directly after refrigeration without affecting the appearance or efficiency of the coating must be evaluated to determine its suitability for pack house conditions. Coating pears when the coating solution temperature is different to that of the fruit temperature may result in whitening of the coating because of moisture condensation on the fruit surface due to the temperature difference. The hydrophobic protein may precipitate out of the coating solution on contact with the water on the fruit surface (Prof. J.R.N. Taylor, Professor, Department of Food Science, University of Pretoria, 2005 – personal communication).

Application of the kafirin coating solution to pears at 20°C also may not be suitable to industrial pack house conditions. Pears would have to be re-warmed and increasing pear temperature to 20°C prior to coating may contribute to temperature fluctuations, which may result in increased respiration and ultimately quality deterioration in the coated fruit. Coating of pears with wax (Drake *et al.*, 1991) and coating of apples (Bai *et al.*, 2003) and tomatoes (Park *et al.*, 1994) with zein protein was conducted only after fruit were equilibrated at ambient temperatures (20 to 25°C) for 24 h. Subsequent to coating the pear, wax-coated pears were dried at 60°C for 2 min (Drake *et al.*, 1991), zein-coated apples were dried at 50°C for 5 min (Bai *et al.*, 2003), while whey protein-coated apples (Cisneros-Zevallos and Krochta, 2003) and zein-coated tomatoes (Park *et al.*, 1994) were dried at 20°C using a fan.

The coating solution would have to be kept warm (approximately 18 to 20°C) and care taken that the ethanol does not evaporate from the solution because this would increase the concentration of the kafirin and the viscosity of the coating solution. This may result in a thicker fruit coating that does not allow for normal, aerobic respiration of the fruit during ripening. As a result, the fruit would remain hard, fail to de-green and off-flavours such as ethanol would be produced (Buchner *et al.*, 2004). The ethanol in the coating solution is a well known fire hazard and special precautions would have to be taken (i.e. in the design of the coating applicator) to ensure the safety of the staff.

The temperature of the pears at the time of coating and its effect on coating efficiency and pear quality, as well as a method for applying the kafirin coating to the pears, are areas that require more research. Following pear coating, the coated pears should be air-dried by a fan with an air temperature of approximately 25°C for 2 to 3 minutes. The advantage of using ethanol as a solvent is that it evaporates quickly once the pears have been coated, thus air drying without increasing the air temperature will be possible. Special fume hoods would have to be constructed to dispose of the ethanol fumes and the fumes would have to be collected (i.e. by blowing it through water) to prevent air pollution. Drying may be followed by blast refrigeration of the coated pears.

5.2.3.4 Handling and storage procedures

Coated pears may require handling and storage procedures that are different to those currently in use. The packing of kafirin coated pears in boxes with polyethylene liners or storage under CA conditions entail the reduction of the O_2 concentration to reduce fruit metabolism. As coated pears would already be exposed to reduced O_2 levels due to the gas barrier properties of the kafirin coating, CA storage or packaging in polyethylene liners may result in anaerobic respiration. As a consequence, pears destined for CA storage would possibly require coating after CA storage and packaging pears without the use polyethylene liners may be required. The kafirin coating was able to reduce the respiration rate of the coated pears while allowing for normal ripening of pears at 20°C under RA conditions, and may thus be a possible substitute for polyethylene liners for creating a modified atmosphere around the pears.

Alternatively, thinner polyethylene liners may be used. However, the O_2 permeability of the kafirin coating and of the polyethylene liner will have to be determined to ensure that anaerobic respiration will not be induced during export. According to Kader (1989), internal O_2 levels below 1% or CO_2 levels above 20%, accompanied by the accumulation of ethanol and acetaldehyde are indications that aerobic respiration has changed to anaerobic respiration. However, polyethylene liners accomplish more than just the reduction of respiration rate. Packing of pears in boxes lined with polyethylene liners is able to prevent excessive transpiration and thus shrivelling whereas the kafirin coating did not seem to have sufficient water barrier properties (Bester, 1973). Shrivelling was only observed over the entire skin surface of the coated pears after 10 days of storage at 20°C. The shrivelling on the surface of the coated pears was noticeably more than on the uncoated pears. It is possible that the soluble cuticular lipids in the cuticle were damaged by the ethanol in the coating formulation, which ultimately resulted in an increased permeability of the cuticle to moisture vapour. Thus, a polyethylene liner or bag may not be able to prevent moisture loss in coated pears sufficiently, especially when pears are unpacked and stored at 20°C.

6. CONCLUSIONS AND RECOMMENDATIONS

The first phase of this study supports the hypothesis that the rate of physiological and biochemical changes, and ultimately quality deterioration of ‘Packham’s Triumph’ pears, will increase as the storage temperature in RA at 95% RH increases. When ‘Packham’s Triumph’ pears are stored in air at 95 to 98% RH under ideal refrigerated temperature (-0.5°C), temperature-abused conditions at the export destination (10°C) and at typical ripening conditions (20°C), pears at 20°C ripen in approximately half the time that pears at 10°C do. Due to their low metabolic activity, pears stored at -0.5°C, do not ripen during RA storage and the quality of the pears changes insignificantly over time. Storage of pears at -0.5 and 10°C causes no significant moisture loss or stem-end shrivelling on the fruit, even though pears at 10°C start to ripen. In contrast, the metabolic activity of pears is accelerated during ripening at 20°C. Consequently, the pears shrivel visibly after only 4 days and lose a significant amount of moisture. Ultimately, pears at 10 and 20°C ripen to similar colour and firmness values when they are fully ripe.

It is also confirmed that ‘Packham’s Triumph’ pears stored at temperature-abused (10°C) conditions prior to ripening, are physiologically more mature and ripen faster at 20°C than pears stored at -0.5°C because storage at 10°C is not sufficient to retard the onset of pear ripening during storage. Pears stored at 10°C reach physiological ripeness, senescence and its corresponding quality attributes faster at 20°C than pears stored at -0.5°C before ripening. Pears stored at 10°C are more yellow and softer after 7 d of storage at 20°C, than pears -0.5°C prior to ripening. In contrast, pears stored at -0.5°C only reached similar levels of flesh softness and skin colour during ripening at 20°C after four weeks of storage at -0.5°C. The rate and intensity of physiological and biochemical changes in pears from -0.5°C is possibly connected to the concentration of ethylene that builds up during cold storage. Conversely, in pears stored at 10°C, the rate and intensity of physiological and biochemical changes are the result of an accelerated pear metabolism that is brought on by the higher storage temperature (10°C) prior to storage at 20°C.

A kafirin protein coating can reduce the rate of quality deterioration in coated ‘Packham’s Triumph’ pears during storage at 20°C (35 to 45% RH), by reducing the

amount of O_2 available for physiological activity, physico-chemical changes and microbiological growth. If pears start to ripen prior to coating, the kafirin coating does not delay climacteric peak, ethylene production and flesh softening. However, in comparison to the uncoated fruit that reach senescence between 3 and 7 d, senescence in the coated fruit is postponed for up to 10 days. In comparison to uncoated pears, de-greening and yellowing of coated pears are postponed by more than 3 weeks at 20°C while moisture loss is insignificantly affected by the coating. However, due to the increased maturity of pears from CA storage (prior to coating) and the dehydrating effect of ethanol, the current coating formulation does not reduce pear shrivelling as well as expected. The hypothesis that a kafirin-based fruit coating will delay senescence and extend the shelf-life of ‘Packham’s Triumph’ pears is thus confirmed.

It is recommended that the kafirin coating formulation be reformulated to improve its moisture barrier properties and reduce surface shrivelling over the entire fruit surface. Application of the coating under circumstances that may be encountered in a typical pack house should be investigated to explore the effect of such conditions on the efficiency of the coating to extend pear shelf-life. If the coating can successfully be applied to export quality pears in an industrial set-up, pear shelf-life can be extended without refrigeration and packaging material can be reduced.

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