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Postharvest quality retention and decay control of South African litchi in modified atmosphere packaging

By

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Submitted in partial fulfilment of the requirements for the degree

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DECLARATION

I, Karen de Reuck, declare that the dissertation, which I hereby submit for the degree Magister Scientiae (Plant Pathology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

....

Karen de Reuck

30 April 2010

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"A man's heart plans his way, but the Lord directs his steps."

\- Proverbs 16:10

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POSTHARVEST QUALITY RETENTION AND DECAY CONTROL OF SOUTH AFRICAN LITCHI IN MODIFIED ATMOSPHERE PACKAGING

Supervisor: **Prof. L Korsten**
Co-supervisor: **Prof. D Sivakumar**

ABSTRACT

Litchi (*Litchi chinensis* Sonn.) is a commercially valued fruit mainly for its attractively red pericarp and exotic taste. However, the market value of the fruit is affected by pericarp browning, desiccation and postharvest decay. Current control measures include sulphur dioxide (SO_2) fumigation, low temperature storage and high relative humidity (RH). Sulphur residues on fruit, moisture loss, altered taste and decay caused by *Penicillium* spp., limit the use of SO_2 fumigation. Technology that can provide a potential alternative method to retain the quality of fruit is modified atmosphere packaging (MAP). In this study (Chapter 3), the effect of active and passive modified atmospheres on quality retention of litchi cultivars 'Mauritius' and 'McLean's Red' was investigated. Results indicated that 'McLean's Red' is more suitable for MAP technology than 'Mauritius'. Lidding film–4 holes significantly reduced activity of oxidation enzymes, polyphenol oxidase (PPO) and peroxidase (POD), and retained higher pericarp colour. Lidding film–10 holes retained soluble solids concentration to titratable acidity ratio (SSC/TA) (~65), thereby preventing the loss of taste and litchi fruit flavour. In order to enhance the MAP technology further (Chapter 4), chitosan coating of fruit was also assessed. Chitosan (1.0 g L^{-1}) combined with MAP effectively prevented decay, browning and pericarp colour loss in 'McLean's Red'. Chitosan (1.0 g L^{-1}) integrated with MAP reduced PPO and POD activity, retained membrane integrity, anthocyanin content and pericarp colour. 'McLean's Red' was found to be more suitable for the chitosan (1.0 g L^{-1}) and MAP integrated treatment than 'Mauritius' in retaining overall quality. In addition, the effect of 1-methylcyclopropene (1-MCP) in combination with MAP was determined for both cultivars (Chapter 5). In this case 1-MCP (300 nL L^{-1}) was most effective in preventing browning and retaining colour in both cultivars after 14 and 21 days of cold storage. The effect of 1-MCP (300 nL L^{-1}) showed more potential on 'McLean's Red' than 'Mauritius'. At higher concentrations (500 and 1000 nL L^{-1}), 1-MCP showed negative effects on membrane integrity, pericarp browning, PPO and POD activity in both cultivars. The effect of integrated postharvest treatments i.e. modified atmosphere packaging combined with chitosan and integrated MAP and 1-MCP as well as MAP and chitosan coating on foodborne bacterial pathogens (*Escherichia coli* O157:H7 and *Staphylococcus aureus*) spike-inoculated on litchi fruit surfaces, and

Penicillium spp. decay were also investigated (Chapter 6). Results showed integrated MAP and chitosan (0.1 g L^{-1} and 1.0 g L^{-1}) treatments significantly reduced high and low inoculums load of *E. coli* O157:H7 and *S. aureus* on litchi fruit after 21 days of cold storage. Integrated MAP and 1000 nL L^{-1} 1-MCP resulted in higher disease severity. Integrated MAP and chitosan (0.1 g L^{-1} and 1.0 g L^{-1}) treatments showed very good decay control. The total microbial population of the litchi fruit surface was also determined. Integrated MAP and 1.0 g L^{-1} significantly reduced the total microbial flora after 21 days of cold storage.

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B. TECHNOLOGY TRANSFER PAPER:

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CHAPTER 1

GENERAL INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a popular non-climacteric tropical to subtropical fruit belonging to the family Sapindaceae; which also includes longan (*Dimocarpus longan* Lour.), rambutan (*Nephelium lappaceum*) and pulasan (*Nephelium mutabile*) (Huang *et al*., 2005). Litchi has originated in the subtropics of China and northern Vietnam, where over 300 cultivars have been cultivated over the past 3,500 years (Lemmer, 2002). Since the late 1700s, various cultivars have been introduced to other countries (Ghosh 2001). Furthermore, litchi fruit is valued for their attractive red skin and exotic flavour and taste, which all contribute to its popularity as an exotic in the European market (Holcroft and Mitcham, 1996). The South Africa litchi industry commercially produces two cultivars i.e. 'Mauritius' and 'McLean's Red'. From late November to early March, both South Africa and Madagascar supply litchi fruit to the European market (Ghosh, 2001).

Several limitations in litchi production and trade prevent the industry from expanding. Postharvest disorders such as skin browning, desiccation and decay affect the cosmetic appearance of the fruit, attaining lower market prices. The South African litchi industry has adopted sulphur dioxide (SO_2) fumigation to prevent pericarp browning and postharvest decay. The SO_2 inhibits oxidative enzymes responsible for skin (pericarp) browning; has antifungal properties and improves the pliability of the litchi skin. However, SO_2 fumigation leaves undesirable residues on the fruit, affecting fruit quality and health of sulphur-sensitive workers and consumers (Lonsdale and Kremer-Köhne, 1991; Koeing *et al*., 1983). Litchi fruit treated with SO_2 followed by acid dip treatments (diluted hypochloric acid (HCl)) resulted in more intensified micro-cracks on the pericarp and contributed the establishment a microbial vacuum on the fructoplane (Sivakumar and Korsten 2004; Korsten, 2006; Lichter *et al*., 2004). Thus, fruit subjected to these treatments are predominantly affected by epiphytic *Penicillium* growth, since this opportunistic organism invades the fructoplane due a microbial vacuum. The lowered pH (pH 2.5) of the pericarp provides an ideal substrate for *Penicillium* growth (Lichter *et al*., 2004; Korsten, 2006; Underhill et al., 1992). Postharvest *Penicillium* decay of litchi occurs during low temperature storage and transport, causing major losses to producers and the industry.

Increase in economic losses and growing concern over food safety and environment pollution, have driven postharvest technology research to develop alternative treatments to replace SO_2 fumigation. In recent years, a number of alternative postharvest treatments have been applied to retain overall fruit quality while controlling the decay during storage and transportation. These include gamma irradiation (Ilangantileke *et al*., 1994); fruit coatings (Duvenhage, 1993; Zhang and Quantick, 1997); heat treatments (Lichter *et al*., 2000); biological control agents (Korsten *et al*., 1993; Jiang *et al*., 2001), alternative postharvest dip treatments (Jiang and Chen, 1995; Jiang *et al*., 2005), controlled atmosphere (CA) (Jiang and Fu, 1999; Mahajan and Goswami, 2004) and modified atmosphere packaging (MAP) (Pesis *et al*., 2002; Sivakumar *et al*., 2005). Among the developed postharvest technologies, MAP has the advantage of low cost and ease of implementation at the commercial level (Flores *et al*., 2004). The MAP maintains a high relative humidity

within the packaging and reduces moisture loss and thereby prevents pericarp browning (Kader, 1994; Lemmer and Kruger, 2000; Pesis *et al*., 2002). A modified atmosphere (17% O_2 and 6% CO_2) created in bioriented polypropylene (BOPP) films enabled the retention of litchi fruit quality during storage by reducing pericarp browning (Sivakumar and Korsten, 2006). However, fruit packed in MAP should be absent of disease and pericarp browning, since pre-sorting of fruit before sale is not practical in large-scale marketing chains.

Modified atmosphere technology can be improved by combining postharvest treatments in order to retain fruit quality and effectively control decay. The application of a 'protectant' such as a biological control agent can be used to reduce postharvest decay. Sivakumar et al. (2007) investigated the effect of integrated treatment of MAP and *Bacillus subtilis* on postharvest decay control and quality retention of litchi during storage. Fruit subjected to this treatment prevented fungal decay at market-shelf conditions (14°C) as well as retaining good pericarp colour and high overall acceptability (Sivakumar *et al*., 2007). Thus, the integration of MAP and other postharvest treatments should be investigated. The application of chitosan coatings on fruit reduce fruit respiration and transpiration rates, induce defense responses in host tissue and showed antimicrobial activity against decay-causing pathogens and foodborne pathogens (El Ghaouth *et al*., 1991, 1992; Devlieghere *et al*., 2004). Chitosan coating in acidic solution minimized litchi pericarp browning (Zhang and Quantick, 1997; Joas *et al*., 2005; Sivakumar *et al*., 2005) reduced the losses of anthocyanin, flavonoids and total phenolic compounds and inhibited the increase of polyphenol oxidase (PPO) and peroxidase (POD) activity in the pericarp (Zhang and Quantick, 1997). Furthermore, Kruger *et al*. (2005) identified the potential of using 1-methylcyclopropene (1-MCP) treatment in combination with MAP on quality retention of 'Mauritius' litchi fruit. The cyclic olefin 1-MCP binds to the ethylene receptor, preventing the signaling mechanisms that activate senescence- and ripening-associated genes (Sisler and Serek, 1997). Qu *et al*. (2006) reported that the application of 1-MCP at 1 nL L^{-1} reduced the browning and disease index in 'Huaizhi' fruit stored at 28 – 33 °C and 95 – 100% RH for six days.

The aim of this study was to find a suitable alternative to replace the currently adopted SO_2 fumigation using novel postharvest applications such as MAP technology and integrated treatments with MAP that will control pericarp browning and decay while retaining the overall litchi fruit quality. In the second chapter, the literature concerning litchi production, fruit physiology and pathology, and alternative postharvest treatments were reviewed. The effect of active and passive modified atmosphere on litchi cultivars 'Mauritius' and 'McLean's Red' was investigated in Chapter 2. As a means to improve MAP, the effect of integrated application of chitosan coating and MAP on overall quality retention of litchi cultivars was investigated in Chapter 4. The effect of integrated treatment of 1-MCP and MAP on overall litchi quality was determined in Chapter 5. The effect of integrated MAP and postharvest treatment on microbial populations, decay control and foodborne pathogen survival was studied in Chapter 6.

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CHAPTER 2

A LITERATURE SURVEY ON LITCHI PHYSIOLOGY, POSTHARVEST CHAIN MANAGEMENT AND ALTERNATIVE TECHNOLOGIES

1. LITCHI PRODUCTION AND TRADE

1.1 LITCHI PRODUCTION AND EXPORT

Litchi is commercially produced in China, South Africa, Israel, Madagascar, Mauritius, Réunion, the United States (Hawaii and Florida), Australia, subtropical parts of India, Pakistan, the Philippines, Thailand, Taiwan, Indonesia, Vietnam and Brazil (Menzel *et al*., 2005). Major litchi cultivars are listed in Table 1 (Menzel *et al*., 2005), followed by a brief description of some commercial cultivars in Table 2 (Menzel *et al*., 2005). Due to low productivity in many countries, inefficient postharvest handling and high domestic demand limit exports (Huang *et al*., 2005). Currently, China is the leading litchi producing country in the world. In 2005, litchi fruit production in China was estimated at 1,440,589 tonnes, which was about 70% of the world's total quantity (Wu *et al*., 2007). However, the annual litchi crop is largely consumed within the Chinese domestic market with less than 2% available for export (Huang *et al*., 2005). China and Taiwan export approximately 12,000 – 15,000 tonnes of litchi to international markets, which mainly include Japan, Hong Kong, Singapore, Europe and North America (Huang *et al*., 2005).

Litchi is also very popular in Europe and fruit are imported from South Africa, Madagascar, Réunion, Mauritius and Australia during the winter and from Thailand, China and Israel during the summer (Huang et al., 2005). Annually, the European markets import approximately 20,000 tonnes of litchi, of which almost half goes to France and the rest is imported mainly by Germany and the United Kingdom. South Africa and Madagascar are the main suppliers for Europe from November to March (Mitra 2006). Counter-seasonal litchi export by countries such as South Africa, Madagascar and Australia to the northern-hemisphere has lead to increased competition among producers, higher grade standards and improved postharvest treatment and quality assurance (Huang et al., 2005).

1.2. LITCHI PRODUCTION IN SOUTH AFRICA

The first litchi trees were imported to South Africa from Mauritius in 1876 and introduced in KwaZulu-Natal, but were later established in the subtropical parts of Mpumalanga and Limpopo (Oosthuizen, 1993) (Fig. 1). Two litchi cultivars are commercially produced in South Africa, namely 'HLH Mauritius' ('Tai So') and 'McLean's Red' ('Bengal'). In 1999, two cultivars, 'Feizixiao' and 'Huaizhi', were added to the list recommended for commercial plantings (Froneman, 2002).

Table 1. Commercial cultivars produced in major litchi producing countries (Menzel *et al*., 2005)

Country	Major cultivars
China	Sanyuehong, Baitanying, Baila, Shuidong, Feizixiao, Haak Yip, Guiwei, Lanzhu, Tai So, Chen Zi (Brewster), Nuomici, Huaizhi
Taiwan	Haak Yip, Nuomici, Guiwei Sah Keng
India	Bedana, Calcutta, Late Bedana, Longia, China and Shahi
Thailand	Chacapat, Haak Yip, Kom, Tai So, Huaizhi
Indonesia	Local Selection
Vietnam	Thieuthauhha
Philippines	Sinco, Mauritius and ULPB Red
South Africa	Mauritius and McLean's Red
Madagascar	Mauritius
Reunion	Mauritius
Mauritius	Mauritius
Australia	Feizixiao, Souey Tung, Kwai May Pink, Salathiel, Huaizhi, Tai So
Israel	Mauritius, Floridian, Brewster
USA	Mauritius, Kaimana, Brewster, Haak Yip

Table 2. Characteristics of popular litchi cultivars

Cultivar	Feizixiao	Mauritius	Nuomici	Guiwei	Huaizhi	McLean's Red
	Fay Zee Siu, "Smile of the Imperial Concubine'	Dazao', 'Tai So', 'Big Crop', 'Hong Huay'	No Mai Chee', 'Glutinous Rice Cake'	Kwai May Red', 'Kwai Mi'	Wai Chee', 'Cherished Litchi'	Bengal'
Country	China, Taiwan, Australia	China, South Africa, Israel, Florida, Australia and most litchi-growing areas of the world	China, Taiwan	China, Taiwan	China, Taiwan, Australia	South Africa
Fruit at maturity	Skin splotchy green, yellow-red with dense sharp-pointed protuberances	Skin thin, red with small sharp-pointed protuberances	Skin thin, bright red and smooth surface	Skin thin, bright red, with swollen segments and sharp-pointed protuberances	Skin dark red, thick and tough, with large flat segments	Skin bright red, thick rough texture, with sharp-pointed to wedge-shape protuberances
Shape	Round to oval	Oblong, with uneven shoulders	Heart-shaped	Round	Round	Lopsided heart-shaped, uneven shoulders
Average Weight	25 g	24 g	27 g	23 g	20 g	23 – 27g
TSS	18%	15%	18%	19%	19%	18%
Acid	0.20%	0.30%	0.25%	0.18%	0.25%	0.28%
Seed size	Variable	Large	Small	Small to medium	Medium	Large
Flesh Recover	80%	75%	85%	80%	70%	70%

Currently, about 3357 ha of litchi trees are planted in the areas of Malelane, Nelspruit, Trichardsdal, Tzaneen, Levubu and the South Coast of KwaZulu Natal (National Agricultural Directory, 2007). In South Africa, the 'HLH Mauritius' cultivar is grown in all production areas, however, 'McLean's Red' is restricted to the high altitude and cool area of Tzaneen in the Limpopo Province (Froneman *et al*., 2002). Annually, more than 7,500 tons of fruit are produced (Lemmer, 2002), of which 'Mauritius' accounts for more than 75% of production and 84% of exports, followed by 'McLean's Red', which contribute to 16% of production and 13% of exports (Ghosh, 2001). In South Africa, harvesting and availability of litchi have a very short marketing period over Christmas and New Year (6 – 8 weeks) (Froneman *et al*., 2002). During this period, South Africa exports large quantities to main exporting destinations such as the UK (29%) and Europe (41%) (France, Germany and the Netherlands) (Table 3) (PPECB, 2008).

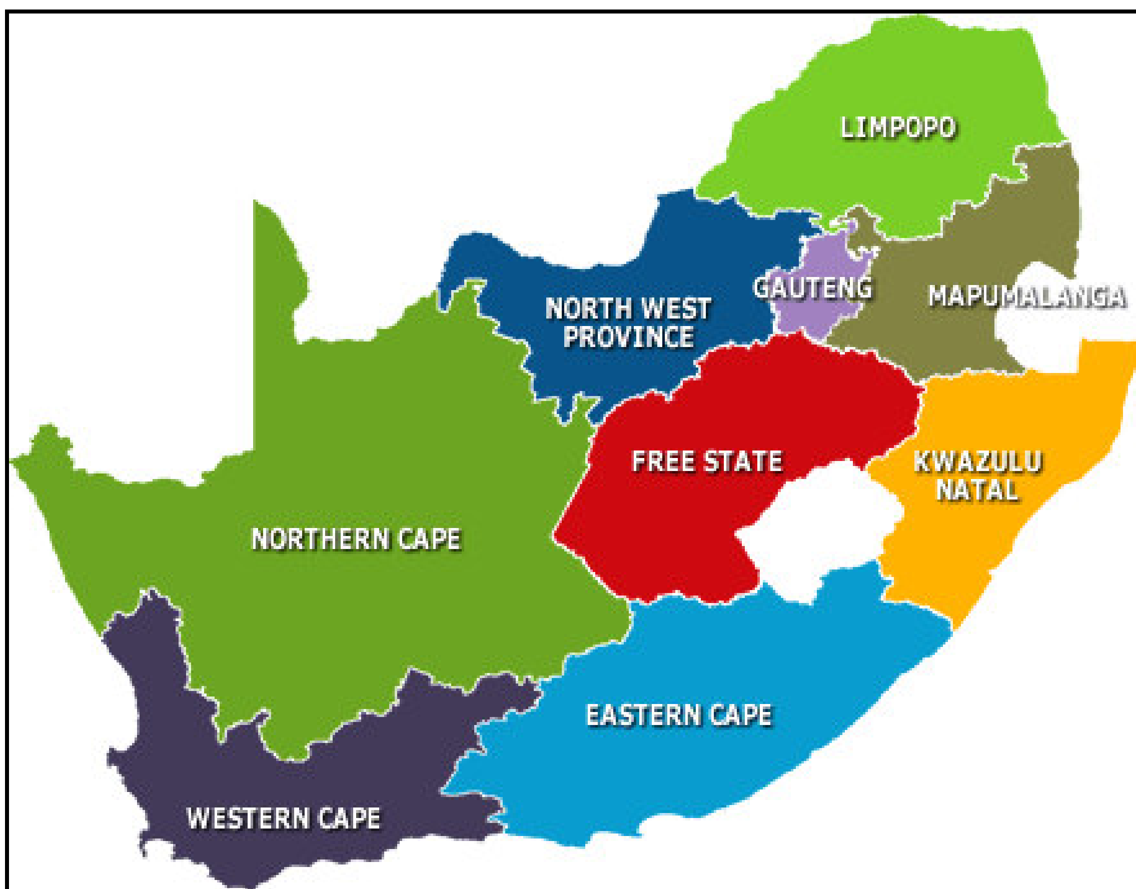

Fig. 1. Main litchi production areas in the Limpopo (lime green) and Mpumalanga (brown) provinces, South Africa.

2. LITCHI TREE MORPHOLOGY, FRUIT MORPHOLOGY AND PHYSIOLOGY

2.1. TREE MORPHOLOGY, INFLORESCENCE AND FLOWERS

Litchi is a medium to large evergreen tree, which can grow up to 10 - 12 m (Subhadrabandhu and Stern, 2005). The crown is generally round, dense, compact and symmetrical. In some cultivars, the branches are tightly curved or twisted and hang down to the ground; some cultivars are more erect than others. Trees normally have a thick, straight, short trunk and dark brown-grey bark; however, branches often have V-shaped crotches and are easily broken off by strong winds. Leaves are pinnately compound with four to seven leaflets about 7.0 cm long. The upper surface of the leaf is glossy dark green and grey-green on the lower surface. Leaflets are usually 5.0 – 15.0 cm long and 2.5 – 4.0 cm wide, and elliptical to lance-shaped. The bases of the blades are wedge-shaped or rounded (Subhadrabandhu and Stern, 2005).

The inflorescence is determinate and composed of several panicles produced on current-season shoots. Panicles are normally produced terminally in clusters of ten or more but in some trees, a high proportion of subterminals or axillaries may be produced. Panicles are generally in a mixed form with the lowest bud producing leaves only; the middle buds producing floral buds in the axils of the leaves, and the topmost buds producing only floral branches and sometimes very small leaves that do not persist (Joubert, 1986). Panicles are 10.0 – 40.0 cm long and produce hundreds of small, white, green or yellow flowers, which produce a distinctive scent when the tree is in full bloom (Stern and Gazit, 2003; Subhadrabandhu and Stern, 2005). The flowers are 3.0 – 6.0 mm wide when fully open and rest on 1.5 mm pedicles. They possess a cup-shaped calyx with four to five short, serrated sepals, but have no petals. Each flower has six to ten stamens. Flowers are usually produced from late autumn to early spring, with three types opening in succession on the same panicle. Flowers vary in sex, length and function of the stamens, as well as development and function of the pistil (Stern and Gazit, 2003).

2.2. GROWING CONDITIONS

Litchis are commercially grown from 17 - 32° latitude, which are usually found at low elevation in the subtropics and from 300 – 600 m in tropical locations; with cool or cold winters and warm to hot summers. Most of the tropical and subtropical litchi-growing areas have winter minima below 20 ºC and 10 – 15 ºC, respectively (Menzel, 2001). The rainfall is generally highest in summer and lowest in winter or spring. Litchis are grown on a range of soils including alluvial sands, rocky soils, loams, heavy clays, and soils with a high content of organic matter or lime (Menzel, 2002).

2.3. PROPOGATION

Litchis are propagated asexually by air-layers (marcots), cutting or grafts. Air-layering has a long history in China and is the most widely used method for propagating litchis. Air-layering involves the production of a plant *in situ* from aerial branches. New plants can also be raised *in vitro* by somatic embryogenesis and shoot-tip culture, although these methods have not been commercialised. Seedlings are not used for raising litchi plantations (Mitra and Ray, 2005).

2.4. FRUIT MORPHOLOGY

Litchi fruit is a drupe or stone fruit. At maturity, the fruit may be either conical, heart or spherical shaped, covered with a thin, leathery, indehiscent pericarp. The colour of the pericarp varies from green, pink to bright red, depending on the maturity stage and cultivar. The litchi aril, which is white to translucent in colour, is an extension of the funiculus or seed stalk that arises from the placenta and surrounds the seed. The seed is a single dicotyledonous seed with a smooth brown testa (Joubert, 1986). Most cultivars have medium to large seeds, with some having small or shrivelled seeds ('chicken tongue'). The pericarp surface is covered in numerous protuberances, resulting in a rough, spiky texture. Mature litchi pericarp (1.0 – 3.0 mm thick) consists of three distinct layers: (1) the outermost epicarp consists of a continuous cuticle (1.0 – 3.0 µm), a uniseriate epidermis and sub-epidermal sclerenchyma; (2) the middle mesocarp is a parenchymatous tissue, which contains chlorophyll and most of the anthocyanins; and (3) the innermost endocarp consists of small, thin-walled, unsuberized epidermal cells. Unlike other fruit, the litchi pericarp cuticle reduces during development from 8.75 µm at 21 days after anthesis to 1.88 µm in the mature fruit. The decrease in pericarp cuticle thickness has been attributed to both rapid expansion of the fruit and a reduction in cuticle synthesis (Underhill and Critchley, 1992).

2.5. FRUIT PHYSIOLOGY

2.5.1. Fruit Growth and Development

Litchi fruit have a sigmoidal growth pattern. In stage I, the seed and pericarp develop, followed by stage II that represents the development of the aril. Fruit takes about 80 – 120 days to develop from anthesis into mature fruit, weighing about 15.0 – 30.0 g, depending on the cultivar (Groff, 1921). An average ripe 'Mauritius' litchi fruit weighs approximately 22 g, of which 70 – 75% is aril, 14 – 16% pericarp and 11 – 14% seed.

The pericarp remains green and photosynthetic until fruit mature; thereafter the chlorophyll content decreases and anthocyanin synthesis is initiated (Underhill and Critchley, 1992). The colour of the pericarp is produced

by the combination of chlorophyll, carotenoids, flavones and anthocyanins (Zhang *et al*., 2000). Anthocyanins are responsible for the red pericarp colour, which are found in the outer mesocarp and exocarp (Underhill and Critchley, 1994). However, chlorophyll degradation is required in order for anthocyanin to form. High concentrations of chlorophylls in the pericarp of 'Feizixiao' (a poor-coloured cultivar), mask anthocynins in displaying the red fruit surface colour, as well as retarding its synthesis (Wang *et al*., 2002). Lee and Wicker (1991) noted seven different types of anthocyanins: cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, malvidin-3-acetylglucoside, pelargonidin-3-lycoside and quercetin 3-rutinoside. Zhang *et al*. (2000) and Sarni-Manchado *et al* (2000), using high-performance liquid chromatography (HPLC), identified the important coloured anthocyanins as cyanindin-3-rutinside, cyaniding-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside.

During fruit development and ripening, the soluble solid concentration (SSC) increases and the titratable acidity (TA) and the organic acids show a remarkable decrease (Joubert, 1986; Paull *et al*., 1984). Fruit pH increases during fruit development and ripening (Paull et al., 1984). Paull et al. (1984) found that total aril phenols decreased early in development and remained low (<1 mg 100 g^{-1}). However, Jaiswal et al. (1986) observed an increase in phenolic content in both the pericarp and aril during ripening, followed by a decrease with advancing senescence. The important phenolic compounds in litchi are tannic, caffeic, vanillic, salicylic, gentisic and fi-hydroxybenzoic acids, and 2-methyl resorcinol (Jaiswal et al., 1986). Titratable acidity and total identified acid decreases during development, while pH increases (Paull *et al*., 1984).

2.5.2. Fruit Composition

2.5.2.1. Sugars and Acids

The total sugar content increases with fruit maturity, but vary between different cultivar types (Wang *et al*., 1996). Sucrose, fructose and glucose have been identified as major sugar components in litchi in different ratios between different litchi cultivars (Paull *et al*., 1984). According to Jiang *et al*. (2006) and Cavaletto (1980) the total sugars in the aril tissue may vary from 55.9 - 61.4% on dry weight basis with 41.5 - 43.5% reducing sugars. Reducing sugars represent more than 70% of the total sugars in the aril (Paull *et al*., 1984; Chan *et al*., 1975; Jiang *et al*., 2006).

Succinic acid is the major organic acid present during fruit development. At maturity, malic acid has been identified as the dominant organic acid (80.0%) (Paull and Chen, 1987; Paull *et al*., 1984; Wang *et al*., 1996), which is accompanied by other relatively minor acid components such as citric, succinic, levulinic, glutaric, malonic and lactic acids (Cavaletto, 1980; Paull *et al*., 1984).

2.5.2.2. Ascorbic Acid and Mineral Content

Litchi is a good source of ascorbic acid (vitamin C) (Deng *et al*., 1999). The early maturing Hawaii litchi 'Kaimana' showed an average vitamin C content of 33.2 mg 100 g $^{-1}$ (Wall, 2006). The vitamin C contents in 'Mauritius' and 'McLean's Red' were 27.4 mg 100 g $^{-1}$ and 21.4 mg 100 g $^{-1}$, respectively (Sivakumar *et al*., 2008). However, ascorbic acid levels decrease in harvested fruit irrespective of storage conditions (Cavaletto, 1980; Lin *et al*., 1988; Paull and Chen, 1987). Potassium, phosphorus, magnesium and calcium accumulate during fruit development (Paull *et al*., 1984). Sivakumar *et al*. (2008) found that the mineral contents N, P, K Ca, Mg, Na and Fe, differed significantly between South African cultivars 'Mauritius' and 'McLean's Red', even though the two cultivars were grown under similar conditions in the same location.

2.5.2.3. Aroma Volatiles

Litchi aroma is often described as rose-floral and citrus-like. An unspecified litchi cultivar from Florida was reported as having a citrus flavour due to the presence of limonene, geranial and neral; its floral note was mainly due to 2-phenylethanol (Johnston et al., 1980). Tulemonde and Beauverd (1985) and Froehlich and Schreider (1986), investigated the headspace and neutral volatiles from an unspecified litchi cultivar imported from South Africa. Among the volatiles identified, limonene, rose oxide, nonanal, decanal, citronellol and geraniol contributed to the significant fruity-floral and citrus notes of litchi fruit. Twenty five volatile compounds including an ester (1), alcohols (14), aldehydes (2), acids (4), ketones (2) and terpenes (2) were reported in a litchi cultivar from Taiwan, with geraniol and geranial being the major volatile compounds (Chyau et al., 2003). Sivakumar et al. (2008) found fourteen volatile compounds, namely three alcohols, one ester, three monoterpenes and seven sesquiterpenes, in harvested 'Mauritius' and 19 volatile compounds consisting of four alcohols, three monoterpenes, two oxides and ten sesquiterpenes in 'McLean's Red'. In litchi 'Mauritius', alcohols represented 50% of the main fraction, followed by sesquiterpene (23%) and monoterpene (21%), an unknown component at 6%, and ester at 0.7% composition. No esters were detected in 'McLean's Red' (Sivakumar et al., 2008). Among the three alcohols, citronellol and geraniol predominated in the aroma profile of 'Mauritius', conferring a characteristic 'floral, rose, citrus and fruity aroma' to the litchi fruit (Chyau et al., 2003). Limonene, rose oxide, citronellol and geraniol were detected at relatively low levels in cv. McLean's Red when compared to 'Mauritius', although no rose oxide was detected in 'Mauritius'. Zingiberene was identified as the predominant compound and terpinolene as an abundant compound among the monoterpenes in 'Mauritius'. In 'McLean's Red', Germacrene D was detected as predominant compound in the sesquiterpene fraction, followed by muurolene, of which both are associated with a woody smell. Therefore, 'McLean's Red' lacks the sweet and rose-like fragrance present in 'Mauritius', which supports consumer preference for 'Mauritius'.

2.6. FRUIT RESPIRATION AND ETHYLENE PRODUCTION

Akamine and Goo (1973) classified litchi as a non-climacteric fruit based on the observations that ripening in 'Tai So' was not accompanied by increased respiration and ethylene production. Lin *et al*. (1990) showed by dipping 'Guiwei' fruit in ethephon (50.0 g L^{-1}) increased pericarp respiration; however, no increase in aril respiration or cyanide-sensitive respiration was observed, a feature of non-climacteric fruit. Jiang *et al*. (1986) observed a continuous decline in respiration rate during fruit ripening. Paull and Chen (1987) reported that the respiration rate of 'Chenzi' fruit declined from 103.0 to 39.0 mg kg^{-1} h^{-1} after 8 days of storage at 22 ºC, whereas Nagar (1994) showed that the respiration rate of 'Calcutta' fruit declined from 36.3 to 18.1 mg kg^{-1} h^{-1} after 6 days of storage at 25 ºC. Different respiration rates of litchi fruit can be attributed to cultivar differences. Litchi fruit has relatively low levels of ethylene production after harvest (<2.8 µL kg^{-1} h^{-1}) (Chen *et al*., 1986; Jiang *et al*., 1986). Fruit does not ripen after harvest and ethylene production remains constant at 1.0 – 3.0 ºC storage temperatures for 30 days (Chen *et al*., 1986).

2.7. MATURITY INDICES AND QUALITY COMPONENT

The non-climacteric nature of litchi fruit does not allow continual ripening and accumulation of sugars after harvest. During fruit development and ripening, the soluble solid concentration (SSC) increases and the titratable acidity (TA) and the organic acids show a remarkable decrease (Joubert 1986). The aril SSC/ TA ratio is traditionally used with pericarp colour as harvesting indices at commercial maturity. For most cultivated litchi, fruit physiological maturity is determined when the soluble solids range from 18.0 to 21.0% and acidity from 0.5 to 1.0% (Paull *et al*., 1984). It has been proven that SSC/TA ratio and TA are good indicators for flavour (Batten, 1989); SSC/TA ratio is a reliable indicator for most litchi cultivars. In South Africa, 'Mauritius' is ready for marketing when the SSC/TA ratio reaches 10:1 (Swarts, 1983). However, certain cultivars produce yellow or green coloured fruit, while other fruit turn red some time after physiological maturity is reached; thus the criteria vary, depending mostly on the cultivar and country as well as the climate area in which it is grown (Sauco and Menini, 1989).

2.8. CODEX ALIMENTARIUS QUALITY STANDARDS FOR EXPORT

The Food and Agriculture Organisation of the United Nations has developed CODEX quality standards for fresh litchi fruit. According to the CODEX Alimentarius standards mature litchi fruit must have a predominant red pericarp with only a small area of green; the diameter of the fruit must be larger than 20.0 mm for standard or second class (classes I and II) fruit; and 33.0 mm for "Extra" (superior) class fruit (Codex Standard, 1996). The soluble solid concentration (SSC) should be ~18.0% and the residue for sulphur in the flesh should not exceed 10.0 µg g^{-1}.

2.9. QUALITY ANALYSIS

Fruit quality evaluations are based on the assessment of fruit size and weight, colour, firmness, the SSC/TA ratio as well as sensory evaluations. Uniform shape and size are important quality characteristics. Misshapen fruit may be susceptible to mechanical injury. The colour of fruit can be subjectively determined by using the human eye to evaluate colour; however, an instrument can provide a specific colour value based on the amount of light reflected off the fruit surface, which ensures lesser variability. A colorimeter expressing the CIELAB, Commission international de I'Eclairage (CIE) colour space L*, a* and b*, are used to determine the pericarp colour of litchi. L* represent lightness of the colour (for black, L* = 0, and for white, L* = 100). The a* axis varies from green (-a*) to red (+a*) and the b* from blue (-b*) to yellow (+b*). Fruit firmness is measured to determine the textural properties of the fruit. Subjective measurement of firmness with the fingers can be useful for a quick measure of gross differences in firmness. However, a firmness tester is more accurate, such hand-held firmness tester.

Sugars are the major soluble solid and therefore soluble solids can be used as an estimate of sugar content. A refractometer is used to determine the soluble solids content (SSC) in fruit juice. The refractometer measures the refractive index, which indicates how much a light beam will be retarded when it passes through the fruit juice. The titratable acidity (TA) can be measured by titrating a known volume of fruit with 0.1 N NaOH (sodium hydroxide) to an end point of pH 8.2 as indicated by a phenolphthalein indicator or by using a pH meter. The TA is expressed as percent malic, citric or tartaric acid. Litchi is calculated as a percentage of citric acid (Mitcham *et al*., 1996). The organoleptic assessment of litchi fruit is determined on a hedonic rating scale given by trained taste panellists. Sensory attributes in terms of the pericarp colour, overall appearance, fruit firmness, juiciness, taste and flavour are scored.

3. LITCHI POSTHARVEST CHAIN MANAGEMENT

3.1. PICKING, IN-FIELD SORTING AND TRANSPORT

Litchi fruit are harvested by breaking or cutting panicles, or cutting or twisting individual fruit from the panicles, and placing them into plastic crates. During the early season, picking must be done selectively to ensure only mature fruit are marketed. Picking must be repeated at regular intervals during the harvest season (Holcroft et al., 2005). Harvesting should be done with care, since mechanical damage will lead to increased fruit desiccation and decay. Fruit should be harvested early in the morning to minimize moisture loss and fruit heating. Shading harvested fruit, rapid transfer of fruit from the orchard to the packinghouse,

and minimal delays between harvesting and cooling, are all important factors in determining postharvest quality (Holcroft *et al*., 2005; Lemmer and Kruger, 2002). Sorting and grading of fruit after harvest should take place in shaded and well-ventilated areas or in temperature-controlled packinghouses. Panicles are split into sub-panicles and leaves and twigs are removed. Fruit that are small, immature, overripe, misshapen, diseased, damaged by insects or split are discarded.

3.2. PRE-COOLING

Management of fruit temperature begins with pre-cooling (7 to 10 °C), an initial cooling stage to remove field heat prior to sulphur dioxide fumigation. Pre-cooling can be carried out as fruit arrive from the field, or after other packinghouse operations such as grading and sorting. Room cooling, forced-air cooling, hydro cooling and room cooling, forced-air cooling, hydro cooling and vacuum cooling are pre-cooling options that can be used for litchi fruit. Hydro cooling utilises cold water dips, sprays or ice slurries to rapidly chill the fruit; resulting in fast field heat removal with minimal loss of moisture. However, free water remaining on the fruit surface can increase postharvest pathogen growth during storage. Forced-air cooling drives air flow in a single direction through a stack of fruit and has substantial benefits compared with simply placing fruit in a cool room. Higher cooling speed is achieved by forcing air between and around fruit. Due to the speed of cooling, forced-air cooling can use low RH air in some cases, without causing significant water loss (Joyce and Patterson, 1994). Forced-air cooling may be a viable alternative for litchi, providing the benefits of rapid cooling, without causing fruit wetness.

3.3. PACKHOUSE TREATMENT

3.3.1. Sulphur Dioxide Fumigation

The SO_2 fumigation is commercially adopted to overcome postharvest browning and infection by postharvest pathogens (Swarts 1983). The SO_2 treatment entails fumigating fruit with SO_2 gas, which is performed by injecting compressed gas directly into a sealed chamber containing the fruit to be treated. Alternatively, sulphur powder can be burned to form a gas: 100 g of 90% sulphur powder per m^3 of fruit at room temperature (25 - 28 °C) for 20 min with no humidity control (Holcroft and Mitcham, 1996; Lemmer and Kruger, 2002).

The SO_2 is an anti-oxidant, which inhibits oxidizing enzymes responsible for pericarp browning; controls saprophytic surface fungal growth and retains the pliability of the skin (Lemmer and Kruger, 2002). Sulphur rapidly bleaches the pericarp surface due to the formation of a colourless anthocyanin-SO_3H complex. The pink or

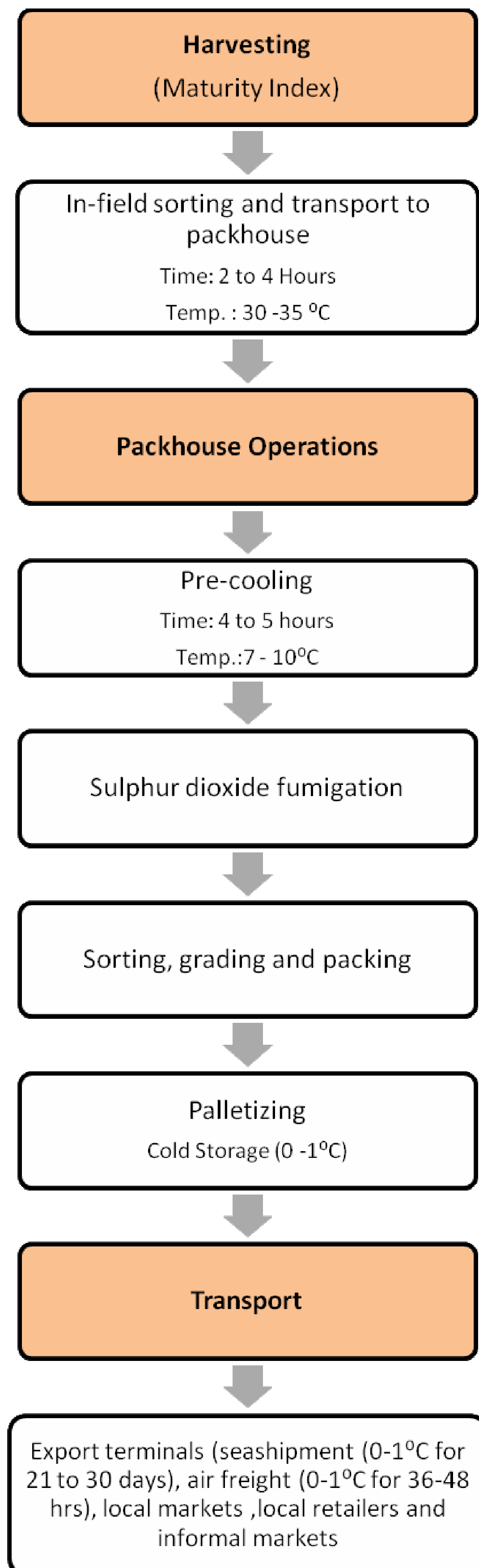

Fig. 2. Litchi postharvest chain operations.

red colour is recovered after 3 – 5 days when kept at 22 °C (Zaubermann *et al* 1990) (Figure 4). The use of excessive sulphur causes the pericarp colour to turn yellow or pale green, which subsequently fails to return to the original pinkish red colour. SO_2 injury may also occur if fruit are fumigated at too low dosage. Symptoms of SO_2 injury are irregular brown lines or patches on the interior of the pericarp, observed at the stem-end especially in over-matured fruit, or thick-skinned or dark-coloured cultivars.

3.3.2. Undesirable effects of SO_2 fumigation

One of the main concerns with SO_2 fumigation is that it has undesirable effects on fruit quality. Fruit taste is altered due to a higher TA and lower pH resulting from direct penetration of SO_2 through the skin into the aril (Lonsdale and Kremer-Kohne, 1991). Evaluation of SO_2 fumigated fruit of different cultivars indicated a 12.0 – 14.0% mass loss during low temperature storage at 1 °C (Lemmer *et al*., 2000; Sivakumar and Korsten, 2006). It is also evident that commercial SO_2 fumigation intensifies micro-cracking of the pericarp (Sivakumar *et al*., 2005). SO_2 fumigation also results in health hazards for packinghouse workers and consumers, causing allergic reactions and respiratory problems (Koeing *et al*., 1983).

Accumulation of SO_2 residues in the pericarp and aril depend on different factors, such as damage to the pericarp, RH and storage temperature. According to Lemmer *et al*. (2000), SO_2 residue levels in the pericarp and aril of six cultivars (‘Wai Chee’, ‘Fay Zee Siu’, ‘Kwai May Pink’, ‘Haak Yip’, ‘HLH Mauritius’ and ‘McLean’s Red’) were observed to be 1000 - 1400 ppm in the pericarp and 10 - 14 ppm in the aril, which soon after SO_2 fumigation declined to 200 - 250 ppm and 8 - 12 ppm, respectively, when kept at low temperature storage (1 °C). The detected SO_2 residues varied between cultivars: higher values were recorded in ‘McLean’s Red’ than ‘Mauritius’. Furthermore, higher SO_2 residues were reported in the arils of ‘McLean’s Red’ than ‘Mauritius’ when subjected to acid dip treatments following SO_2 fumigation. Lemmer and Kruger (2000) also explained that peel injury caused by low pH treatment, could facilitate an increased diffusion rate into the aril, leaving less residues in the peel and consequently higher residues in the aril. Lemmer and Kruger (2000) found that more mature fruit (higher SSC: TA ratio), were likely to have higher SO_2 residue ratios between the aril and pericarp due to the initiation of pericarp degradation as a result of the senescence process. These factors lead to a higher SO_2 diffusion rate through the pericarp, resulting in higher residues in the aril. Storage temperature and RH also influenced the movement and absorption of SO_2 in the fruit; higher storage temperatures with low RH favoured the build-up of SO_2 residues in the aril (Lemmer and Kruger, 2000). The time lapse between harvesting and fumigation also influenced the SO_2 residue build-up in the aril (Lemmer and Kruger, 2000). All these undesirable effects of SO_2 fumigation have necessitated the development of alternative postharvest treatments to maintain overall quality during storage and transportation.

3.3.3. Acid dip treatment

In Israel and South Africa, sulphur treated fruit is also subjected to dipping in diluted hypochloric acid (HCl pH 0.9 to 1.0) to restore the red pericarp colour (Zaubermann *et al*.; 1990, 1991; Lemmer and Kruger, 2002). The SO_2 bleaches the litchi skin and by treating fruit with acid restores the skin colour by converting the anthocyanin to the red flavylium ion, which predominates at low pH. Furthermore, SO_2 fumigation increases the permeability of the plasma membrane, which allows the acid to reach the vacuoles, where the anthocyanins are situated (Zauberman *et al*., 1990, 1991). The pericarp colour fades with extended storage probably due to the increase in the pH of the cell plasma (Zauberman *et al*., 1990, 1991).

3.4. SORTING, GRADING AND PACKING

Fruit should be graded to suit their intended markets (Holcroft *et al*., 2005) and packed into plastic crates, fibreboard cartons or polystyrene boxes lined with polyethylene for export or long-distance transportation. Export fruit are packed in two-kilogram cartons. Cartons must be well ventilated to ensure the gradual dissipation of SO_2 from the pericarp and the pulp. Wooden pallets should be dry, free of bark and mould growth, as this may pose a phytosanitary risk to importing countries (Lemmer and Kruger, 2002).

3.5. TEMPERATURE MANAGEMENT

Pre-cooling followed by low temperature storage of fruit reduces water loss and disease development. Storing of fruit at low temperatures also decrease PPO activity, reduce changes in TSS, TA and ascorbic acid concentrations, as well as delay pericarp browning and aril breakdown (Paull and Chen, 1997). Minimum temperature recommendations vary from 0.0 – 7.0 °C, depending on the length of storage. Swarts and Anderson (1980) recommended 0.0 – 1.0°C for up to 30 days of storage for export litchi from South Africa to destination sites. In addition, by maintaining RH of 90-95%, moisture loss can be minimised. Maintenance of the cold chain from packinghouse, road transport and shipping is essential to ensure product quality.

3.6. CONSTRAINS IN THE LITCHI EXPORT CHAIN

Pericarp browning is the main postharvest problem associated with exported litchi fruit. The bright red coloured litchi pericarp rapidly turns brown within 1 - 2 days at ambient temperature (Jiang and Fu, 1998; Zhang and Quantick, 1997). Moisture loss, desiccation, mechanical injury, chilling injury, heat stress, pathogen or pest attack and senescence can result in the browning of the pericarp (Jiang and Fu, 1999; Scott *et al*., 1982; Holcroft and Mitcham, 1996). Browning caused by temperature stress, decay and senescence (Bagshaw *et al*., 1995), is evident as typical dark and water-soaked areas on the pericarp, whereas browning due to desiccation is differentiated by a pale-dry appearance. Browning initiates from the protuberances of

the pericarp and thereafter, extends over the entire pericarp surface, until the pericarp eventually becomes dry and brittle (Underhill and Critchley, 1995). Although pericarp browning does not affect the eating quality of the aril, the cosmetic appearance of the fruit is affected, selling at lower market value.

3.6.1. Moisture Loss

Fruit moisture loss occurs due to a water potential gradient that draws water vapour into the atmosphere. Moisture loss is encouraged by poor skin resistance to water vapour movement, air currents, warm temperature, low RH and temperature gradients between air and fruit. Dehydration of fresh produce causes weight loss, decline in appearance and textural quality. Litchi moisture loss has been shown to result in increased conductivity of pericarp tissues and loss of membrane integrity (Chen and Hong, 1992). Membrane conductivity ranged from 50 - 100 µS in hydrated fruit, and increased to 100 - 150 µS at 10% loss of pericarp weight, and 150 - 250 µS at 20% loss of skin weight (Bryant, 2004). Browning of the pericarp correlates with moisture loss, with both Hunter a* values (redness) and visual colour (Underhill and Critchley 1994). It has been suggested that browning can become apparent when as little as 2% of the pericarp moisture is lost after harvest (Underhill and Critchley 1994).

3.6.2. Desiccation

Desiccation is one of the main factors causing browning of the fruit (Scott *et al*., 1982). During storage, as desiccation occur, the pH of the pericarp homogenate increase and the permeability of cell membranes changed to influence the micro-structure of pericarp cells (Underhill and Critchley, 1994). Desiccation leads to the breakdown of vacuoles, leakage of anthocyanin and destroy compartmentation of browning-related enzymes and their substrates (Chen and Hong, 1992; Underhill and Critchley 1994).

3.6.3. Mechanical Damage

Mechanical damage lowers the cosmetic appearance of the fruit and accelerates moisture loss and browning, increase fruit respiration and risk of pathogen invasion. Bryant (2004) observed that injury inflicted by impact caused darkening of the protuberance tip, cracking of the pericarp and skin colour deterioration. Compression also caused tip darkening, and severe loads were capable of puncture, shape distortion and skin cracking of the fruit. Abrasion and vibration injuries were characterised by strong yellowing of the pericarp colour (Bryant, 2004). The effects of mechanical injury are often latent.

3.6.4. Micro Cracking and Fruit Cracking

Litchi micro cracking was reported by Underhill and Simons (1993) and suggested that it is caused due to desiccation. Micro cracking is also one of the causes of pericarp browning (Huang *et al*., 2004). According to Underhill and Simons (1993), the micro-cracks observed prior to harvest intensified during storage. Micro cracking of the pericarp occurs at the initial stage of fruit development due to the rapid expansion of the aril

(Huang and Xhu, 1983). According to Joubert (1986), the expanding aril exerts an increased stress or turgor pressure against the pre-grown pericarp. Drought is another major cause of pericarp cracking during fruit development, which leads to loss of pericarp extensibility (Li *et al*., 2001). In addition, due to handling or packing line operations micro cracking can be induced (Sivakumar and Korsten, 2004). Spongy tissue, mainly responsible for gas exchange in the pericarp, was thought to be responsible for water loss (Deng, 1997). However, the findings of Huang *et al*. (2004) showed that 'Huaizhi', which had a thicker, spongy layer, showed less desiccation. Huang *et al*. (2004) further showed that the cuticle accumulation pattern might help to explain the susceptibility or resistance to micro cracking in different cultivars.

Fruit cracking also affects the cosmetic appearance of the fruit at the domestic or export market. Fruit cracking is the result of rapid aril expansion during fruit development; the aril exerts pressure on the pericarp, which has ceased to forming new tissue. The degree of severity or damage, depending on the cultivar, is known to intensify with desiccation. Fluctuation of wet and dry periods at late fruit developmental stages can also aggravate fruit cracking. Qiu *et al*. (1999) reported a relationship between fruit cracking and endogenous hormones or mineral nutrition (Ca, Mg and B) in cultivar 'Nuomoci'. Calcium contribution to cracking resistance by its structural role in cell walls; thus, calcium availability is important during early fruit development (Huang *et al*., 2005).

3.6.5. Chilling Injury

Tongdee *et al*. (1982) reported chilling damage to benomyl-treated fruit packed in punnets and wrapped in PVC film, after 30 days at 0 and 5 °C. Fruit treated in the same manner but stored at 20 °C remained in a good condition for 11 days while untreated fruit turned brown after 1-2 days. However, there is some uncertainty regarding the susceptibility of litchi to chilling injury since it is difficult to separate the symptoms of chilling from those of desiccation. Reported differences in susceptibility to chilling injury can be complicated by time and temperature interactions and genetic differences between cultivars. Symptoms of chilling injury are reported as uniform browning of the pericarp at 0 °C and irregular brown patches at 5 °C, followed by rapid breakdown of the fruit when returned to room temperature (Tongdee *et al*., 1982).

3.6.6. Biochemistry of Pericarp Browning

During pericarp browning, anthocyanins are prone to non-enzymatic and enzymatic degradation. This observation was supported by Zhang *et al*. (2000) who noted a decline in cyanidin-3-glucoside (major anthocyanin, representing 91.9% of the total anthocyanin) with increasing severity of browning during storage. Simpson *et al*. (1976), proposed two possible mechanisms for the non-enzymatic degradation of anthocyanin: 1) the hydrolysis of the 3-glycosidic linkages to produce the more labile aglucone, and 2) hydrolytic opening of the pyrylium ring to form a substituted chalcone. Enzymatic degradation of anthocyanin by polyphenol oxidase (PPO) and peroxidase (POD) enzymes cause the formation of polymeric

browning pigments, or *o*-quinones (Huang *et al*., 1990; Lee and Wicker, 1990; Zauberman *et al*., 1991). Anthocyanins are predominantly found in the epicarp and mesocarp of the pericarp, and high PPO activity observed in these two layers, led to the conclusion that the involvement of PPO activity in desiccation mediated browning (Underhill and Critchley, 1995).

Desiccation or moisture loss from the pericarp tends to increase the pericarp pH 4.15 - 4.52 over 48 h at 25 °C and 60% RH (Underhill and Critchley, 1994), converting anthocyanin to carbinol (colourless form). Jiang and Fu (1999), reported that the moisture loss and increased pH in the pericarp tissue were directly related to the PPO activity: the PPO activity was observed to increase at higher pH (7-7.4) and decrease at lower pH, whereas no activity was observed below pH 4.2 (Jiang *et al*., 1997). Underhill and Critchley (1994) concluded that the increase in pH from 4.15 to 4.52 during desiccation could probably stimulate PPO activity. Water loss or dehydration causes rapid loss of membrane integrity, bringing the PPO in close contact with the substrate (-) epicatechin to initiate the browning reaction (Jiang and Fu, 1999; Sun *et al*., 2006). However, PPO has a low affinity for anthocyanins, which is probably due to the presence of the sugar moiety causing steric hindrance (Kader *et al*., 1998; Sarni *et al*., 1995). Jiang (2000) reported that litchi anthocyanins degraded more rapidly in the presence of phenol extract and PPO, thereby suggesting the involvement of an anthocyanin-PPO-phenol system.

Peroxidase is an oxidative enzyme in the litchi pericarp, and Lin *et al*. (1988) and Underhill and Critchley (1995) have observed increased POD activity during pericarp browning. Gong and Tian (2002) found that partially purified POD rapidly oxidized 4-methylcatechol in the presence of H_2O_2, forming polymeric pigments. Zhang *et al*. (2005) concluded that although POD could not directly catalyse anthocyanin degradation in presence of H_2O_2, anthocyanin could be rapidly degraded by POD when both H_2O_2 and simple phenols, such as guaiacol, were present.

Anthocyanase (anthocyanin-β-glucosidase) can also play a role in removing the sugar groups, leading to anthocyanin decolourisation (Huang, 1955). Zhang *et al*. (2001) found high anthocyanase activity in the litchi pericarp (Zhang *et al*., 2001), and Jiang *et al*. (2004) suggested that the anthocyanase catalyses the hydrolysis of sugar moieties from anthocyanins to anthocyanidins, rendering it more accessible to PPO and POD. The findings by Jiang *et al*. (2004) suggested that the anthocyanase-anthocyanin-PPO reactions take place in the pericarp cells. Litchi enzymatic browning by POD could also involve anthocyanase-anthocyanin-phenolic-H_2O_2 reaction (Zhang *et al*., 2005).

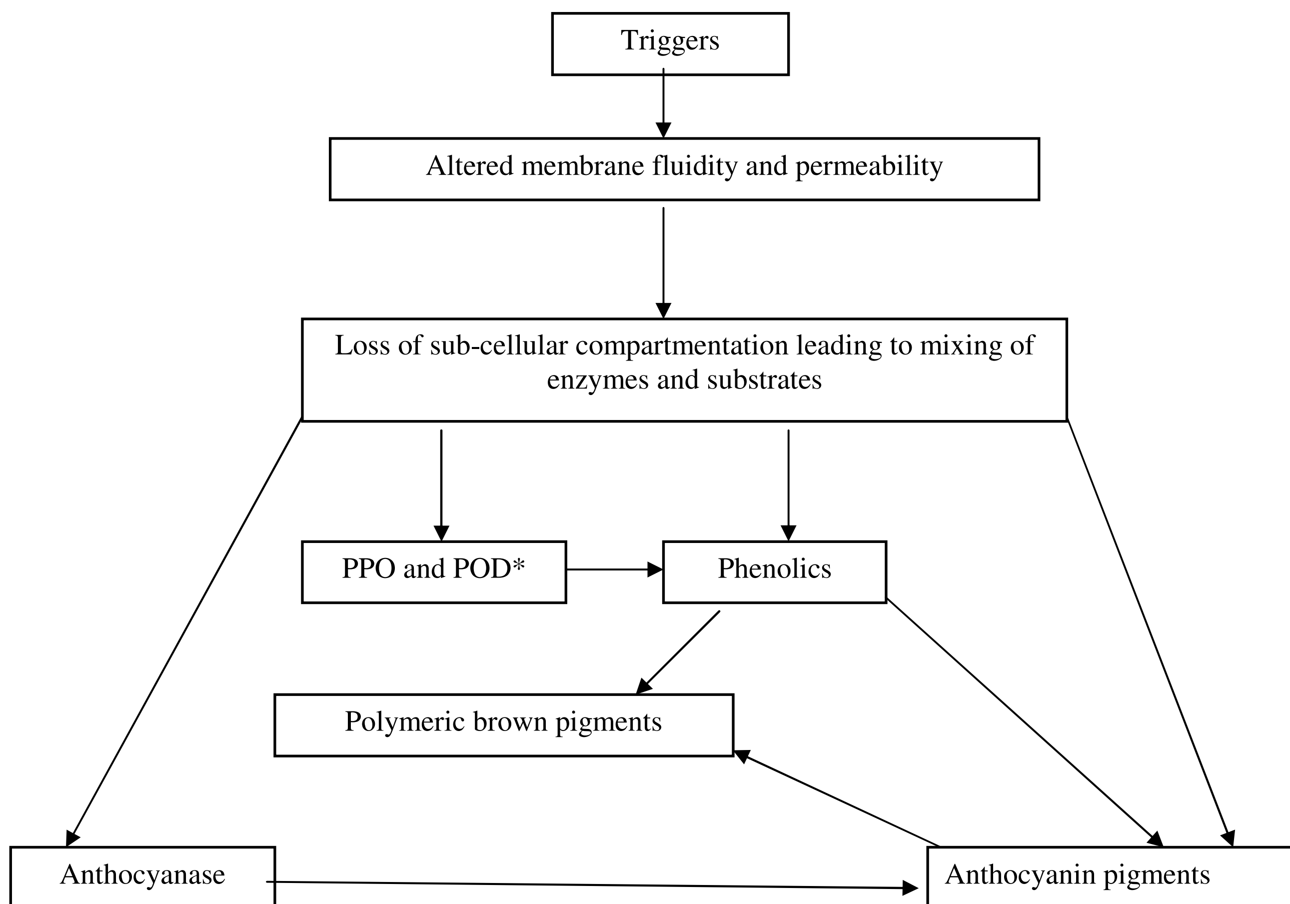

Fig. 3. Schematic diagram of enzymatic browning (based on Jiang *et al*., 2004). Mechanical injury, chilling injury, decay, dehydration or senescence can trigger browning during extended storage of litchi fruit. The membrane integrity is altered and the loss of sub-cellular compartmentation subsequently promotes contact between the phenolic substrate and enzyme. *PPO: polyphenol oxidase, POD: peroxidase.

3.6.7. Postharvest Decay

Postharvest decay is one of the major obstacles in the postharvest fruit chain. Predominant fungal genera associated with litchi in South Africa are *Penicillium, Phomopsis, Pestalotiopsis, Trichoderma, Alternaria, Botryosphaeria* and *Fusarium* spp. (De Jager *et al*., 2003). Most decaying organisms are controlled by SO_2 fumigation and acid treatment; however, treated fruits have an altered microflora on the fructoplane as well as a lowered pericarp pH, causing fruit to be highly sensitive to the invasion of *Penicillium* species (De Jager *et al*., 2003; Korsten, 2006; Lichter *et al*., 2004).

The rough texture of the litchi pericarp provides an ideal surface for conidial attachment and fungal growth (Undehill and Simons, 1993). Litchi aril has a low pH, high sugar content and nutrients, which are ideal to promote fungal growth, in particular that of *Penicillium* spp. (Lichter *et al*., 2004). Epiphytic growth of the fungus on the fruit surface affects the cosmetic appearance; however, the internal integrity of the fruit remains unchanged. Initial decay appears as white foci that develop into typical *Penicillium* decaying symptoms: a blue-green powdery mould growth (Coates *et al*., 2005), (Fuchs *et al*., 1993). Alternatively, pathogenic infection of fruit by *Penicillium* species is characterised by the physical invasion of the tissue, causing severe pericarp browning and aril softening. Micro cracks and wounds, introduced during handling, are ideal entry ports for *Penicillium* invasion.

Penicillium spores are very resistant and light-weight, and are easily disturbed into the air. Environments with a high RH, such as packinghouses, provide suitable conditions for the growth and spread of *Penicillium* conidia. Cross-contamination may occur between stored fruit, as well as between surrounding environments (Coates *et al*., 1995). Unhygienic packinghouses and storage harbours are good sources for the introduction of *Penicillium* spores to the litchi consignment. Long sea shipment periods or prolonged cold storage by importers provide a sufficient incubation period for *Penicillium* decay (Lemmer and Kruger, 2002).

3.7. FOOD SAFETY IN THE LITCHI SUPPLY CHAIN

In the past two decades the number of outbreaks foodborne illnesses due to the consumption of fresh fruit and vegetables has been observed. Influencing factors that may attribute to the increase in outbreaks have been identified as: (i) the increasing trend to centralise production and distribute produce over greater distances, (ii) importing fresh produce from abroad, (iii) increased world-wide consumption of fresh fruit and vegetables, (iv) increased exposure of people to new pathogens through global trade and international travel, (v) inappropriate storage and handling practices in food preparation areas and (vi) improved scientific identification methods to track the source of pathogens (Beuchat, 1996). In some study cases the sources of the outbreaks have been be traced back to the point where produce have been contaminated in the pre- or postharvest environment (Beuchat 1996, 2002). Several foodborne pathogen outbreaks have been associated with fresh- and processed fruit. *Salmonella* spp., *Shigella flexneri, Vibrio* cholera, viruses and protozoan parasites have been isolated from watermelon, cantaloupe, fruit salad, coconut milk, fresh and frozen strawberries and raspberries (Gaylor *et al*., 1995; O'Brien, 1998; Centers for Disease Control and Prevention (CDC) 1991, 1997; Niu et al., 1992; Ponka et al., 1999; Herwalt and Ackers, 1997). Up to date, no disease outbreaks have been linked to the consumption of fresh litchi fruit, proper agricultural and handling practices need to be adhered to during the supply chain stages of fresh fruit (Beuchat, 2002). The introduction of contaminating organisms is mostly accidental due to the use of untreated manure, contaminated irrigation

water during cultivation and/or unhygienic handling or conditions during packaging or transport. The uneven litchi pericarp and micro-cracks, exposing the fruit flesh, may provide ideal surfaces for the attachment of foodborne pathogens.

4. ALTERNATIVE POSTHARVEST TECHNOLOGIES

Increase in economical losses and a growing concern over food safety awareness and environment pollution have become the main driving forces for developing alternative postharvest treatments that are environmentally friendly, safe and economically acceptable. Alternative postharvest treatments that have been studied in recent years include: gamma irradiation (Ilangantileke *et al*., 1994); fruit coatings (Duvenhage, 1993; Zhang and Quantick, 1997); heat treatments (Lichter *et al*., 2000); biological control agents (Korsten *et al*., 1993), alternative postharvest dip treatments (Sivakumar *et al*., 2005) and controlled atmosphere and modified atmosphere packaging (Jiang and Fu, 1999; Sivakumar and Korsten, 2006) (Table 4). However, despite the various advances in postharvest technology research, none has been successfully adopted by the industry due to insufficient control of postharvest disorders, high implementation costs and impracticality of treating large volumes of fruit. However, despite the various advances in postharvest technology research, none has been commercially adopted. Therefore, a suitable alternative postharvest treatment that ensures overall fruit quality retention with shelf-life extension, still needs further investigation. An integrated approach involving all role players in the production chain will also be needed to assure viability, adoptability and sustainability of alternative technology in the commercial sector.

4.1. MODIFIED ATMOSPHERE PACKAGING TECHNOLOGY

Among the postharvest technologies available for overall fruit quality retention, MAP technology has the advantage of low cost and being easy to implementation at the commercial level (Flores *et al*., 2004). The successful use of MAP is based on the specific permeation properties of polymer films to O_2 and CO_2 to generate atmospheres that are suitable for the postharvest life of many horticultural commodities (Pesis *et al*., 2002). The MAP design is determined based on the product mass, product respiratory activity, the recommended gas atmosphere, the packaging material's permeability to gases and its dependence on temperature, the respiration rate of the commodity as affected by the gas composition and temperature (Talasila *et al*., 1995; Artes and Martinez, 1998, Beaudry, 2007). The MAP maintains a high humidity environment, which is essential for preventing water loss and browning of the litchi pericarp (Kader, 1994; Lemmer and Kruger, 2000; Pesis *et al*., 2002). A modified atmosphere (17% O_2 and 6% CO_2) created in bioriented polypropylene (BOPP) films retained litchi fruit quality during cold storage by reducing pericarp

browning (Sivakumar and Korsten, 2006). The successful implementation of MAP technology depends on cultivar type, fruit maturity, in terms of early and late harvested fruit, absence of decay as well as storage temperature management (Pesis *et al*., 2002; Lichter *et al*., 2000).

Lichter *et al*. (2000) investigated the effect of hot water brushing treatment on litchi 'Mauritius', followed by a 2 min HCl dip treatment (4%), amended with prochloraz and packed in laminated polyethylene bags (BoxiBag®, Atifon, Israel) with either of two types of perforations (micro and macro). The results showed higher CO_2, acetaldehyde and ethanol production in late harvested fruit than the early-seasonal fruit in micro-perforated packaging at the end of storage (1.5 °C for 4 weeks and 20 °C for 3 days) (Lichter *et al*., 2000). This indicate that fruit maturity, in terms of late or early harvested fruit, plays a crucial role in the successful usage of MAP technology. Anaerobic respiration due to micro-perforation indicated the importance of selecting suitable films with specific permeance to create a desirable atmosphere around the fruit. Tian *et al*., (2005) reported ethanol production by fruit in MAP (15 - 19% O_2 + 2 - 4% CO_2) during low temperature storage in cv. 'Heiye'.

Litchi fruit 'McLean's Red' dipped in hot water at 55 °C for 2 min, packed in Xtend® or BOPP (bioriented polypropylene), showed an increase in CO_2 composition around the fruit with a decrease in weight loss and fruit firmness, lower chroma, higher postharvest decay and browning during low temperature storage (Sivakumar and Korsten, 2006a). Different types of packaging (Xtend® or BOPP) or the same type of packaging (BOPP) with different perforations showed different gas compositions and RH around the litchi fruit, which affected their overall quality during long-term storage (Sivakumar and Korsten 2006a; 2006b). The BOPP with 17% O_2, 6% CO_2 and ~90% RH around the early seasonal litchi fruit 'Mauritius' reduced the rate of respiration, thereby preventing the browning related enzymatic mechanism, colour deterioration and weight loss, while retaining the overall fruit quality up to 34 days at 2 °C and 3 days at 14 °C (Sivakumar and Korsten, 2006b). Furthermore, the sensory panellists did not detect ethanol or acetaldehyde related off-flavours in fruits. MAP has the potential to be implemented as a partial alternative to SO_2 due to good quality retention by maintaining high humidity, essential for prevention of moisture loss and browning of the litchi pericarp (Kader, 1994). However, maintaining the cold chain is vital in the success of MAP technology. A negative impact on fruit quality can be encountered when the fruit in MAP is subjected to temperature fluctuations during shipping, handling or at the retail display. In MAP packaging, it is essential that the fruits are almost 100% disease free, since the pre-sorting of fruits before sale is not practicable in the large scale marketing chain. The presence of postharvest pathogens can act as inoculum and contaminate surrounding fruits in the packaging.

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Table 3. Development of postharvest treatments as an alternative to sulphur dioxide fumigation

Postharvest Treatment	Control Parameters		Drawbacks	Reference
	Physiological	Microbial		
Sulphur dioxide fumigation (*Commercial treatment*)	Pericarp browning control; Improve pliability of skin	Reduce decay	Altered taste, fruit weight loss; health risk; *Penicillium* decay	Lonsdale and Kremer-Kohne 1991
HCl treatment	Pericarp colour retention	Reduce decay	*Penicillium* decay	
Gamma Irradiation	Pericarp colour retention		Ineffective for long-term storage (<16 days)	Ilangantileke *et al*., 1993
<u>Heat Treatment</u>				
Steam, low pH	Pericarp colour retention	-	Aril discolouration	Kaser *et al*., 1995
Vapour	Uniform colour retention good eating qualities	Reduce decay	Increased pericarp browning	Jacobi *et al*., 1993
Hot water brushing + (HCl and Prochloraz	-	Reduce decay	Prochloraz unsuitable for commercial application	Lichter *et al*., 2000
Hot water dips / sprays	Pericarp colour retention	-	Fungal rot	Olesen *et al*., 2004
<u>Dip Treatment</u>				
Polyamines (putrescine, spermine or spermidine)	Pericarp colour retention	Reduce decay	-	Jiang and Chen, 1995
Chitosan coating	Reduce pericarp browning,	Reduce decay	Storage up to 21 days only	Jiang *et al*., 2005
<u>Biocontrol Agent</u>				
Bacillus subtilis	Moderate pericarp browning	Reduce decay	Fruit integrity affected	Korsten *et al*., 1993; Jiang *et al*., 2001
Modified Atmosphere Packaging (MAP)	Reduce pericarp browning; weight loss	-	Not suitable for late seasonal fruit	Sivukamar *et al*., 2005
Controlled Atmosphere Storage and 1-MCP	Reduce pericarp browning up to 21 days	Reduced decay incidence	-	Sivakumar and Korsten, 2010

Table 3. Development of postharvest treatments as an alternative to sulphur dioxide fumigation (Continue)

Postharvest Treatments	**Control Parameters**		**Drawbacks**	**References**
	Physiological	**Microbial**		
Integrated treatments				
Hot water + MAP	Decrease weight loss, fruit firmness	-	Increased pericarp browning; funga decay	Sivakumar and Korsten, 2006
Bacillus subtilis + MAP	Pericarp colour retention	Reduce decay	-	Sivakumar *et al*., 2007
Controlled Atmosphere	Pericarp colour retention; quality	-	-	Jiang and Fu, 1999
Superatmosphere O_2	Pericarp browning control; quality	-		Duan *et al*., 2004

The MAP has the effect of reducing the rate of respiration, retarding the ripening process and increasing the shelf life of the produce. Changing the atmosphere the packaging environment can also have an effect on bacterial survival or growth. However, these effects appear to be variable depending on the organism, produce type and the concentrations of O_2 and CO_2. On endive, *Listeria monocytogenes* has been shown to survive and grow, while *Bacillus cereus* was reduced (Gorris, 1994). Other studies have been cited as showing that MAP has no effect on *Escherichia coli* O157:H7, mesophilic bacteria and *L. monocytogenes* in vegetables (Zagory, 1999). There is a concern that MAP may actually increase the risk from pathogens in that the normal process of spoilage is delayed, allowing more time for the growth of pathogens (Francis *et al*., 1999). Under conditions of temperature abuse, the atmosphere could also favour the growth of anaerobic organisms.

4.2. CHITOSAN COATING

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin, a major component of crustacean shells (No and Meyers, 1995). Chitosan is water-insoluble but soluble in weak organic acid solutions. The antimicrobial activity of chitosan against a range of foodborne filamentous fungi, yeast and bacteria, has attracted attention as a potential preservative of natural origin (Sagoo *et al*., 2002). Chitosan was observed to show antimicrobial activity against important bacterial foodborne pathogens, *Listeria monocytogenes* (Devlieghere *et al*., 2004), *Salmonella* Typhimurium (Park *et al*., 1999), *Staphylococcus aureus* (Park *et al*., 1999), as well as decaying fungi *Penicillium chrysogenum*, *P. digitatum*, *P. expansum*, *P italicum* and *P. notatum* (Chien and Chou 2006; Chien *et al*., 2007). Chitosan has also been documented to possess a film-forming property for the use as edible film or coatings (Butler *et al*., 1996). The application of an edible coating, followed by cold storage, is a potential approach to extend the storage of fruit (Park *et al*., 2005). The edible coating can be used as a protective barrier to reduce respiration and transpiration rates through the fruit surface. Chitosan coating modify the internal atmosphere without causing anaerobic respiration, since chitosan films are more selectively permeable to O_2 than to CO_2 (Bai *et al*., 1988). El Ghaouth *et al*. (1991, 1992) investigated the effect of chitosan coating on decay and quality of strawberries at 13 °C. Strawberry fruits were inoculated with *Botrytis cinerea* (El Ghaouth *et al*., 1991, 1992) or *Rhizopus stolonifer* (El Ghaouth *et al*., 1992) and subsequently dipped in chitosan solutions (1.0% and 1.5% in 0.25 N HCl). In both studies, chitosan coating significantly reduced the decay of strawberries compared to the control. However, there was no added benefit to decay control by increasing concentration of chitosan from 1.0% to 1.5%. During storage at 4 °C, chitosan coated berries were firmer, had higher TA and synthesized anthocyanin at a slower rate than the control and the fungicide, Rovral®-treated berries (El Ghaouth *et al*., 1991). The control of decay in strawberries could be attributed either to the fungistatic property of chitosan or to its ability to induce defense enzymes or a combination (El Ghaouth *et al*., 1991, 1992).

Zhang and Quantick (1997) reported that chitosan coating, irrespective of concentration (1% and 2% dissolved in 2% glutamic acid), delayed changes in contents of anthocyanins, flavonoids, and total phenolics contents. It also delayed the increase in PPO activity and partially inhibited the increase in POD activity. Jiang *et al*. (2005) also similarly observed that chitosan (2% in 5% acetic acid) coating delayed the decrease in anthocyanin content and the increase in PPO activity. Thus, chitosan might form a protective barrier on the fruit surface, reducing weight loss and browning. Chitosan coating partially controlled decay of stored litchi fruit (Jiang *et al*., 2005; Zhang and Quantick, 1997).

4.3. 1-METHYLCYCLOPROPENE

1-Methylcyclopropene (1-MCP) is a synthetic cyclic olefin that inhibits ethylene by blocking access to the ethylene-binding receptor (Sisler and Serek, 1997). The effectiveness of ripening and/or ripening inhibition is dependent on the 1-MCP concentration as well as the saturation of the binding sites. The extent and longevity of 1-MCP action is determined by the species, cultivar tissue and mode of ethylene biosynthesis. 1-Methylcyclopropene influences respiratory metabolism (respiration rate, soluble solids concentration and titratable acidity), pigments (chlorophyll degradation or anthocyanin accumulation), the total phenolic content by decreasing the phenylalanine ammonia lyase (PAL) activity and polyphenol oxidase (PPO) activity, the cell wall metabolism, as well as increase the susceptibility to pathogen and fungal growth (Watkins, 2006). 1-Methylcyclopropene has been used extensively in the apple industry since 2002 as a means to retain fruit firmness and skin colour during cold storage and extend shelf life. The application of 1-MCP at 30 – 70 nL L^{-1} for 24 h significantly delayed the onset of ripening of avocado when the treatment was applied at the preclimacteric stage (Feng *et al*., 2000). Jiang and Joyce (2000), observed that by treating mango with 25 – 100 µL L^{-1} 1-MCP for 14 h at 20 ºC delayed fruit softening, colour change and ripening. However, the treatment resulted in higher fruit decay (Jiang and Joyce, 2000). On non-climacteric fruit, such as strawberry, Ku *et al*. (1999) observed that 1-MCP at 5 – 15 nL L^{-1} concentrations, doubled postharvest life at 5 °C, but reduced storage life was observed at higher concentrations (50 – 500 nL L^{-1}). In another study by Jiang *et al*. (2001), the use of 150 and 250 nL L^{-1} 1-MCP reduced decay Rhizopus rot on strawberry, but decay increased with the application of 500 and 1000 nL L^{-1} 1-MCP. Qu *et al*. (2006) reported that the application of 1-MCP at 1 nL L^{-1} reduced the browning and disease index in 'Huaizhi' fruit stored at 28 - 33 °C and 95 – 100% RH for 6 d. 1-MCP in combination with MAP was shown as a potential postharvest treatment to retain litchi quality (Kruger *et al*., 2005). The findings by Jomori *et al*. (2003) showed that treating 'Tahiti' lime with 1.0 uL L^{-1} 1-MCP followed by low temperature storage (5 or 10 ºC) for 30, 60 and 90 days, retained the green skin colour, lowered fruit respiration as well as reduced chilling injuries (40 to 45%) after 60 days.

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CHAPTER 3

EFFECT OF PASSIVE AND ACTIVE MODIFIED ATMOSPHERE PACKAGING ON QUALITY RETENTION OF TWO CULTIVARS OF LITCHI (*LITCHI CHINENSIS* SONN.)

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ABSTRACT

Modified atmosphere packaging (MAP) has been considered beneficial to maintain high humidity, prevention of water loss and browning of litchi pericarp and is also considered a convenient method to market value added small fruits. 'Mauritius' and 'McLean's Red' were packed in active (5% O_2 and 5% CO_2) or passive modified atmosphere in polypropylene punnets. Lidding film with ten and four holes was sealed onto the punnets during the packing process. Packed fruits were stored at 2 ºC and 95% relative humidity up to 21 days. Observations indicated that 'McLean's Red' was more suited for MAP technology than 'Mauritius'. Lidding film-four holes showed ~7% O_2 and 9% CO_2, significantly reduced the activity of oxidation enzymes and retained higher pericarp colour by retaining the hunter color values L*, C*, h* in 'McLean's Red'. Lidding film-ten holes showed ~17% O_2 and ~5% CO_2, retained the soluble solids concentration to titratable acidity ratio (~65), thereby preventing the loss of taste and litchi fruit flavour with acceptable pericarp colour. The use of an active modified atmosphere does not change the composition of the gases at equilibrium but lessens the time necessary to reach this equilibrium. The outcome of this study provides a partial solution for small and medium scale litchi growers and exporters for marketing chains for up to 21 days, especially for early season 'McLean's Red' litchi.

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit with an attractive red pericarp and white-translucent aril. The anthocyanin pigment is responsible for the attractive skin colour of the litchi fruit. However, this highly commercial-valued fruit has a limited shelf life (2 – 3 days) when stored at 25 - 30 ˚C, mainly due to rapid pericarp browning, desiccation and decay after harvest. The browning mechanism in the litchi pericarp is due to the oxidation of anthocyanin by polyphenol oxidase (PPO) (Jiang, 2000; Huang *et al.*, 1990) or peroxidase (POD) (Zhang *et al*., 2005). Commercially a strong antioxidant, sulphur dioxide (SO_2) fumigation is used to inhibit the oxidation reactions involved in pericarp browning and to prevent decay due to its antimicrobial properties (Swarts, 1985). However, according to Lonsdale and Kremer-Köhne (1991), SO_2 fumigation increases the acidity levels in the fruit and results in altered taste. The European Community permits a maximum concentration of 10 μg g^{-1} in the edible portion of the fruit due to reported harmful effects on human health and well-being (Ducamp-Collin, 2004).

Among the proposed alternative postharvest technologies, modified atmosphere packaging (MAP) has been considered beneficial for litchi (Sivakumar and Korsten, 2006). In a MAP, different atmospheres are attained according to the film permeability and the respiratory intensity of the fruit or cultivar types, and these affect the physiological activity of the fruits. When an internal atmosphere is passively modified by the respiration of a commodity, it takes some days or weeks, to reach the gas equilibrium concentration. It is assumed that the gas composition inside the bag is at equilibrium when the quantity of gas exchanged through the fruit surface is similar to that exchanged through the film (packaging) and the equilibrium is attained when the gas composition inside the package stabilises (Zagory and Kader, 1988). An active modified atmosphere is created by the addition of certain gas compositions inside the bag at the moment of fruit packaging, which can provide an earlier state of equilibrium and help to keep the desired atmosphere (gas composition) for a longer period around the fruit. According to Yahia and Gonzalez-Aguilar (1998), the active modified atmosphere contributes to the extension of the shelf life of fruits. Therefore, for horticultural products with low respiratory rates, that take a long period of time to reach atmosphere equilibrium in MAP, the injection of CO_2 into the packaging during the packing process could shorten this period of time.

The aim of this study was to investigate the effect of passive and active atmospheres on oxidation enzymes (PPO and POD) and fruit quality parameters during storage (2 ºC, 90% RH) up to 21 days. We also investigated the evolution of the atmospheric composition in passive and active from (O_2 and CO_2) inside packages made with different perforations on a specific film for the two South African export litchi cultivars 'Mauritius' and 'McLean's Red'.

2. MATERIALS AND METHODS

2.1. FRUIT AND PACKAGING MATERIAL

Litchi cvs. 'Mauritius' and 'McLean's Red' were harvested early morning at optimum maturity from commercial orchards in the Mpumalanga and Limpopo regions, South Africa. Fruits of uniform color, size and weight, as well as the absence of disease and damage, were used in this study. A set of 450 g (15 - 16 fruits) fruits were transferred into polypropylene punnets (without perforations) and the punnet was sealed with a polyester lidding film (64 µm, O_2 and water vapour transmission rate of 4.3 cm^3/m^2/day/bar at 23 ºC and 85% RH and 2.3 g/m^2/day, respectively) with different perforations as described in Table 1.

Table 1. Treatment codes and perforations

Treatment Code	Perforation combination
P1-A (A)	Ten perforation holes (0.6 mm diameter) and 5%O_2: 5%CO_2: 90%N_2 (active atmosphere)
P1-P (B)	Only ten perforation holes (passive atmosphere)
P2-A (C)	Four perforation holes (0.6 mm diameter) and 5%O_2: 5%CO_2: 90%N_2 (active atmosphere)
P2-P (D)	Only four perforation holes (0.6 mm diameter) (passive atmosphere)
NP2 (E)	No perforations

A tray sealer (Multivac tray sealer model T200, Wolfertschwenden, Germany) was used to seal the lidding film to the punnet. Passive modified atmosphere was created by sealing the punnets with perforated film; an active modified atmosphere was achieved by flushing food grade gases 5% O_2, 5% CO_2 and 90% N_2, (Air Products Pty, ltd.; Kempton Park, South Africa) into the punnets and sealing with perforated-film. The punnet with unperforated film (NP2 (F)) was included as control in this study. Ten replicates of each treatment were stored at 2 ºC and 90% RH for 14 and 21 days. Replicates of each treatment was removed from cold storage after designated storage time to evaluate: (1) the fruit quality parameters: pericarp browning index (BI), pericarp colour, SSC/TA ratio; (2) biochemical parameters: anthocyanin content, polyphenol oxidase and peroxidase activity, relative leakage; and (3) organoleptic parameters: skin color, taste and flavour. An additional three replicates per treatment were used to determine the gas composition from 0 – 10 days. Thereafter, the gas composition measurements were taken from three replicates per treatment after 14 and 21 days cold storage. The gas composition measurements were recorded by using a PBI Dansensor CO_2/ O_2 gas analyzer (Checkmate 9900; Ringsted, Denmark).

2.2. MEASUREMENT OF FRUIT QUALITY

2.2.1. *Browning and decay index*

The severity of pericarp browning was assessed visually according to a browning index (BI) according to Sivakumar and Korsten (2006) as: 1= no browning; 2= 1 – 2 brown spots; 3= some spots with browning; 4= 50%; 5= 75% entire fruit surface brown. The browning index was calculated according to Zhang and Quantick (1997). Decay index was assessed on a 1 – 5 scale, describing the severity of postharvest fungal decay: 1= no disease; 2= ¼; 3= ½; 4= ¾ of the fruit's surface; 5= entire fruit decayed due to disease (Sivakumar and Korsten 2006).

2.2.2. *Colour*

Fruit surface skin colour (L, chroma, hue angle; L^* = lightness coefficient, C^* = chroma and h° = hue angle) was measured on two sides per fruit unit (opposite sides) (16 fruits per replicate per treatment) according to Sivakumar and Korsten (2006) using a Minolta chromameter (Minolta CR-300 Colorimeter, Osaka, Japan).

2.2.3. *Fruit firmness*

Fruit firmness was measured on opposite sides of each fruit unit (16 fruits per replicate per treatment) by a hand-held firmness tester (Chatillon and Sons, New York, USA). Fruit firmness was expressed in Newton (N) units.

2.2.4. *Soluble solids content and titratable acidity*

A total of 16 fruit per replicate per treatment was selected to determine soluble solids concentration (SSC) according to Sivakumar and Korsten (2006). The aril of peeled fruit was pressed through layers of gauze and the SSC was determined with a digital refractometer (Pr-100 Atago, Tokyo, Japan) and expressed in percentages. Percentage titratable acidity (TA) was determined by titration of 10 mL juice with 0.05 M NaOH and calculated as citric acid equivalent.

2.3. MEASUREMENT OF BIOCHEMICAL PARAMETERS

2.3.1. *Anthocyanin content*

Pericarp (1 g) of ten fruits, selected from three replicates per treatment, was peeled and extracted with 15 mL HCl-methanol (0.15% HCl: 95% methanol = 15: 85) for 4 h. The extract was filtered and its absorbance was determined at 530, 620 and 650nm using a spectrophotometer (Carl Zeis (Jena), Jena, Germany). The anthocyanin content was determined according to Zheng and Tian (2005) based on the formula: Δ A/g FW = $(A_{530}-A_{620}) - 0.1(A_{650}-A_{620})$.

2.3.2. *Measurement of oxidation enzymes activity and relative leakage*

Litchi pericarp (10 g) from 16 fruits per treatment was homogenised in 20 mL of 0.05 M potassium phosphate buffer (pH 6.8) and 0.6 g Polyvinylpyrrolidone (Sigma Chemicals, Johannesburg) at 4 ºC according to Jiang (2000). The filtered homogenate was centrifuged for 15 min at 19,000 g (Ortoalresa, Alvarez Redondo S.A., Madrid Spain model DIGICEN 20-R) at 4 ºC. The supernatant was collected to determine the polyphenol oxidase (PPO) enzyme activity. The PPO activity was assayed according to Jiang (2000), using a reaction mixture of 0.05 M sodium phosphate buffer (pH 6.8) containing 0.2 M 4-methylcatechol (0.2 mL) and crude enzyme (0.5 mL). Tubes were incubated for 5 min at 30 ºC and the absorbance was measured at 410 nm by visible spectrophotometry (Carl Zeis (Jena), Jena, Germany).

The peroxidase activity was measured according to Zhang *et al*. (2005). Litchi pericarp (2 g) taken from each treatment was ground with 20 mL of 0.05 M sodium phosphate buffer and 0.6 g polyvinylpyrrolidone, and then centrifuged for 15 min at 19, 000 g at 4 ºC. A reaction mixture consisting of 2 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol were added to 1 mL of enzyme extract and the POD activity was measured at 470 nm. The protein content was determined according to Bradford (1976). One unit of enzyme activities was defined as an increase in absorbance unit per minute at 25 ºC. The oxidation enzymes activities were measured from three replicates per treatment.

Membrane permeability, expressed by relative leakage rate, was measured according to Lichter *et al*. (2000). Thirty peel discs were cut from 16 fruits peels per replicate per treatment using a 10 mm cork borer. Pericarp discs were prepared and electrolyte leakage was determined with a conductivity meter (H176300 EC214, Hanna Instruments, Johannesburg). Relative leakage rate was expressed as percentage of total electrolytes.

2.4. ORGANOLEPTIC EVALUATIONS

Eating quality was evaluated by a panel of ten members familiar with the organoleptic properties of fresh litchi fruit. Evaluations were based on a 1 - 4 scale basis considering fruit decay, pericarp browning, colour, appearance and eating quality: 1 = decayed and browned, 2 = fair; limited marketability; 3 = good, acceptable marketability; 4 = excellent.

2.5. STATISTICAL ANALYSIS

The experiment was repeated twice adopting complete randomized design and the data of each cultivar was analysed separately. Statistical evaluation of the differences was performed using analysis of variance (ANOVA). The mean values of the significant interactions were compared by Fisher's protected *t*-test L.S.D (least significant difference) at the 1% level using statistical program GenStat (2005).

3. RESULTS AND DISCUSSION

3.1. CHANGES OF THE IN-PACKAGE GAS COMPOSITION

The Figs. 1 A – D show the evolution of the internal atmosphere of the punnets made with different perforations on the lidding film with 'Mauritius' and 'McLean's Red' respectively. The temperature, fruit weight and exchange area through the film remained constant and therefore, the change of the internal atmosphere inside the bag and the time necessary to reach equilibrium were controlled by the permeability of the plastic film and the respiration rate of the litchi fruit. It was observed that as the permeability increased (number of perforations) the CO_2 concentrations at equilibrium were lower and the O_2 concentration higher. The punnets that had ten perforations on the lidding film [P1-A (A) and P1-P (B)] showed ~16% O_2 and 6% CO_2 for 'Mauritius' and ~17% O_2 and ~5% CO_2 for 'McLean's Red'. It is also evident from this observation that the internal atmosphere at equilibrium depended on the type of cultivar due to their different respiration rates. Similar observations were reported on Beliana and Polonias apricot varieties (Pretel *et al*., 2000). The equilibrium in internal atmosphere (passive) was attained for 'Mauritius' after eight days in punnets [P1-P (B)] with 10 holes in the lidding film. Whereas in punnets with four holes [P2-P (D)] in the lidding film attained the equilibrium atmosphere after 10 days. However, with 'McLean's Red' the equilibrium atmosphere was attained after six days in punnets [P1-P (B)] with 10 holes and after eight days in punnets [P2-P (D)] with four holes on the lidding. The punnets with the highest film permeability reached a rapid equilibrium with less substantially modified CO_2 and O_2 concentrations (Gil-Izquierdo *et al*., 2002). A mixture of 5% O_2 and 5% CO_2 was flushed into the punnets at the moment of packaging replacing the initial atmosphere (air). The evolution of the internal atmosphere indicated that the flushing of gas mixture helped to minimise the time taken to reach the equilibrium state in both types of cultivars and it was established almost from the first day of storage. During the steady state, litchi respiration (O_2 consumption and CO_2 production) was balanced by O_2 and CO_2 diffusion through the film. Fruit packed in punnet NP2 (E) showed accumulation of higher CO_2 concentration ('Mauritius' $> 40\%$ CO_2 and 'McLean's red' $< 40\%$ CO_2) probably as a result of anaerobic respiration. A similar trend in increase of CO_2 concentration was observed in strawberries packed in cups without micro perforations (Almenar *et al*., 2007).

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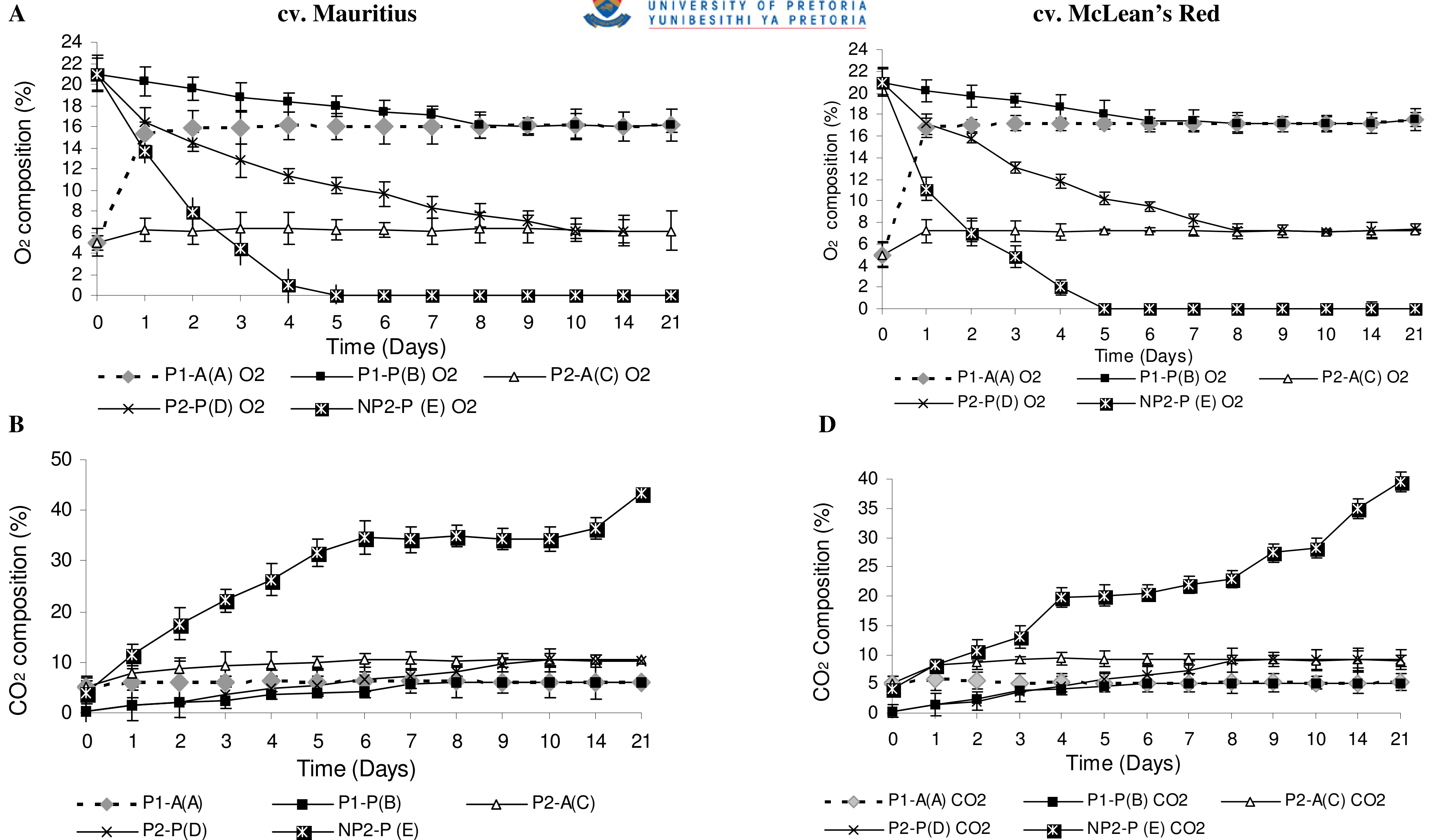

Fig. 1. Changes of the in-package gas composition in punnets packed with 'Mauritius' (A) O_2 composition (B) CO_2 composition; and 'McLean's Red; (C) O_2 composition (D) CO_2 composition. Error bars represent the SD (Standard deviations). Punnet P1-A (A) – Ten perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P1-P (B) – Only ten perforation holes (passive atmosphere); P2-A (C) – Four perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P2-P (D) – Only four perforation holes (0.6 mm diameter) (passive atmosphere); NP2 (E) – no perforations. Data was the mean of three replicates.

3.2. EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON PERICARP DECAY, BROWNING AND PERICARP COLOUR

Decay was absent in all packaging treatments adopted in this study. The browning index (BI) increased with storage time (Fig. 2A). However, fruits of both cultivars in NP2 –P(E) punnets showed significantly ($P < 0.001$) higher BI due to the CO_2 accumulation to injurious levels (CO_2 injury) within the punnets (Figs. 1B and D). Similar observation was reported by Almenar *et al*. (2007) in wild strawberries packed in sealed cups. Fruits packed punnets P2-A(C) and P2-P(D) did not show significant ($P < 0.001$) browning in both cultivars. 'McLean's Red' packed in P2-A(C) and P2-P(D) punnets showed absence of pericarp browning. However, 'Mauritius' in punnet P1-A(A) and P1-P(B) showed significantly ($P < 0.001$) higher BI ~ 3.0 by showing some brown spots with limited marketability. 'McLean's Red' fruits in P1-A(A) and P1-P(B) revealed BI ~2 with 1 – 2 brown spots with acceptable marketability. The hunter values L^*, C^* and h^o were found to decrease with storage time with all packaging treatments in both cultivars. Fruits of both cultivars in punnet NP2(E) turned deep brownish red color and showed a significant ($P < 0.001$) decline in L^*, C^* and h^o values due to CO_2 injury after cold storage for 21 days. Almenar *et al*. (2007) reported similar effect on L^* value in presence of higher CO_2 concentration in wild strawberries packed in sealed. However, fruits of both cultivars in P2-A(C) and P2-P(D), showed moderately higher L^* than the fruits in P1-A(A) and P1-P(B) after 14 and 21 days cold storage (data not shown). Decrease in C^* indicates loss of red color. Loss of C^* was related to the permeability of the material. Fruit in low permeability punnet [P2-A(C) and P2-P(D)] showed higher C^* than the fruit stored in higher permeability punnet [P1-A(A) and P1-P(B)] (Fig. 2 B) which is in agreement with the effect observed by Watkins *et al.* (1999) in strawberries stored in high-CO_2 atmospheres. Although fruit of both cultivars in punnets, P2-A(C) and P2-P(D) showed higher C^* and $h°$ values than the fruits in punnets P1-A(A) and P1-P(B), the McLean's Red in punnet P1-A(A) and P1-P(B) showed acceptable C^* and $h°$ after 21 days cold storage (Figs. 2B and C).

3.3. EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON ANTHOCYANIN CONTENT, OXIDATION ENZYME ACTIVITY AND RELATIVE MEMBRANE LEAKAGE

Anthocyanin content decreased continuously with increasing storage time (Fig. 3A). Decrease in anthocyanin content was most effectively delayed in fruits packed in punnets P2-A (C) and P2-P(D) than in punnets P1-A(A) and P1-P(B). Fruits of both types of cultivar packed in NP2 –P(E) showed a significant ($P < 0.001$) decline in anthocyanin content during storage. Furthermore, higher anthocyanin content was observed in 'McLean's Red' than in 'Mauritius' with respect to all packaging treatments adopted in this study. The observed differences in anthocyanin concentration between the two cultivars were noted in freshly harvest fruit (Sivakumar *et al*., 2008).

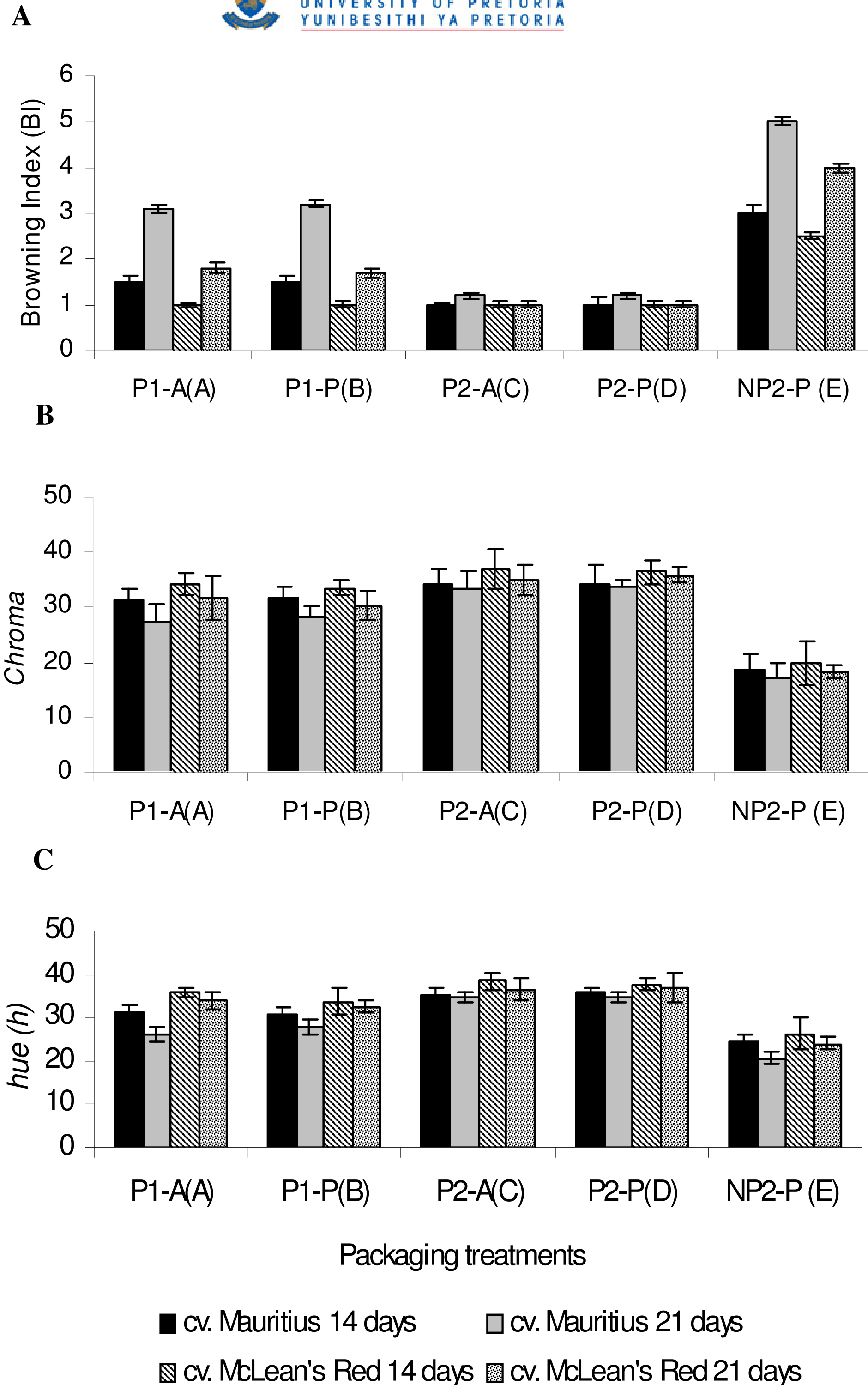

Fig. 2. Effect of modified atmosphere packaging on: (A) pericarp browning, (B) Chroma and (C) Hue of litchi cultivars 'Mauritius' and 'McLean's Red'. Error bars represent the SD (standard deviation). Punnet P1-A (A) – Ten perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P1-P (B) – Only ten perforation holes (passive atmosphere); P2-A (C) – Four perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P2-P (D) – Only four perforation holes (0.6 mm diameter) (passive atmosphere); NP2 (E) – no perforations. Data was the mean of ten replicates per packaging treatment per storage time.

In both cultivars, the polyphenol oxidase activity was high up to 14 days, thereafter it decreased significantly ($P < 0.001$) (Fig. 3B). Fruit stored in punnets, P2-A(C) and P2-P(D) showed significantly ($P < 0.001$) lower PPO activities than the fruit in P1-A (A), P1-P(B) for both cultivars. Whereas highest PPO activity was observed in fruit packed in NP2 –P(E) for both cultivars. The PPO activity in both cultivars was lower than the POD activity which confirms the observations of Zauberman *et al.* (1991), Zhang and Quantick (1997) and Ducamp-Collin *et al*. (2008). Initially, the peroxidase activity was lower for both cultivars up to 14 days. Thereafter, a significant ($P < 0.001$) increase in POD activity was noted after 21 days storage. Although fruit in punnets P1-A(A) and P1-P(B), showed higher POD activity than the fruit in punnets P2-A(C) and P2-P(D), the fruit in NP2 –P(E) punnet revealed the highest POD activity after 21 days storage (Fig. 3C).

Higher PPO activity was reported in the pericarp before the browning sets in (Tian *et al*., 2002). Jiang and Fu (1999) reported that the POD activity increased with prolonged storage time. Furthermore, the higher oxidation enzyme activities can be related to the higher BI observed in punnets NP2 –P(E). The observed reduction in anthocyanin content in both cultivars packed in punnets P1-A(A) and P1-P(B), could be attributed to the increased oxidation enzyme activities as illustrated in Figs. 3B and C. The increased oxidation enzyme activities would have favored by the higher O_2 composition (~17%) around the fruit. The PPO and POD activity was observed to be higher in 'Mauritius' than 'McLean's Red' and similar observations were reported with respect to Chinese cultivar 'Nuomici' that browns easily, which showed higher PPO activity than 'Guiwei', a cultivar that browns more slowly (Chen *et al*., 2001). The observed higher oxidation enzymes activity in explain the loss of anthocyanin content and BI noted in 'Mauritius'.

According to Tian et al. (2002), the integrity of membrane systems can be expressed as relative leakage rate. With increasing storage time, the integrity of pericarp cells changes due to senescence process and thereby increases the permeability of cells (Duan *et al*., 2007). The relative leakage rate increased in both cultivars during storage (Fig. 3D). Fruit of both cultivars in P2-A(C) and P2-P(D) showed lower relative leakage than the fruit in P1-A(A) and P1-P(B). Pericarp browning is associated with the loss of membrane integrity that occurs during tissue deterioration and senescence (Duan *et al*., 2004). Fruit in punnet NP2 –P(E) had the highest relative leakage, indicating membrane disintegration and association with pericarp browning. Since membrane disintegration leads to the decompartmentalisation of enzymes and substrates that led to enhanced substrate-enzyme contact and accelerated anthocyanin degradation as stated by Jiang and Fu (1999). Furthermore, the observed differences in relative membrane leakage in the two cultivars could be due to the differences in the resistance of membrane system.

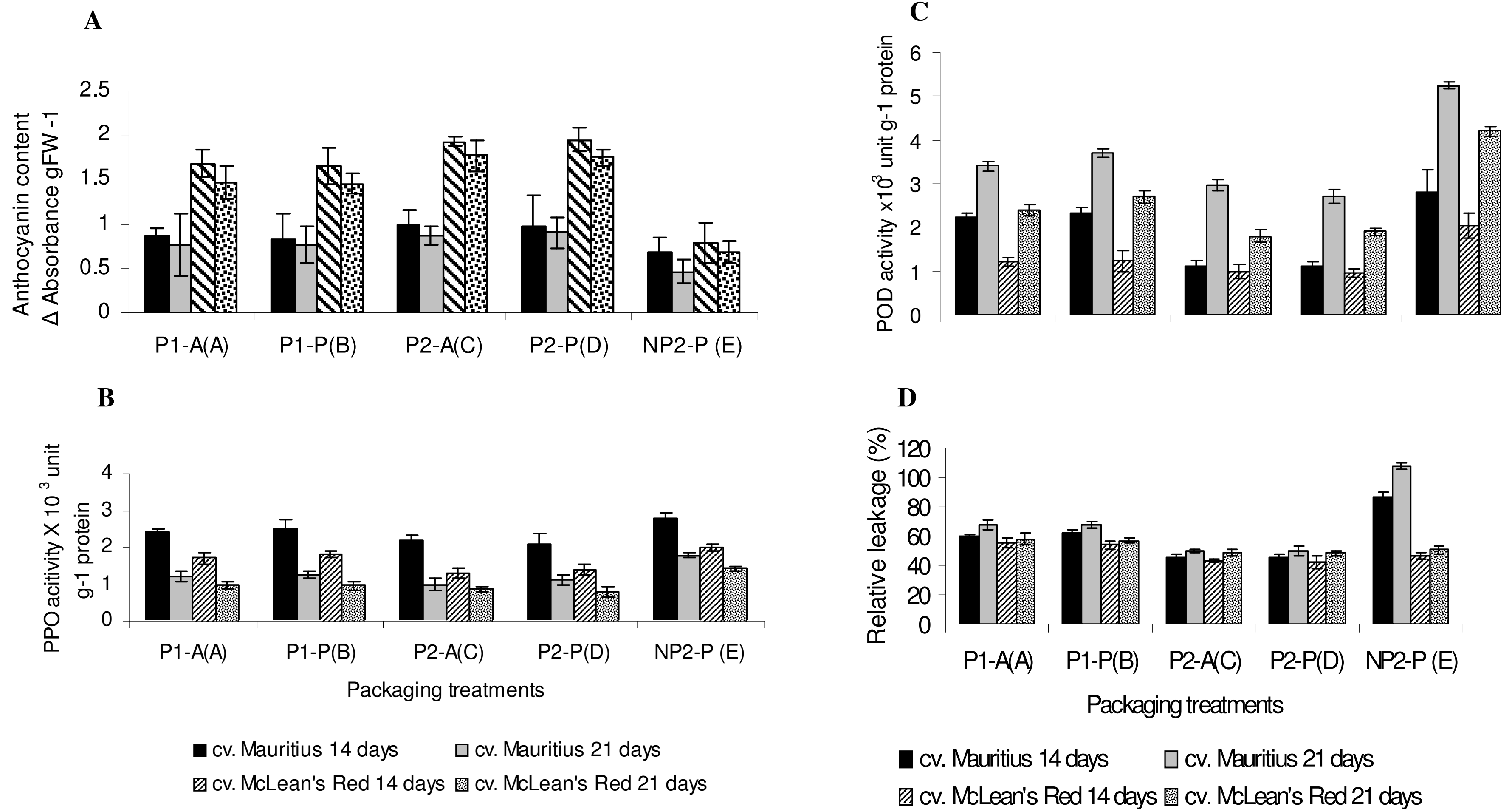

Fig. 3. Effect of modified atmosphere packaging on: (A) anthocyanin content (B) PPO activity (C) POD activity (D) relative leakage of litchi cultivars 'Mauritius' and 'McLean's Red'. Punnet P1-A (A) – Ten perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P1-P (B) – Only ten perforation holes (passive atmosphere); P2-A (C) – Four perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P2-P (D) – Only four perforation holes (0.6 mm diameter) (passive atmosphere); NP2 (E) – no perforations. Data was the mean of ten replicate per packaging treatment per storage time. Error bars represent the SD (Standard deviations).

3.4. EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON SSC/TA AND ORGANOLEPTIC EVALUATIONS

'McLean's Red' showed a higher SSC/TA ratio than 'Mauritius' with respect to P1-A(A), P1-P(B) P2-A(C) and P2-P(D) punnet treatments. Acceptable SSC/TA ratio (50 – 65) was detected in both cultivars packed in punnets P1-A(A) and P1-P(B) after 21 days of storage (Fig. 4). Fruits packed in P2-A(C) and P2-P(D) showed lower SSC/TA than the fruits in punnets P1-A(A) and P1-P(B). However, ($P < 0.001$) lower SSC/TA was observed in fruits packed in punnet NP2 –P(E) was due to increase in acidity caused by fermentation. This can be further explained by the observed higher CO_2 composition within the punnet NP2 –P(E). Furthermore, fruit subjected to MAP treatments in this study retained fruit firmness and prevented the weight loss due to the presence of high RH (90%) around the fruits (data not shown). The organoleptic observation revealed although the fruit skin color was significantly ($P < 0.001$) higher in fruits packed in punnets P2-A(C) and P2-P(D) for both cultivars, the taste, flavor were higher in fruits packed in punnets P1-A(A) and P1-P(B) after 21 days cold storage (Figs 5A and B).

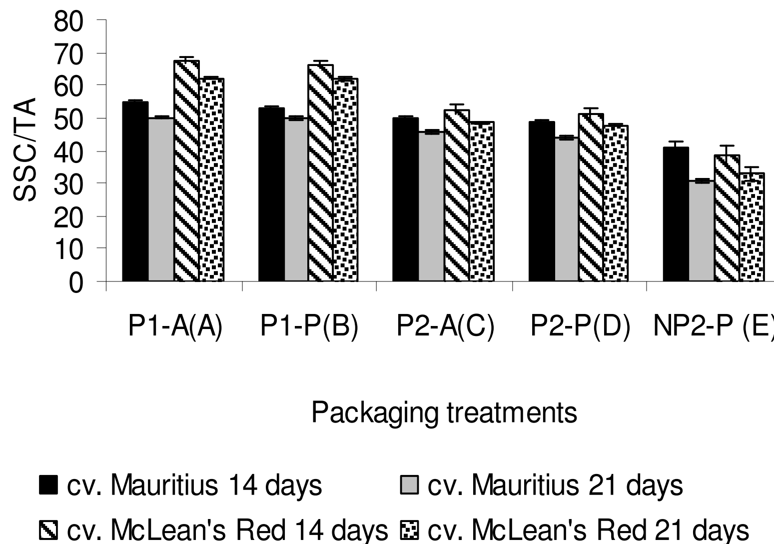

Fig. 4. Effect of modified atmosphere packaging on SCC/TA of 'Mauritius' and McLean's Red'. Error bars represent the SD (Standard deviations). Punnet P1-A (A) – Ten perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P1-P (B) – Only ten perforation holes (passive atmosphere); P2-A (C) – Four perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P2-P (D) – Only four perforation holes (0.6 mm diameter) (passive atmosphere); NP2 (E) – no perforations. Data was the mean of ten replicate per packaging treatment per storage time.

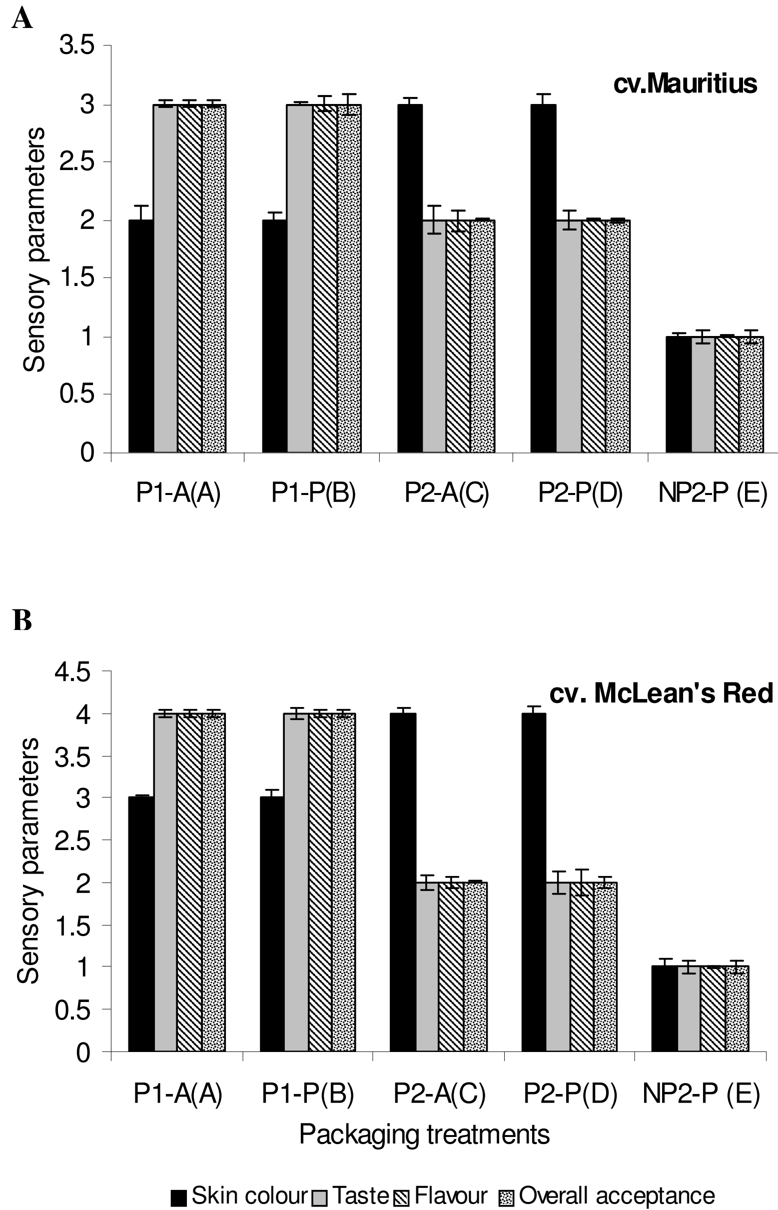

Fig. 4. Effect of modified atmosphere packaging on (A) sensory parameters of 'Mauritius' and (B) sensory parameters of 'McLean's Red'. Error bars represent the SD (Standard deviations). Punnet P1-A (A) – Ten perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P1-P (B) – Only ten perforation holes (passive atmosphere); P2-A (C) – Four perforation holes (0.6 mm diameter) and 5% O_2: 5% CO_2: 90% N_2 (active modified atmosphere); P2-P (D) – Only four perforation holes (0.6 mm diameter) (passive atmosphere); NP2 (E) – no perforations. Data was the mean of ten replicate per packaging treatment per storage time.

4. CONCLUSION

Our observations demonstrated cultivar performance with respect to different MAP treatments. Based on the organoleptic assessments, biochemical and fruit quality parameters, it is concluded that the MAP technology was observed to be more beneficial for 'McLean's Red' than 'Mauritius'. The 'McLean's Red' can be packed in punnets P1-A(A) and P1-P(B) since the skin colour, taste and flavour were retained. Also, the use of an active modified atmosphere does not change the composition of the gases at equilibrium but lessens the time necessary to reach this equilibrium.

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CHAPTER 4

EFFECT OF INTEGRATED APPLICATION OF CHITOSAN COATING AND MODIFIED ATMOSPHERE PACKAGING ON OVERALL QUALITY RETENTION IN LITCHI CULTIVARS

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ABSTRACT

The storage life of litchi is limited due to pericarp browning and decay. Modified atmosphere packaging (MAP) showed promising results for ensuring quality retention. However, to improve the efficiency of MAP the integrated treatment of a chitosan coating and MAP was investigated. The effect of chitosan (1.0 g L-1) + MAP was compared with MAP (control), and was effective in preventing decay, browning and retaining the pericarp colour in the cultivar 'McLean's Red'. Chitosan (1.0 g L-1) + MAP significantly reduced polyphenol oxidase (PPO) and peroxidase (POD) activity, retained membrane integrity, anthocyanin content and prevented the decline of pericarp colour values during storage. The POD activity was greater than the PPO activity in the cultivar 'McLean's Red' and 'Mauritius'. The two cultivars differed in anthocyanin content and the activity of oxidation enzymes. The gas compositions within the packages were compared between chitosan at 1.0 g L^{-1} and 20.0 g L^{-1} concentration for both cultivars. Chitosan (20.0 g L^{-1}) + MAP lowered the respiration during storage in both cultivars compared to 1.0 g L^{-1} + MAP. The 'McLean's Red' cultivar is better suited for chitosan (1.0 g L^{-1}) + MAP integrated treatment than is 'Mauritius' in retaining overall quality.

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a popular subtropical fruit that is of high commercial value in the European markets for its attractive red colour and exotic flavour. The anthocyanin pigment is responsible for the attractive red colouration of the litchi skin. However, rapid pericarp browning and decay limit the storage life of litchis. The browning mechanism in litchi is reported to be a result of oxidation of anthocyanins by polyphenol oxidase (PPO) (Huang *et al*., 1990; Jiang 2000) or peroxidase (POD) (Zhang *et al*., 2005). Commercially a strong antioxidative treatment, sulphur dioxide (SO_2) fumigation, has been adopted to block oxidation reactions involved in browning. Due to harmful effects caused by the allergic reaction of sulphur residues, the European Community permits a maximum concentration of only 10 µg g^{-1} in the edible portion of the fruit (Ducamp-Collin, 2004).

An alternative technology to consider is modified atmosphere packaging (MAP), since it maintain high humidity, prevent of water loss and browning of litchi pericarp (Kader, 1994). The 17% O_2 and 6% CO_2 created in bioriented polypropylene (BOPP) MAP helped to retain quality of litchi fruit and reduced pericarp browning (Sivakumar and Korsten, 2006). However, in MAP it is essential that the fruits are almost 100% free of browning and disease because pre-sorting before sale is not practicable in the large-scale marketing chain. Therefore, there is a need to improve the application of MAP technology for litchi fruits in order to use it as a partial alternative to SO_2 fumigation. According to the observations of chapter 3 indicated that 'McLean's Red' was more suited for MAP technology than 'Mauritius' and the lidding film-ten holes showed ~17% O_2 and ~5% $CO_{2,}$ retained taste and litchi fruit flavour with acceptable pericarp colour, of McLean's Red'. Therefore, the reason for carrying out this investigation is to see whether the combined application of chitosan coating and MAP will help to retain overall quality of both South African export litchi cultivars 'Mauritius' and 'McLean's Red' during long term storage and transportation.

Chitosan coating in acidic solution minimised pericarp browning (Zhang and Quantick, 1997; Joas *et al*., 2005; Caro and Joas, 2005; Sivakumar *et al*., 2005). The treatment reduced the losses of anthocyanin, flavonoids and total phenolic compounds and inhibited the increase of polyphenol oxidase and peroxidase activity in the pericarp (Zhang and Quantick, 1997). Chitosan inhibits the growth of decay causing fungi and induces defence response in host tissues (Allan and Hadwiger, 1979; Shibuya and Minami, 2001). Therefore, an integrated application of a chitosan coating and MAP would be beneficial to obtain fruit that was free of 100% browning and disease for export purposes. According to Romanazzi *et al*. (2007) the use of lower concentrations of chitosan would be beneficial in order to reduce the cost of production and for easy application due to its lower viscosity. The objective of this study was to investigate the potential of a low chitosan-concentration coating integrated with MAP (bioriented polypropylene) to extend the storage life and

quality retention of two South African litchi cultivars, 'Mauritius' and 'McLean's Red'. The BOPP was selected from the previous findings (Sivakumar and Korsten, 2006) carried out at the Plant Pathology Laboratories, University of Pretoria. According to Sivakumar and Korsten (2006) the BOPP has the potential for commercialisation because it is easy to implement at the domestic supper market chains, economical and cost effective than using the tray packaging and the Multivac machinery.

2. MATERIALS AND METHODS

2.1. FRUIT

Early season litchi fruit 'Mauritius' and 'McLean's Red' were picked at commercial maturity from Geldenhuys plantations in Tzaneen, Northern Province of South Africa during the 2005 – 2006 and 2006 – 2007 growing seasons. It must be noted that, although the fruiting pattern is different in both cultivars, the trials were conducted during the same growing seasons. After harvest, fruits were transported to the on-farm laboratory and were sorted for uniform size, colour stage and absence of mechanical damage.

2.2. CHITOSAN SOLUTION

Chitosan at a concentration of 20.0 g L^{-1} (Crab-shell chitosan, Sigma Chemicals) was prepared by dissolving under continuous stirring, in deionised water and glacial acetic acid according to Jiang *et al*. (2005). After preparation, the chitosan solution was cooled to 25 ºC. Chitosan 1.0 g L^{-1} was obtained by dilution with deionised water. A total volume of 6000 mL chitosan solution was used for fruit dipping treatment and Tween 80 (0.1%) was added to the solution to improve wetability.

2.3. INTEGRATED TREATMENT OF CHITOSAN SOLUTION AND MODIFIED ATMOSPHERE PACKAGING

A complete randomised design was used in this experiment. Fruits were divided into two sets, each containing 650 fruits, and each set was divided into 13 replicates, each replicate containing 50 fruit per treatment per cultivar. Within 2 – 3 h after harvest, fruits were dipped in chitosan solution (1.0 g L^{-1}) for approximately 5 min and allowed to air dry for 45 min (Sivakumar *et al*., 2005). Thereafter, a set of 50 fruits was packed in bioriented polypropylene packages (MAP; Knilam Packaging, Cape Town) and sealed with a heat sealer to create a passive modified atmosphere around the fruits. The thickness of the bag was 35 µm (size 40 cm x 18 cm) and sealed with a heat sealer (Multivac C200; Multivac, Heidelberg). The O_2, CO_2 and water vapour transmission rates of the film were 1100 cm^3 m^{-2} atm^{-1} and 4.5 g^{-2} day^{-1}, respectively

(Sivakumar and Korsten, 2006). Fruits subjected to an integrated treatment with chitosan + MAP and stand-alone MAP (control) were stored at 2 ºC and at 90 % RH for 21 days and thereafter at 14 ºC, 75 – 78 % RH for 48 h to simulate market shelf condition. The gas composition within packages was determined after 21 days from three replicates per cultivar using a PBI Dansensor CO_2/O_2 gas analyser (Checkmate 9900, Ringsted, Denmark). Ten replicates of each treatment were removed from the simulated market shelf conditions and the effect of all treatments on decay incidence, browning index, PPO and POD activity, measurement of relative leakage, and fruit quality parameters, pericarp colour, weight loss, soluble solids concentration (SSC) and titratable acidity (TA), were assessed. Fruits of both cultivars dipped in 20.0 g L^{-1} chitosan solution were packed in MAP (three replicates per cultivar) and stored in cold storage. Head-space gas analysis was determined after 21 days.

2.4. MEASUREMENT OF FRUIT QUALITY

2.4.1. *Decay and browning index*

The severity of postharvest disease was assessed on a scale of 1 – 5, which describes the severity of postharvest fungal decay as: 1 = no disease; 2 = 25 %; 3 = 50 %; and 4 = 75 % of fruit surface affected and 5 = entire fruit decayed due to disease (Sivakumar and Korsten, 2006). The severity of pericarp browning was assessed visually as: 1 = no browning; 2 = 1 – 2 brown spots; 3 = some spots with browning; 4 = 50 %; 5 = 75 % and entire fruit surface brown (Sivakumar and Korsten, 2006). The browning index (BI) was calculated according to Zhang and Quantick (1997).

2.4.2. *Colour*

Fruit pericarp colour was measured (20 fruits per replicate per treatment) using a Minolta Chromameter (model CR-300; Minolta, Osaka, Japan), expressing the CIELAB, Commission international de I'Eclairage (CIE) colour space, L*, a* and b*. The coordinates a and b were analysed in order to express the red colour and the effect of BI. Although L C h was used in the previous investigation (in Chapter 1), the colour coordinate a was selected here because the colour value *a** directly relates to the red colour of the pericarp according to Ducamp-Collin *et al*. (2008).

2.4.3. *Soluble solids concentration and titratable acidity*

A set of 20 fruits per replicate per treatment was randomly selected from each for the determination of soluble solids concentrations (SSC) using a digital refractometer (Atago Co., Tokyo, Japan) and expressed by titrating 10 mL of fruit juice with 0.01 mol L-1 NaOH and the result was calculated as citric acid equivalent (Sivakumar and Korsten, 2006).

2.5. MEASUREMENT OF BIOCHEMICAL PARAMETERS

2.5.1. *Anthocyanin*

Pericarp (10 g) from 20 fruits was sliced and extracted with 15 mL HCl-methanol (0.15% HCl: 95% methanol, 15:85) for 4 h. The extract was filtered and its absorbance determined at 530, 620 and 650 nm, respectively. The anthocyanin content measurement was carried out according to Zheng and Tian. , (2005) using the formula: $\Delta A/\ gFW = (A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$. The absorbance measurement were obtained using a spectrophotometer (Carl Zeiss, Jena, Germany), where FW is the fresh weight, in grams, and ΔA is the difference in absorbance.

2.5.2. *Measurement of polyphenol oxidase and peroxidase activity and relative leakage*

Pericarp tissues (10 g) from 20 fruits per treatment per replicate were homogenised in 20 mL of 0.05 mol L-1 potassium phosphate buffer (pH 6.8) and 0.6 g of polyvinylpyrrolidone (Sigma Chemicals) at 4 ºC according to Jiang (2000). After filtering the homogenate through a cotton cloth, the filtrate was centrifuged fro 20 min at 19 000 x g and 4 ºC. The supernatant was then collected as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate according to the method of Jiang (2000) at 410 nm. POD activity, using guaiacol as a substrate, was assayed according to Zhang *et al*. (2005), in a reaction mixture of 3 mL containing 25 µL of enzyme extract, 2 mL of 0.05 mol L-1 phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol. The increase in the absorbance at 470 nm, due to guaiacol oxidation, was recorded for 2 min. Protein content was determined according to Bradford (1976). One unit of enzyme activity was defined as an increase in absorbance unit per minute at 25 ºC. There were three replicates per treatment.

A set of 30 peel discs from 20 fruits per replicate per treatment were cut using a 10 mm cork borer from the equatorial region of the fruit pericarp and the conductivity was measured using a conductivity meter (H176300 EC214; Hanna Instruments) according to Lichter *et al*. (2000).

2.6. STATISTICAL ANALYSIS

The effect of treatment on each cultivar was analysed separately for each growing season by analysis of variance (ANOVA) using MINTAB Version 14 (Minitab Inc., State College, PA, USA). Since there is no significant effect of the year in the ANOVA, the results from the two growing seasons (2005 – 2006 and 2006 – 2007) were combined in the analysis.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENT ON THE INCIDENCE OF DECAY AND BROWNING

It is evident from this study that integrated treatment with chitosan + MAP revealed an absence of decay in both cultivars. 'Mauritius' fruits in stand-alone MAP showed 11.3% and 10.5% decay incidence with severity 3 and 2, respectively. This observation showed that the chitosan coating can be used as a protectant to prevent decay under market-shelf conditions. Reduced fructoplane population showed the antimicrobial property of chitosan on litchi surfaces (Sivakumar *et al*., 2005). Furthermore, chitosan significantly reduced the decay incidence from 65.5% to 28 – 26% up to 21 days at 4 ºC (Zhang and Quantick, 1997). Chitosan inhibits the growth of decay-causing fungi and induces defence response in host tissues. Romanazzi *et al*. (2007) reported that acetic acid was the best solvent for chitosan, since it activated its antimicrobial and eliciting properties.

Pericarp browning was not observed in 'McLean's Red' in the chitosan + MAP treatment (Figs 1 and 2). Although 'Mauritius' subjected to chitosan + MAP treatment showed a browning index (BI) of 1 – 2 spots, this treatment will not be beneficial for 'Mauritius' since fruit sorting is not possible in MAP. However, 'Mauritius' fruits in the stand-alone treatment showed higher BI (~3). In 'McLean's Red', the stand-alone treatment showed a BI of 1 – 2 spots. The effect of chitosan in delaying pericarp browning as reported by Zhang and Quantick (1997) and Jiang *et al*. (2005) in 'Huaizhi' litchi. The integrated treatment of chitosan with MAP showed a synergistic effect on inhibition of pericarp browning in this study. The observed differences in severity of browning (the BI) in 'Mauritius' and 'McLean's Red' in chitosan + MAP or stand-alone MAP revealed that the effectiveness of the treatment is cultivar dependent.

3.2. EFFECT OF CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENT ON GAS COMPOSITION AROUND THE FRUIT

The CO_2 and O_2 gas composition within the packaging was observed after 21 days. Both cultivars showed similar patterns of CO_2 and O_2 levels within the packaging with respect to different treatments adopted in this study (Fig. 3). 'Mauritius' showed slightly higher CO_2 composition within the packaging with respect to all treatments adopted in this study than 'McLean's Red'. This observation indicates that 'Mauritius' showed higher respiration than 'McLean's Red' during storage. No significant differences were observed in the CO_2 or O_2 concentrations between chitosan + MAP and the stand-alone MAP in both cultivars. This finding coincides with the observation of Romanazzi *et al*. (2005) on grapes 'Thompson Seedless' and in 'Autumn

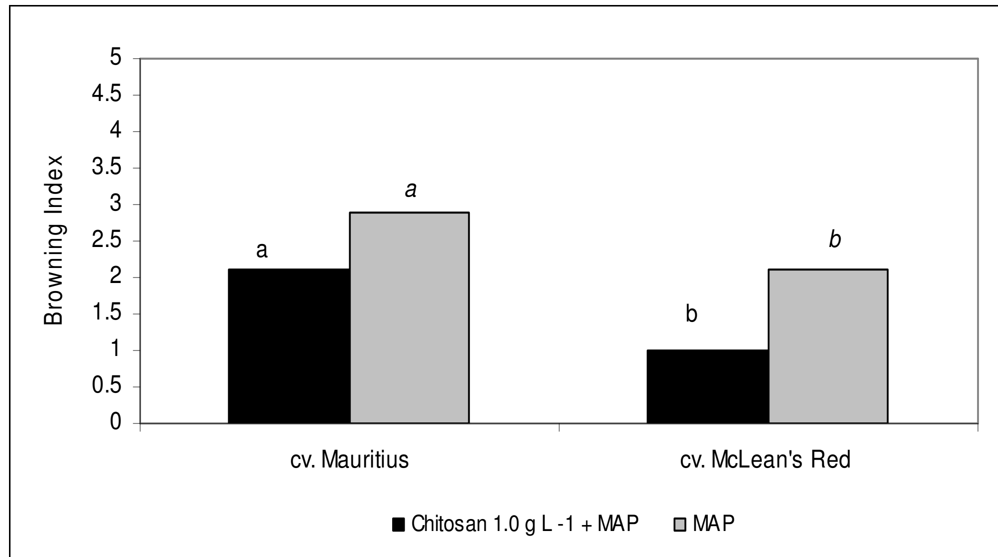

Fig. 1. Effect of integrated treatment with chitosan (1.0 g L^{-1}) + modified atmosphere packaging (MAP) on browning index (BI) of 'Mauritius' and 'McLean's Red' litchi pericarp after 21 days at 2 °C and at 14 °C for 2 days. MAP, modified atmosphere packaging (control). Means in each bar with the same type of letter are not significantly different, P = 0.05. The one-way ANOVA as carried out for each cultivar separately.

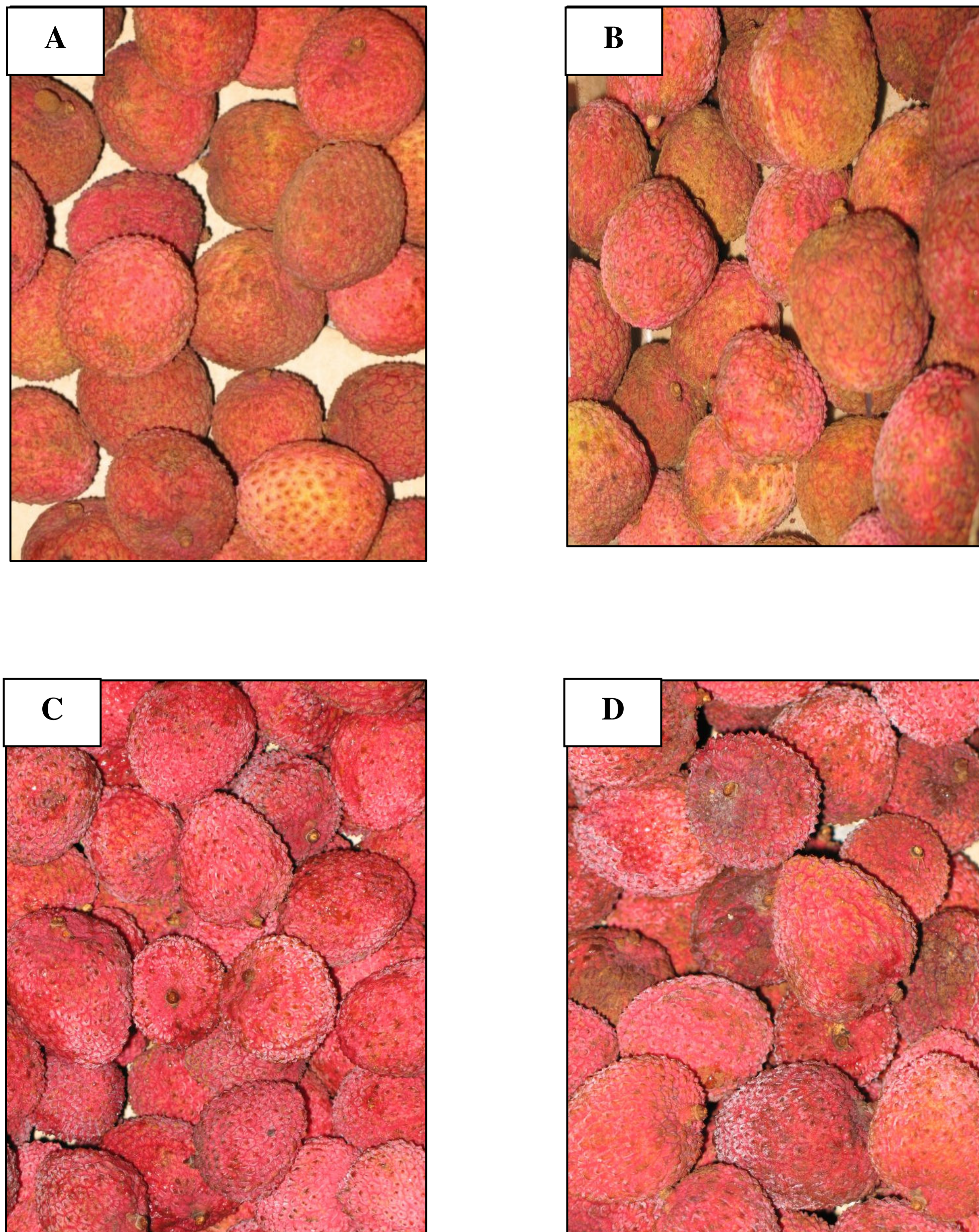

Fig. 2. Effect of integrated treatment with chitosan (1.0 g L^{-1}) + modifed atmosphere packaging (MAP) on skin colour retention in 'Mauritius' and 'McLean's Red' litchi after 21 days at 2 °C and at 14 °C for two days. (A) 'Mauritius' treated with chitosan 1.0 g L-1 and MAP; (B) 'Mauritius' in MAP; (C) 'McLean's Red' treated with chitosan 1.0 g L-1 and MAP; and (D) 'McLean's Red' in MAP.

Seedless' grapes, where a similar trend in reduction in respiration was observed with 5.0 g L^{-1} chitosan coating.

However, chitosan (20 g L^{-1}) + MAP showed higher O_2 composition within the packaging than chitosan (1.0 g L^{-1}) + MAP and stand–alone MAP suggest reduced respiration in chitosan (2 %) + MAP packed fruits (Fig. 3). The observed reduction in respiration in both cultivars coincides with the finding in chitosan coated (20 g L^{-1}) 'Huaizhi' litchi (Jiang *et al*., 2005) and tables grapes (10 g L^{-1} chitosan) (Romanazzi *et al*., 2005). Furthermore, application of chitosan coating inhibited respiration rates of peaches, Japanese pear and kiwifruit (Du *et al*., 1997). Chitosan coating on both cultivars have reduced the fruit respiration in MAP probably by modifying the endogenous CO_2 and O_2 levels due to its semi permeable filmogenic property, which as reported on litchi (Sivakumar *et al*., 2005). Modification of endogenous CO_2 and O_2 levels were shown in chitosan coated papaya (Sivakumar *et al*., 2005).

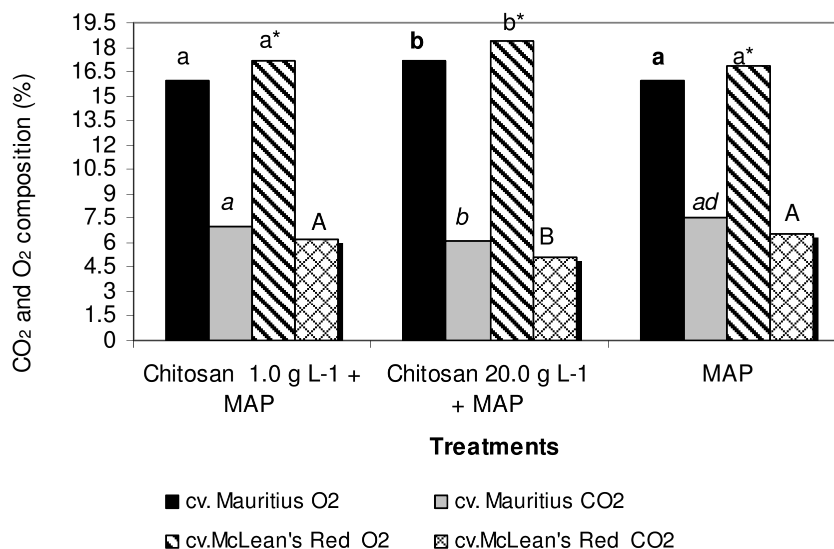

Fig. 3. Effect of integrated treatment with chitosan (1.0 g L-1) + modifed atmosphere packaging on gas composition around 'Mauritius' and 'McLean's Red' litchi fruit in the packages after 21 days at 2 ºC. MAP, modified atmosphere packaging (control). Means in each bar with the same type of letter are not significantly different, P = 0.05. The one way ANOVA as carried out for each cultivar separately.

3.3. EFFECT OF CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENT ON PERICARP COLOUR AND ANTHOCYANIN CONTENT

Changes in colour parameters during storage were cultivar dependent. Freshly harvested 'McLean's Red' showed higher L^*, a and b values than 'Mauritius' (freshly harvested 'Mauritius', L^* = 38.2 – 40.12, a* = 28.42 – 27.03, b* = 26.3 – 27.6; 'McLean's Red', L^* = 41.23 – 42.3, a* = 33.89 – 30.34, b* = 27.5 – 28.24).

A significantly lower (P<0.001) L* value was noted after 21 days cold storage and simulated market shelf condition for 2 days in both cultivars with respect to stand-alone MAP treatment (Fig. 4A). However, chitosan + MAP prevented the decline of L* value (brighter) in both cultivars. The colour value a* directly relates to the red colour of the pericarp and b* expresses the colour variation due to BI (a lower b* indicates higher browning). Both cultivars treated with chitosan + MAP showed higher a* and b* value after simulated market shelf condition (Fig. 4 B and C). However, the effect of chitosan + MAP on retention of a* and b* value was higher in 'McLean's Red'. In both cultivars, stand-alone MAP showed a decline in a*, b* values with increasing BI after 21 days cold storage. The effect of chitosan treatment in MAP on retention of red colour in the pericarp during storage depended on a* value at harvest, therefore, the efficacy of chitosan treatment was higher in 'McLean's Red' since the a* value was higher at harvest.

The anthocyanin content was observed to decline during storage in both cultivars subjected to chitosan + MAP and stand-alone MAP, when compared to the freshly harvested fruits (freshly harvested 'Mauritius' = 1.85 – 2.05 ΔA/g FW^{-1}, McLean's Red = 3.12 – 3.50 ΔA/g FW^{-1}). However, chitosan + MAP retained higher anthocyanin content than stand-alone MAP in both cultivars (Fig. 5). The 'McLean's Red' showed higher retention of anthocyanin content after 21 days storage than 'Mauritius'. The observed difference in anthocyanin content between the two types of cultivars was observed at freshly harvested stage (data not presented). Although the two types of cultivars were grown under similar conditions in the same orchard, the observed difference in anthocyanin concentration could be due to the differences in genetic makeup involved in controlling anthocyanin levels (Matthew *et al*., 2005). According to Underhill and Critchley (1994), the expression of red colour of anthocyanin is pH depended and the colour value a* can be correlated to anthocyanin content and BI. However, previous studies with acetic acid treatments and MAP failed to retain the characteristic red litchi colour during storage, which suggests the red colour retention observed here is attributed to the chitosan acetic acid dip treatment (Sivakumar, personal communication). Ducamp-Collin *et al*. (2008) reported that chitosan citric acid combination showed higher anthocyanin concentration and increase in a* value in storage in litchi 'Kwai may' and 'Wai chee'. In addition, the MAP retained ~80-90% RH during storage and market shelf (simulated), respectively, would have further prevented the desiccation related pH change in the pericarp. The chitosan + MAP integrated treatment showed a synergistic effect on anthocyanin content and colour retention. According to this study in terms of retention of anthocyanin content and colour retention 'McLean's Red' is more suited for chitosan + MAP integrated treatment than 'Mauritius' (Fig. 2).

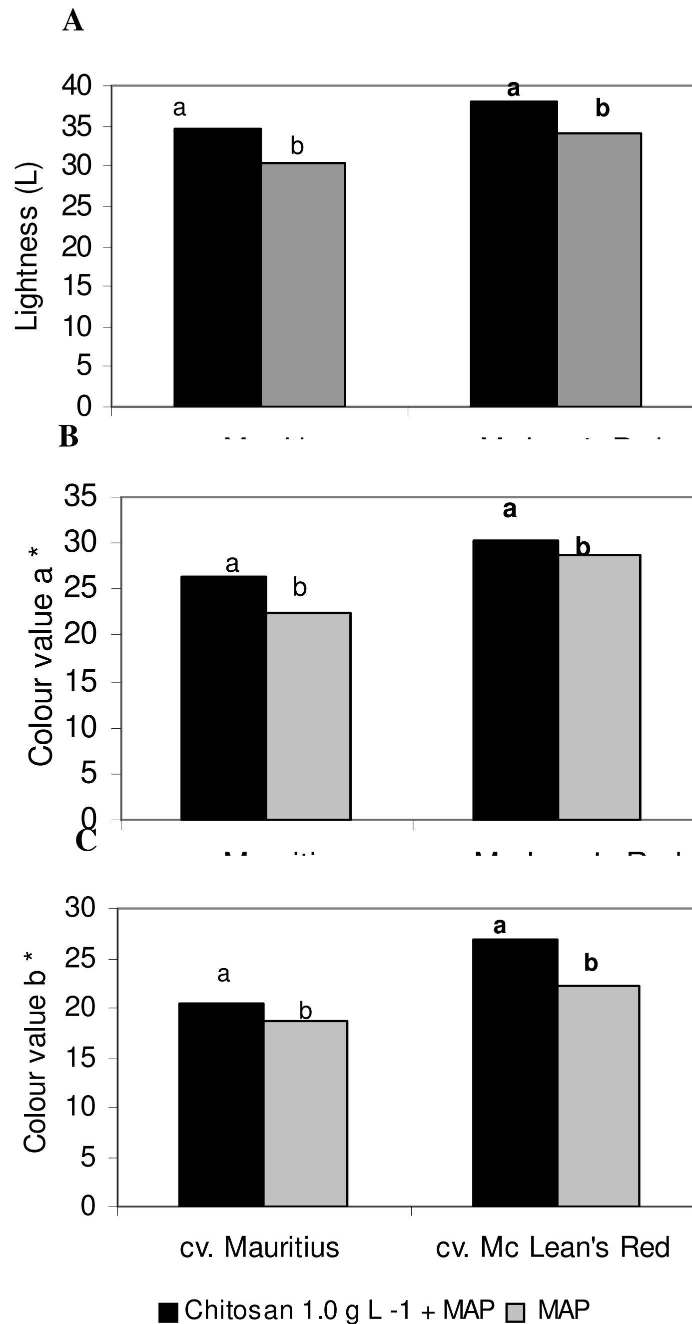

Fig. 4. Effect of integrated treatment with chitosan (1.0 g L^{-1}) + modifed atmosphere packaging. (A) Lightness, *L**; (B) colour value *a**; (C) value *b** in 'Mauritius' and 'McLean's Red' litchi pericarp after 21 days at 2 °C and at 14 ºC for two days. MAP, modified atmosphere packaging (control). Means in each bar with the same type of letter are not significantly different P = 0.05. The one-way ANOVA was carried out for each cultivar separately.

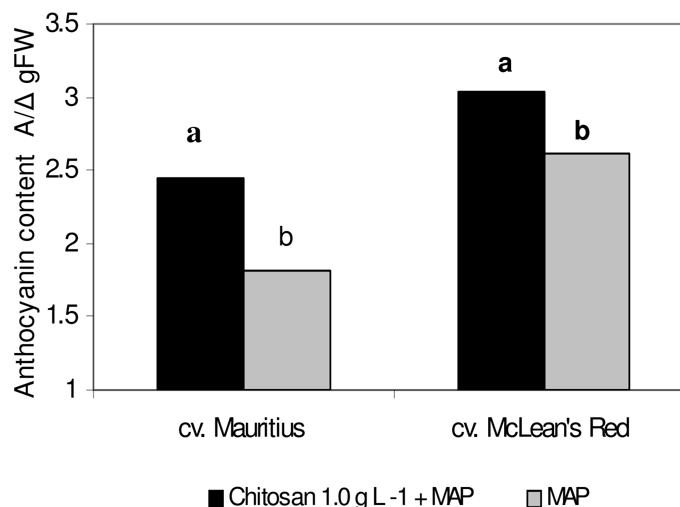

Fig. 5. Effect of integrated treatment with chitosan (1.0 g L^{-1}) + modifed atmosphere packaging on anthocyanin content in 'Mauritius' and 'McLean's Red' litchi pericarp after 21 days at 2 °C and at 14 °C for two days. MAP, modified atmosphere packaging (control). Means in each bar with the same type of letter are not significantly different P = 0.05. The one-way ANOVA was carried out for each cultivar separately.

3.4. EFFECT OF CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENT ON OXIDATION ENZYME ACTIVITY AND RELATIVE MEMBRANE LEAKAGE

The PPO activity was observed to be higher in 'Mauritius' than 'McLean's Red'. The PPO activity in both cultivars was lower than the POD activity (Fig. 6A) which confirms the observations of Zauberman *et al.* (1991), Zhang and Quantick (1997) and Ducamp-Collin *et al.* (2008). The loss of anthocyanin content and the BI appeared to increase with the PPO and POD activity in 'Mauritius'. Zhang and Quantick (1997) reported that chitosan coating inhibited the PPO and POD activity and retained the anthocyanin content in litchi ('Huaizhi') pericarp up to 14 to 15 days and the oxidative enzymes activity increased after 21 days in storage. However, integrated treatment, chitosan + MAP significantly decreased the PPO and POD activity in both cultivars compared to stand-alone MAP. The inhibitory effect of chitosan + MAP treatment on the PPO and POD activity was higher in 'McLean's Red' than in 'Mauritius'.

The integrity of membrane systems can be expressed as relative leakage rate (Jiang and Fu, 1999). Fruit subjected to chitosan + MAP treatment showed lower relative leakage rate than stand-alone MAP. The integrity of membrane system was higher in 'McLean's Red' than 'Mauritius' as shown by the higher leakage rate in 'Mauritius' in stand-alone MAP (Fig. 6B). The MAP maintains higher RH (~ 90%) around the fruit and prevented pericarp desiccation when compared to the fruits stored without MAP in previous studies

(Sivakumar and Korsten, 2006). However, under long-term storage conditions, loss of integrity of the membrane was reported as a result of pericarp senescence (Duan *et al*., 2004) and shown in stand-alone MAP treatment. According to Qu et al. (2006), the loss of integrity of cell membrane is known as a result of "malfunction of membrane lipid biosynthesis". Membrane repair needs ATP and as a result of ATP shortage and malfunction of the membrane system, ion leakage and cellular decompartmentalisation occurs (Qu *et al*., 2006) and consequently, the browning reactions take place when the anthocyanins come into contact with the oxidizing enzymes PPO and POD. According to the observations on membrane integrity, it could be suggested that 'McLean's Red' has a more resistant cellular membrane system than does 'Mauritius'. The chitosan application in MAP helped to reduce the leakage rate by preventing the loss of membrane integrity in 'McLean's Red'.

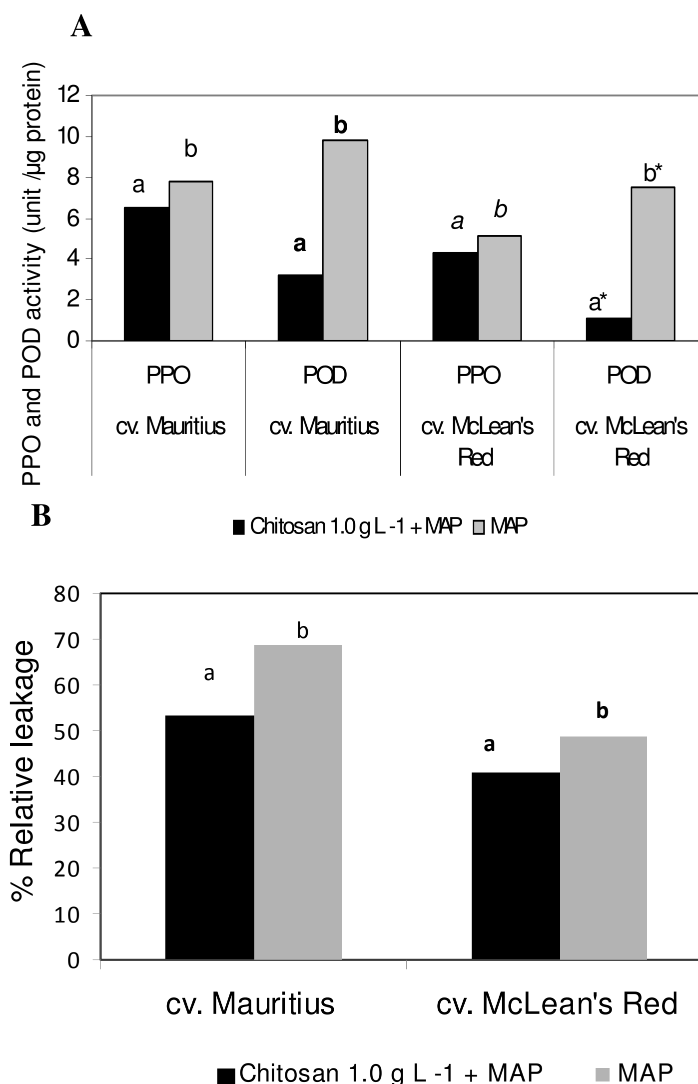

Fig. 6. Effect of integrated treatment with chitosan (1.0 g L^{-1}) + modifed atmosphere packaging on (A) PPO and POD activity; and (B) relative leakage in 'Mauritius' and 'McLean's Red' litchi pericarp after 21 days at 2 °C and at 14 °C for two days. MAP, modified atmosphere packaging (control). Means in each bar with the same type of letter are not significantly different P = 0.05. The one-way ANOVA was carried out for each cultivar separately.

3.5. EFFECT OF CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENT ON SSC, TA AND EATING QUALITY

The chitosan (20 g L^{-1}) + MAP and stand-alone MAP minimized the increase in SSC/TA by keeping the SSC unchanged by reducing the respiration in both TA cultivars (data not presented). Similar observations were reported by Jiang *et al*. (2005) on chitosan-coated 'Huaizhi' litchi. A slight decline in SSC/TA ('Mauritius' 57.4, and 'McLean's Red' 50.5) was observed in chitosan (1.0 g L^{-1}) + MAP in both cultivars after simulated market-shelf condition when compared to freshly harvested fruits. The SSC/TA was slightly higher at harvest in 'Mauritius' (~60 – 62.6) than 'McLean's Red' (~58.6 – 55.5). The high RH (80-90%) within the MAP helped to reduce the weight loss in all treatments (data not shown). Meanwhile non significant difference in firmness was observed with respect to all treatments. The SSC/TA ratio determines the taste and flavour of the litchi fruits. Both cultivars subjected to chitosan (0.1%) + MAP treatment showed acceptable taste and flavour (data not presented).

The integrated application of reduced concentration of chitosan and MAP proposed in this study can overcome problems associated with high viscosity of chitosan at higher concentration (20 g L^{-1}). The 1.0 g L^{-1} chitosan used in this study showed 8.49 cP viscosity (Romanazzi *et al*., 2007). Our previous study with potassium metabisulphite and 1.0 g L^{-1} chitosan without MAP during 21 days storage showed a loss of *a** value, anthocyanin content and eating quality in the 'Mauritius' (Sivakumar *et al*., 2005). The chitosan + MAP helped to improve the quality retention of 'Mauritius' to an acceptable level of marketability. However, chitosan + MAP treatment can be applied for 'Mauritius' for domestic marketing chains that take 14-16 days (unpublished data). The cultivar-dependent response for integrated treatment with chitosan + MAP was clearly stated in this study. The response to chitosan treatment was reported by Ducamp-Collin *et al*. (2008) and 'Kwai May' was better suited to chitosan citric acid treatment than 'Wai chee', which shows that different cultivars respond differently to the chitosan dip treatment. Chitosan solution was prepared in different acids, citric acid, oxalic acid and tartaric acid. Acetic acid was used to prepare the solution in this study, since it is the best acid to activate chitosan antimicrobial and eliciting properties to prevent decay (Romanazzi *et al*., 2005). The integrated application of chitosan at lower concentration with MAP added value to the findings of Zhang and Quantick (1997), Jiang *et al*. (2005) and Ducamp-Collin *et al*. (2008) for commercial application.

4. CONCLUSION

Our observations demonstrated the potential of the integrated treatment of chitosan and MAP for commercial treatment to replace SO_2 fumigation during export for 'McLean's Red'. The follow-up study carried out clearly demonstrated that the storage life of 'McLean's Red' can be extended over 21 days with the chitosan + MAP treatment, without affecting the eating quality. However a semi-commercial trial shipment has to be carried out to the export destinations in the EU to access the viability of this integrated treatment under commercial transport and handling conditions. The treatment was applied on early seasonal fruits in this study and future research will be focused on late seasonal fruits to confirm the integrated treatment of chitosan + MAP on 'McLean's Red'.

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CHAPTER 5

INTEGRATED APPLICATION OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING TO IMPROVE QUALITY RETENTION OF LITCHI CULTIVARS DURING STORAGE

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ABSTRACT

The effect of 1-methylcyclopropene (1-MCP) application on overall quality retention of 'Mauritius' and 'McLean's Red' litchi under modified atmosphere packaging (MAP) was investigated. Fruit was packed in bioriented polypropylene bags and exposed to different concentrations of 1-MCP (300, 500 and 1000 nL L^{-1}) within the packaging, heat sealed and stored at 2 ºC for 14 and 21 days. Of the three concentrations, 300 nL L^{-1} was most effective in preventing browning and retention of colour in both cultivars after 14 and 21 days of cold storage. The effect of 1-MCP (300 nL L^{-1}) was more promising on 'McLean's Red' than 'Mauritius'. 1-MCP (300 nL L^{-1}) significantly reduced the polyphenol oxidase (PPO) and peroxidase (POD) activity, retained membrane integrity, anthocyanin content and prevented the decline of pericarp colour values, L*, a* and b* during storage. At higher concentrations, 1-MCP showed negative effects on membrane integrity, pericarp browning, PPO and POD activity in both cultivars. 1-MCP (1000 nL L^{-1}) significantly suppressed fruit respiration and retained the SSC/TA and firmness. Thus, application of 1-MCP in combination with the use of MAP can extend the storage life of 'McLean's Red' up to 21 days.

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a popular subtropical fruit of high commercial value and anthocyanin pigment is responsible for the attractive red colouration of the litchi skin. Pericarp browning and decay limit the storage life of litchis. The browning mechanism in litchi is reported as a result of oxidation of anthocyanin by polyphenol oxidase (PPO) (Huang et al., 1990; Jiang, 2000) or peroxidase (POD) (Zhang et al., 2005). Commercially a strong antioxidant, sulphur dioxide (SO_2) fumigation is adopted to block the oxidation reactions involved in browning. Due to the harmful effects caused by the allergic reactions of sulphur residues, the European Community permits a maximum concentration of only 10µg g^{-1} in the edible portion of the fruit (Ducamp-Collin, 2004).

Modified atmosphere packaging (MAP) can be beneficial to maintain high humidity essential for prevention of water loss and browning of the litchi pericarp (Kader, 1994). A modified atmosphere (17% O_2 and 6% CO_2) created in bioriented polypropylene films has enabled the retention of litchi fruit quality during storage by reducing pericarp browning (Sivakumar and Korsten, 2006). In MAP it is essential that there is no disease or pericarp browning of the fruit, since pre-sorting of fruit before sale is not practicable in large-scale marketing chains. Kruger *et al*. (2005) identified the potential of using 1-methylcyclopropene (MCP) treatment in combination with MAP on quality retention of 'Mauritius' litchi. 1-methylcyclopropene is an inhibitor of ethylene, which irreversibly binds to the ethylene –binding protein (Sisler and Serek, 1997), and has been commercially adopted to control ethylene-dependent postharvest disorders. Selvarajah *et al*. (2001) observed that 1-MCP reduces the incidence of internal browning in pineapple stored at 10°C after 4 weeks. The basis for this treatment was that chilling injury is involved with ethylene synthesis, even in non-climacteric fruit (Ben-Amor *et al*., 1999; McCollum and McDonald, 1991). Litchi is considered to be a non-climacteric fruit and a study conducted by Qu *et al*. (2006) showed that activities of phenylalanine ammonia-lyase (PAL), PPO and POD, as well as an higher pericarp browning index were observed when litchi Huaizhi' fruit were treated with ethylene. Qu *et al*. (2006) further reported that the application of 1-MCP at 1mL L^{-1} reduced browning and disease index in 'Huaizhi' fruit stored at 28 – 33 ºC and 95 – 100% RH for 6 days. Thus, the application of 1-MCP on litchi will be beneficial since it reduces enzyme activity of PAL and browning enzymes, thereby lowering the pericarp browning (Watkins, 2006). All these observations support the use of 1-MCP application on litchi quality retention during storage. Kruger *et al*. (2005) used a prochloraz® treatment with 1-MCP and MAP (punnets) to prevent fruit decay during storage. However, the use of chemical fungicides can result in unfavourable effects on public health, the environment and could induce resistance in pathogens. Furthermore, investigations by Kruger *et al*. (2005) did not include changes in litchi fruit physiology due to different concentrations of 1-MCP treatments.

The objective of this study was to investigate the potential of 1-MCP as an integrated treatment with MAP (bioriented polypropylene) to extend the storage life and quality retention for up to 21 days of fruit of two cultivars, 'Mauritius' and 'McLean's Red' grown in South Africa.

2. MATERIALS AND METHODS

2.1. FRUIT TREATMENT AND STORAGE

Early season litchi fruit, 'Mauritius' and 'McLean's Red', were picked at commercial maturity from Geldenhuys plantations in Tzaneen, South Africa. Although the fruiting pattern is different in both cultivars, the trials were conducted during the same growing seasons. After harvest, fruit ere sorted for uniform size, colour stage and absence of mechanical damage. A completely randomized design was used in this experiment. Fruit were divided into four lots each of 500 fruit, and each lot was divided into 10 replicates each containing 50 fruit per designated storage time per cultivar.

Within three hours after harvest, fruit (50) were placed in the bioriented polypropylene bag (MAP; 35 µm thickness; size 40 cm x 18 cm; O_2 permeance 38 x 10^{-14} mol s^{-1} m^{-2} Pa^{-1} at 23 ºC according to the manufacturer's information) (Sivakumar and Korsten, 2006). However, the gas permeability of the MAP depended on the degree of perforation that expresses the total area of the pores as a percentage of the film surface. For each cultivar, a set of 10 replicates (MAP) was given one of four treatments in the MAP: (1) 300 nL L^{-1} 1-MCP, (2) 500 nL L^{-1} 1-MCP, (3) 1000 nL L^{-1} 1-MCP and (4) untreated control. 1-MCP was released into the MAP according to Kruger *et al*. (2005) from 1.5 mL capped eppendorf vials (the caps were perforated with needles, five perforations per cap) containing weighed amounts of SmartFresh™ powder (0.14% active ingredient; Rohm and Hass, South Africa) by adding warm water as droplets (30 ºC). Each vial as vortexed and therefore, the MAP was sealed with a heat sealer (multivac C200, Multivac, Heidelberg, South Africa) to create a passive modified atmosphere around the fruit. The 1-MCP concentration was confirmed by means of gas chromatography, using isobutylene as standard (Jiang *et al*., 2001). Fruit packed in MAP without 1-MCP treatment as included as a control (stand-alone MAP).

At the completion of each designated storage time (14 or 21 days) five replicates were removed from cold storage from each treatment. The effect of all treatments on gas composition around the fruit within the packaging, fruit quality parameters (including decay incidence, browning index (BI), weight loss, fruit firmness, pericarp colour, anthocyanin content, soluble solids concentration (SSC) and titratable acidity (TA), activities of oxidative enzymes PPO and POD, and pericarp relative leakage were determined. Another set of

30 replicates of different 1-MCP concentrations + MAP and stand-alone MAP was packed for each cultivar type as mentioned before. These fruit were stored at low temperature and the gas analysis was carried out from three replicates for 0 – 10 days. Head-space gases CO_2 and O_2 were measured using a PBI Dansensor CO_2/O_2 gas analyser (Checkmate 9900, Ringsted, Denmark) after removal from cold storage.

2.2. MEASUREMENT OF FRUIT QUALITY

2.2.1. *Browning and decay index*

Severity of pericarp browning was assessed visually as: 1 = no browning; 2 = 1 – 2 brown spots; 3 = some spots with browning; 4 = 50 %; 5 = 75 % and entire fruit surface brown (Sivakumar and Korsten, 2006). The browning index (BI) was calculated according to Zhang and Quantick (1997). Severity of postharvest disease was assessed on a scale of 1 – 5, which describes the severity of postharvest fungal decay as: 1 = no disease; 2 = 25 %; 3 = 50 %; and 4 = 75 % of fruit surface affected, and 5 = entire fruit decayed due to disease (Sivakumar and Korsten, 2006).

2.2.2. *Fruit firmness*

The fruit subjected to all treatments mentioned above were weighed before and after 14 and 21 days storage and data expressed as percentage weight loss. Fruit firmness as measured on opposite sides of each fruit (20 fruits per replicate per treatment) by a hand-held firmness tester (Bareiss Prüfgerätebau GmbH, DKD-Kalibrierlaboratorium, Germany) (Sivakumar and Korsten, 2006). (A different firmness tester was used due to technical reasons (Chatillon and Sons, New York, USA)). Fruit firmness was measured in Newton (N) units.

2.2.3. *Colour*

Fruit pericarp colour was measured (20 fruits per replicate per treatment) using a Minolta Chromameter (model CR-300; Minolta, Osaka, Japan), expressing the CIELAB, Commission international de I'Eclairage (CIE) colour space, L*, a* and b*. Although L C h was used in the previous investigation in chapter 1, the colour coordinate a was selected here because the colour value *a** directly relates to the red colour of the pericarp according to Ducamp-Collin *et al*. (2008). Two spots on opposite sides of the fruit ere measured and the mean of the two measurements considered as one reading.

2.2.4. *Soluble solid concentration and titratable acidity*

A set of 20 fruits per replicate per treatment was randomly selected for SSC determination, using a digital refractometer (Atago Co., Tokyo, Japan) and expressed in percentages (Sivakumar and Korsten, 2006). The TA was determined by titrating 10 mL of fruit juice with 0.01 M NaOH. The result was calculated as a citric acid equivalent from 20 g aril obtained from 15 to 20 fruit per replicate per treatment (Sivakumar and Korsten, 2006).

2.3. MEASUREMENT OF BIOCHEMICAL PARAMETERS

2.3.1. *Anthocyanin*

Anthocyanin content was determined from pericarp (10 g) peeled from 20 fruits. Pericarp tissue was quickly sliced and extracted with 15 mL HCl-methanol (0.15% HCl: 95% methanol, 15:85) for 4 hours. The extract was filtered and its absorbance determined at 530, 620 and 650 nm, respectively. The anthocyanin content measurement was based on the formula: $\Delta A/ \text{gFW} = (A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$ using a spectrophotometer (Carl Zeiss, Jena, Germany) (Zheng and Tian, 2005).

2.3.2. *Measurement of oxidative enzymes activity and relative leakage*

Pericarp tissues (10 g) from 20 fruits per treatment per replicate were homogenised in 20 mL of 0.05 M potassium phosphate buffer (pH 6.8) and 0.6 g of polyvinylpyrrolidone (Sigma) at 4 ºC. After filtration of the homogenate through a cotton cloth, the filtrate was then centrifuged for 20 min at 19,000 x *g* and 4 ºC. The supernatant was then collected as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate according to the method of Jiang (2000) at 410 nm. POD activity was assayed according to Zhang *et al*. (2005) in a reaction mixture of 3 mL containing 25 µL of enzyme extract, 2 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of guaiacol. The increase in the absorbance at 470 nm, due to guaiacol oxidation, was recorded for 2 min. Protein content was determined according to Bradford (1976). One unit of enzyme activity was defined as an increase in absorbance unit per minute at 25 ºC. There were three replicates per treatment.

Pericarp from 20 fruit per replicate per treatment and a set of 30 peel discs were cut using a 10 mm cork borer from the equatorial region of the fruit pericarp. The pericarp peel discs were prepared and the conductivity was measured using a conductivity meter (H176300 EC214; Hanna Instruments, Johannesburg, South Africa) according to Lichter *et al*. (2000).

2.4. STATISTICAL ANALYSIS

The experiment was repeated twice and the data of each cultivar analysed separately using a bifactorial model (time of storage x kind of treatment with respect to 1-MCP concentration) ANOVA. The mean values of the significant interactions were compared by Fisher's projected *t*-test L.S.D. (least significant difference) at the 1% level using the statistical program GenStat (2005). Pearson's correlation coefficients were calculated to determine the strength of the linear relationship between browning index, PPO, POD activity, anthocyanin content, Hunter colour values and the 1-MCP concentrations separately per cultivar and per day of storage.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENTS ON GAS COMPOSITION AROUND THE FRUIT

Both cultivars showed similar patterns of CO_2 and O_2 levels within the packaging with respect to different treatments (Fig. 1). The equilibrium-modified atmosphere (steady state) was attained within the packaging after 5 days in 'Mauritius' and 3 days in 'McLean's Red'. 'Mauritius' showed slightly higher CO_2 concentrations within the packaging than 'McLean's Red', indicating that 'Mauritius' has a higher rate of respiration. Fruit treated with 1-MCP at higher concentrations (500 or 1000 nL L^{-1}) showed higher O_2 compositions within the packaging. Most climacteric fruit display decreased respiration rates upon application of 1-MCP, although higher respiration rates have been detected in 1-MCP treated ripe figs (Sozzi *et al*., 2005). During the steady state, litchi respiration (O_2 consumption and CO_2 production) was balanced by O_2 and CO_2 diffusion through the film: the O_2 and CO_2 concentrations reached values of ~18% and ~4% ('McLean's Red'), and ~17% and ~5% ('Mauritius'), respectively in 1000 nL L^{-1} 1-MCP + MAP.

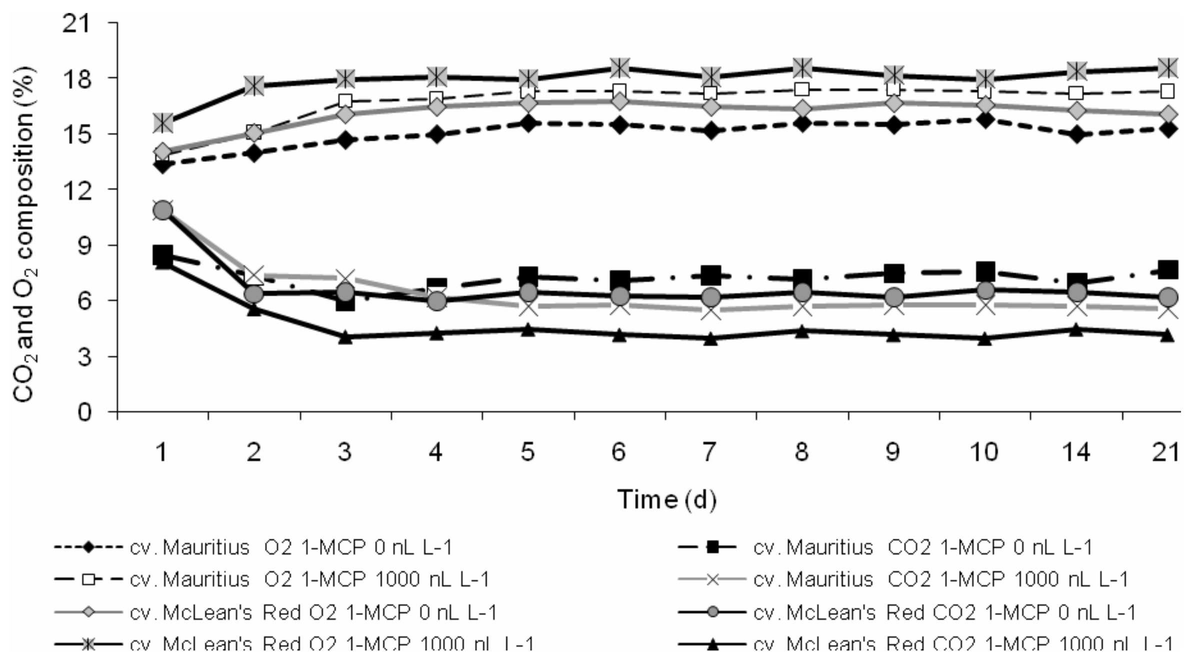

Fig. 1. Effect of 1-MCP on gas composition around (A) 'Mauritius' and (B) 'McLean's Red' litchi fruit at 2 °C. Values represent the means of gas measurements in three replicate bags. Means within 95% confidence interval.

3.2. EFFECT OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENTS ON INCIDENCE OF BROWNING AND DECAY

It is evident from this study that integrated treatments of 1-MCP (300 nL L^{-1}) + MAP revealed an absence of pericarp browning in 'McLean's Red' after 14 and 21 days storage at 2ºC (Fig. 2). However, pericarp browning was not observed in 'Mauritius' in 1-MCP (300 nL L^{-1}) + MAP or stand-alone MAP after 14 days. Pericarp browning increased with increasing concentration of 1-MCP in 'Mauritius' in MAP after 14 days. Although BI was observed to increase in fruit in the integrated treatment with higher 1-MCP concentration in both cultivars after 21 days, 'Mauritius' showed higher pericarp BI than 'McLean's Red'. In 'Mauritius' the pericarp browning was expressed as more yellowish brown. In both cultivars, the stand-alone MAP showed significantly ($P < 0.001$) reduced BI than 1-MCP (500 or 1000 nL L^{-1}) + MAP integrated treatment after 21 days' cold storage.

Both cultivars had disease free fruit after 14 and 21 days' cold storage in 1-MCP (300 and 500 and 1000 nL L^{-1}) + MAP and stand-alone MAP. It should be noted that 'Mauritius' packed in stand-alone MAP at market-shelf conditions (14ºC) during experiments in 2004 did not show decay (Sivakumar and Korsten, 2006). However, during experiments conducted in 2005, 'McLean's Red' in stand-alone MAP revealed ~11.5% decay incidence in market-shelf conditions (Sivakumar *et al*., 2007). Although stand-alone MAP can reduce decay incidence during simulated marketing conditions, a 'protectant' is needed to protect the fruit from decay during temperature changes from 2 - 14 °C. Different integrated treatments have been investigated with anti-browning and biocontrol agents to protect fruit from decay in MAP (Sivakumar *et al*., 2008), and these treatments were effective for domestic marketing chains up to 18 days. Furthermore, mixed observations were reported on the association of 1-MCP and fruit decay. Absence of decay was shown in 1-MCP treated plums (Valero *et al*., 2003), but 1-MCP application increased decay incidence in strawberry (Jiang *et al*., 2001). However, according to Ku and Wills (1999), the induction of decay in strawberry was dosage dependent and the higher concentrations of 1-MCP (500 and 1000 nL L^{-1}) induced decay incidence by inhibiting the beneficial metabolic pathway by lowering phenolic compounds that contributed to the natural defence mechanisms. Meanwhile, increasing the concentration of 1-MCP to 500 and 1000 nL L^{-1} + MAP resulted in progressive decay incidence with a severity score '2' when the storage life was extended to 30 days in 'Mauritius' (data not shown). This might be attributed to the inhibition of defence mechanisms mediated by phenylalanine ammonia-lyase enzyme (PAL) activity. Furthermore, Qu *et al*. (2006) reported the inhibition of PAL enzyme activity in 'Huaizhi' with 1 mL L^{-1} 1-MCP application. It is evident from the reports of Qu *et al*. (2006) that the disease index was low when PAL enzyme activity was high. However, further investigation is needed with respect to different concentrations of 1-MCP treatments and PAL enzyme activity.

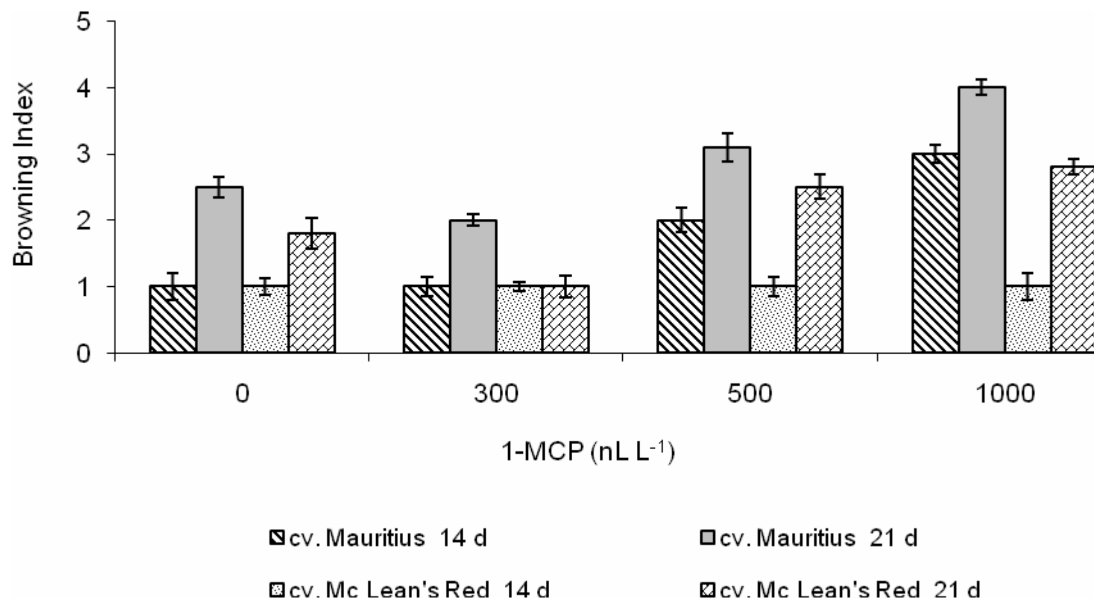

Fig. 2. Effect of 1-MCP + modifed atmosphere packaging on browning index of 'Mauritius' and 'McLean's Red' litchi pericarp at 2 °C. Values represent the means of five replicate bags each containing 50 fruit, and vertical bars indicate standard deviation of the means.

3.3. EFFECTS OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENTS ON WEIGHT LOSS, FIRMNESS AND SSC/TA

The high RH (85 – 90%) within the MAP enabled the reduction of weight loss in all treatments (data not shown). Integrated treatments with 1-MCP (500 or 1000 nL L^{-1}) + MAP showed significantly ($P < 0.001$) higher firmness than 1-MCP (300 nL L^{-1}) + MAP and stand-alone MAP after 21 days' storage in both cultivars (Fig. 3A). Although litchi is a non-climacteric fruit and ethylene does not play a major role in postharvest fruit ripening and fruit softening, 1-MCP (500 or 1000 nL L^{-1}) reduced the loss of firmness after 21 days' storage in both cultivars. Fruit from the 1-MCP (300 nL L^{-1}) and stand-alone MAP showed acceptable firmness. The fruit firmness observed in fruit subjected to 1-MCP (300 nL L^{-1}) + MAP treatment was higher than that in SO_2 fumigated fruit (Sivakumar and Korsten, 2006). Reduction in loss of firmness with 1-MCP treatment has been shown in guava (Bassetto *et al*., 2005), tomato (Guillen *et al*., 2006), and other climacteric fruit.

Non-significant variation in SSC/TA was observed with respect to all treatments in both cultivars stored up to 14 days. However, after 21 days' storage the SSC/TA significantly ($P < 0.001$) increased. This effect was higher in 'Mauritius' than in 'McLean's Red' (Fig. 3B). Slightly higher SSC/TA in ' Mauritius' than in

'McLean's Red' at harvest explains the difference observed after 21 days with respect to all treatments. In both cultivars, 1-MCP (500 or 1000 nL L^{-1}) treatments prevented the increase in SSC/TA after 21 days. The 1-MCP (500 and 1000 nL L^{-1}) reduced respiration, resulting in a decline of SSC/TA by keeping the SSC unchanged. However, 1-MCP (300 nL L^{-1}) retained the SCC/TA better than the stand-alone MAP as shown in Fig. 3B. It was reported in our previous studies that the SSC increased up to 19 – 20% and TA increased to 0.8 – 1% in SO_2 fumigated fruit, resulting in a lower SSC/TA ratio (Sivakumar and Korsten, 2006). The SSC/TA ratio indicates litchi taste and flavour: fruit subjected to 1-MCP (300 nL L^{-1}) +MAP treatment retained their good taste and flavour (data not presented).

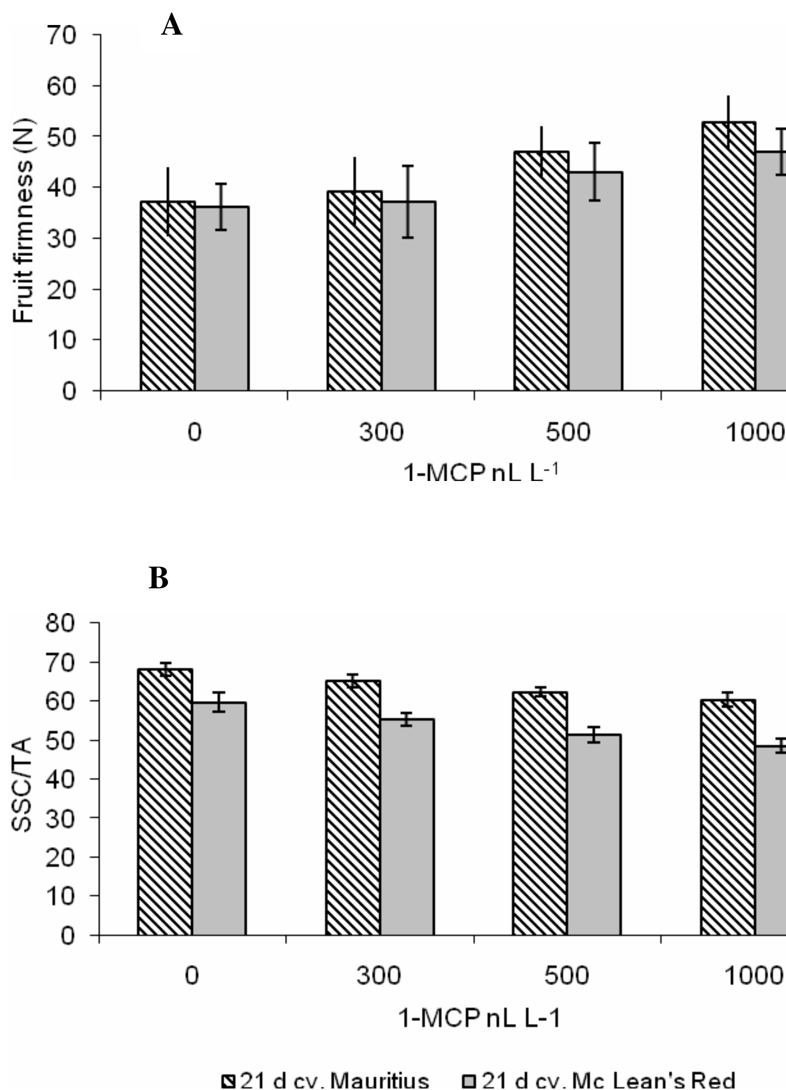

Fig. 3. Effect of 1-MCP + modifed atmosphere packaging on (A) firmness and (B) SSC/TA in 'Mauritius and 'McLean's Red' litchi fruit at 2 ºC. Values represent the means of five replicate bags [from 20 fruit (pericarp) per replicate per treatment] and vertical bars indicate standard deviation of the means.

3.4. EFFECT OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENTS ON PERICARP COLOUR AND ANTHOCYANIN CONTENT

Changes in colour parameters during storage were cultivar dependent. The freshly harvested 'McLean's Red' showed higher L*, a* and b* values than 'Mauritius' fruit (Table 1). The L* value showed significant differences for both cultivars ($P < 0.001$) after 14 days of cold storage with respect to different treatments; the L* value also decreased with time of storage (data not shown). After 21 days' cold storage, 'Mauritius' and 'McLean's Red' showed a significant ($P < 0.001$) decline in L* (darker fruit) in 1-MCP (500 or 1000 nL L^{-1}) + MAP or stand-alone MAP. However, the 1-MCP (300 nL L^{-1}) + MAP showed significantly ($P < 0.001$) higher L* (brighter) in both cultivars. A significant ($P < 0.001$) decrease in colour a* and b* was observed in both cultivars after 14 days' storage in 1-MCP (500 or 1000 nL L^{-1}) + MAP. Both cultivars in 1-MCP (300 nL L^{-1}) + MAP showed higher a* and b* values after 14 days. Although changes in a* and b* were observed in 'McLean's Red' after 14 days' storage, these changes were not observed visually. During long-term storage (21 days) both cultivars showed a decline in a* and b* values. Both cultivars showed a significant ($P < 0.001$) decline in a* and b* and higher BI after 21 days' cold storage in 1-MCP (500 or 1000 nL L^{-1}) + MAP. Changes in a* values with respect to integrated treatments and storage time are given for both cultivars in Fig. 4A, because the a* value was considered to represent the red colour of the pericarp (Ducamp-Collin *et al*., 2008). The magnitude of 1-MCP on colour loss during storage depended on the a* value at harvest, therefore, the efficacy of 1-MCP at higher concentrations was lower in 'McLean's Red' since the colour values were higher at harvest (Table 1). The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for a*, b* and L* values in both cultivars. Colour changes with respect to a* and b* were reduced after 21 days in both cultivars with 1-MCP (300 nL L^{-1}) + MAP. This observation supports the findings of Guillen *et al*. (2006) on tomatoes, where the lower 1-MCP concentration (0.5 μL L^{-1}) reduced the colour changes with respect to a* during storage. However, different observations were reported with respect to colour changes and 1-MCP treatments. In sweet cherries, 1-MCP did not influence postharvest colour changes or stem browning (Gong *et al*., 2002). In, apricots (climacteric fruit), 1-MCP treated fruit were greener and exhibited less colour change than untreated controls (Fan *et al*., 2000). A similar observation was reported in peaches by Kluge and Jacomino (2002). Furthermore, 1-MCP treatment did not affect the colour changes in apricot and plums (Dong *et al*., 2002).

The anthocyanin content was reduced during storage for both cultivars (Fig. 4B). However, 'McLean's Red' showed higher anthocyanin content after 14 and 21 days' storage than 'Mauritius'. The difference between the cultivars was observed at the freshly harvested stage (Table 1). Although both cultivars were grown under similar conditions in the same orchard, the difference in anthocyanin concentration could be due to the genetic control anthocyanin levels (Matthew *et al*., 2005). The anthocyanin content declined with increasing

BI in both cultivars. This decline was higher in 'Mauritius' after 14 and 21 days, and in 'McLean's Red' after 21 days. The 1-MCP treatment at 300 nL L^{-1} significantly ($P < 0.001$) reduced the loss of anthocyanin content in the pericarp of both cultivars during storage. However, a significant ($P < 0.001$) decrease in anthocyanin content was observed in both cultivars in the integrated treatments with higher concentrations of 1-MCP (500 or 1000 nL L^{-1}). The anthocyanin content in the integrated treatments with 1-MCP at higher concentrations was lower than in the stand-alone MAP. The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for anthocyanin content in both cultivars. The strawberry fruit, the anthocyanin content usually increases during storage (3 days at 20 ºC). However, the application of 1-MCP 1000 nL L^{-1} can reduce the increase in anthocyanin content (Jiang *et al*., 2001). This finding supports the observed negative effect of 1-MCP at higher concentrations on anthocyanin content in both litchi cultivars during storage. The inhibitory effect of 1-MCP at 1000 nL L^{-1} could be due to a lower PAL enzyme activity (Jiang *et al*., 2001), a key enzyme in the biosynthesis of phenolics (Cheng and Breen, 1991). Furthermore, Qu *et al*. (2006) observed an increase in BI while the PAL enzyme activity declined in 'Huaizhi' litchi stored at 28 – 33 ºC. Therefore, the reduction in anthocyanin content in 'Mauritius' and 'McLean's Red' fruit could be due to a reduction in PAL enzyme activity. However, further investigations on PAL enzyme activity in both cultivars with respect to different concentrations of 1-MCP would be beneficial to explain the retention of anthocyanin content at 300 nL L^{-1} 1-MCP application.

Table 1. Fruit properties at harvest for the two South African litchi cultivars in this study.

Parameters	Mauritius	McLean's Red
Weight (g)	23.8	20.2
Colour L^*	38.2-40.12	41.23-42.3
Colour a^*	28.42	33.89
Colour b^*	26.3-27.6	27.5-28.24
Fruit firmness (Kg)	5.0-5.8	3.7-3.8
SSC /TA	60-55.6	50.68-47.5
Anthocyanin content (ΔA/g FW $^{-1}$)	1.85-2.25	3.12-4.5

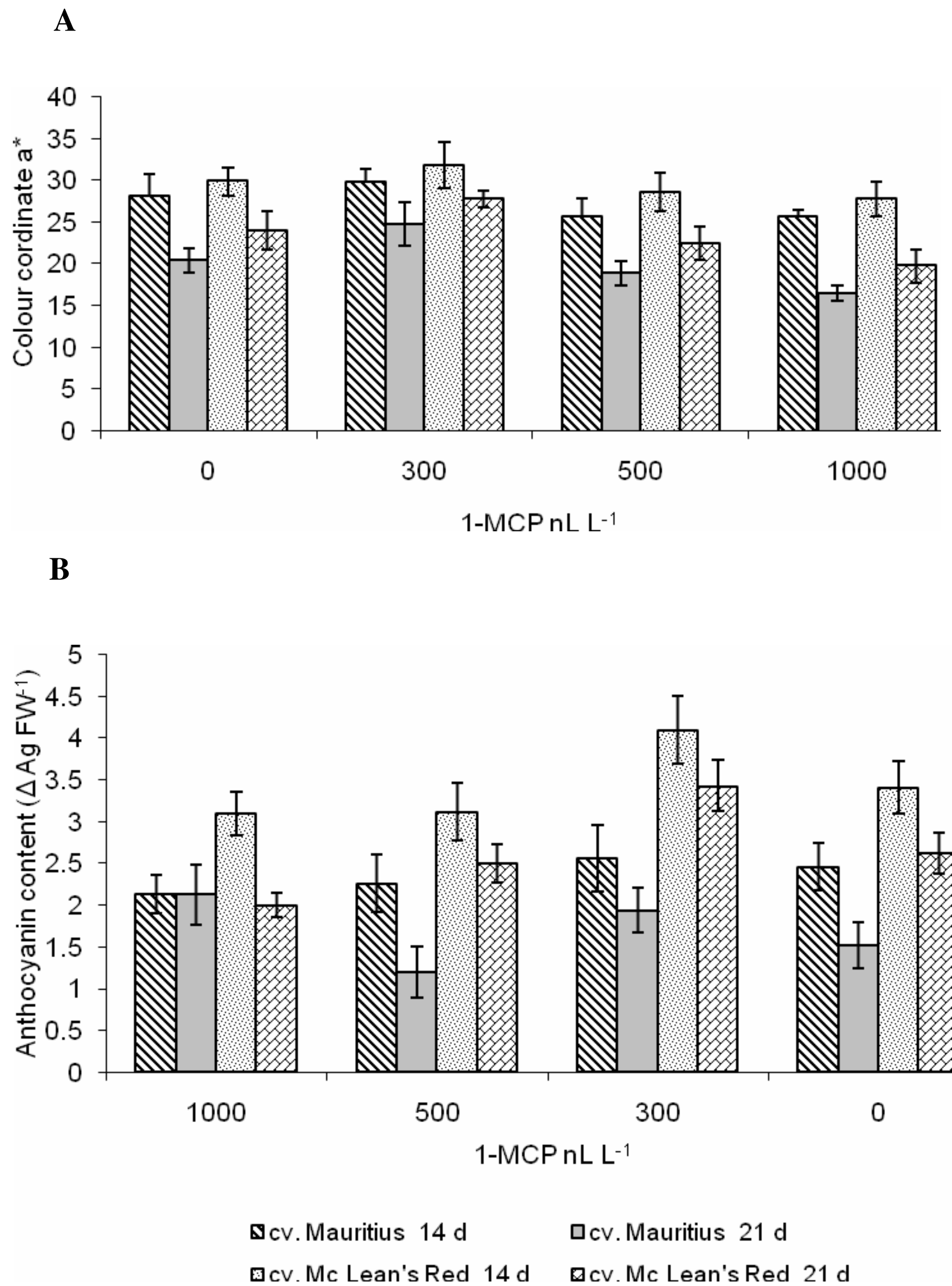

Fig. 4. Effect of 1-MCP + modifed atmosphere packaging on (A) colour a* coordinate and (B) anthocyanin content of 'Mauritius' and 'McLean's Red' litchi pericarp at 2 ºC. Values represent the means of five replicate bags, a* was measured from 20 fruits in each replicate bag per treatment. Anthocyanin content was measured from five replicate carrier bags [from 20 fruit (pericarp) per replicate per treatment] and vertical bars indicate standard deviation of the means.

3.5. EFFECT OF 1-METHYLCYCLOPROPENE AND MODIFIED ATMOSPHERE PACKAGING INTEGRATED TREATMENTS ON OXIDATION ENZYMES ACTIVITY AND INTEGRITY OF THE PERICARP MEMBRANE SYSTEM

PPO activity increased up to 14 days, and thereafter a decline in PPO activity was observed in both cultivars (Fig. 5A). This observation supports the finding of Tian *et al*. (2002) in an unspecified Chinese cultivar, where PPO activity of the litchi pericarp was shown to be higher before pericarp browning occurred and then decreased rapidly during storage. The PPO activity was ~50% higher in 'Mauritius' than 'McLean's Red' after 14 days' storage in 1-MCP (300 nL L^{-1}) + MAP. The integrated 1-MCP treatments with 500 and 1000 nL L^{-1} 1-MCP showed significant ($P < 0.001$) higher PPO activity in fruit stored up to 14 and 21 days in both cultivars. It is also evident from the findings of Qu *et al*. (2006) that 'Huaizhi' fruit treated with 1 mL L^{-1} 1-MCP, showed higher PPO activity than the untreated control fruit during storage at 28 – 33 º C for 6 days. The PPO activity in both cultivars was lower than the POD activity. This observation supports the findings of Ducamp-Collin *et al*. (2008). The POD activity was low up to 14 days and its activity increased after 21 days (Fig. 5B), showed similar trends to PPO in 1-MCP + MAP integrated treatments. Fruit in 1-MCP (300 nL L^{-1}) + MAP had lower POD activity than other treatments, The interaction of storage and 1-MCP treatment was significant ($P < 0.001$) for PPO and POD activity in both cultivars. Two different biochemical mechanisms contribute to browning: one involves the action of enzymes; the other involves changes in the red pigment molecules.

The integrity of membrane systems can be expressed as relative leakage rate (Jiang and Fu, 1999). The increased relative leakage rate observed with storage time shown in Fig. 5C is due to the senescence of the pericarp. However, fruit subjected to 1-MCP (300 nL L^{-1}) + MAP showed a lower relative leakage rate than other treatments. The effect of 1-MCP (300 nL L^{-1}) + MAP on membrane system integrity were higher in 'McLean's Red' than 'Mauritius' (Fig. 5C). The relative leakage increased in the pericarp in integrated treatments with increasing 1-MCP concentrations. The integrated 1-MCP treatments with 500 and 1000 nL L^{-1} 1-MCP showed a higher relative leakage rate than stand-alone MAP treatment in 'Mauritius' after 14 days storage. After 21 days' storage, 1000 nL L^{-1} 1-MCP showed higher a higher relative leakage rate in both types of cultivars; and 'Mauritius' showed a higher relative leakage rate than 'McLean's Red'. The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for relative leakage in both cultivars. Under long-term storage conditions, loss of membrane integrity was reported as a result of pericarp senescence by Duan *et al*. (2004). The loss of cell membrane integrity is known to be a result of 'malfunction of membrane' due to lack of lipid biosynthesis. According to Qu et al. (2006), the membrane repair will be affected due to shortage of ATP and resulting in ion leakage and cellular decompartmentalisation. Consequently, browning reactions will take place when anthocyanins are exposed to the oxidizing enzymes PPO and POD. According to Qu *et al*. (2006), a stable energy charge

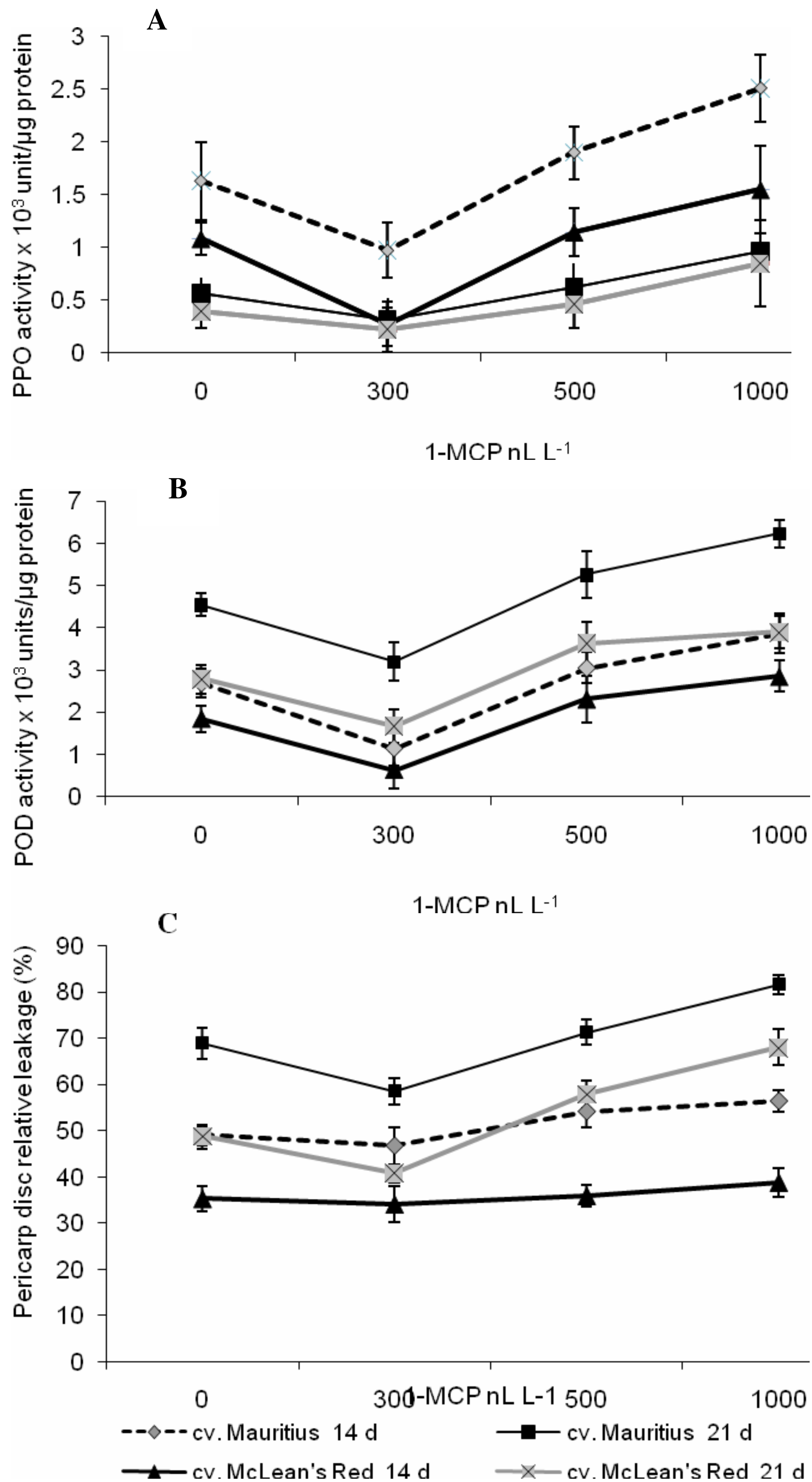

Fig. 5. Effect of 1-MCP + modifed atmosphere packaging on (A) PPO, (B) POD and (C) pericarp relative leakage in 'Mauritius' and 'McLean's Red' litchi pericarp at 2 ºC. Values represent the means of five replicate carrier bags and vertical bars indicate standard deviation of the means.

is essential to maintain normal metabolism in harvested litchi fruit: the application of 1-MCP at 1 mL L^{-1} helped minimize the change in energy charge during storage compared to untreated control fruit. However, this study reveals that 1-MCP is effective at a lower concentration (300 nL L^{-1}) in maintaining membrane integrity. Further studies are needed to determine the ATP:ADP ratio and energy charge during 300 nL L^{-1} 1-MCP application in 'Mauritius' and 'McLean's Red' to conclude its effect on the retention of membrane integrity.

3.6. CORRELATION ANALYSIS

The correlation analysis data obtained for 21 days storage revealed linear relationship between the 1-MCP concentration and BI, relative leakage, PPO and POD activity for both cultivars (Table 2). However, negative correlations were observed between 1-MCP concentrations and the colour value L*, a*, b* and anthocyanin content.

Although there is uncertainty about the role of C_2H_4 in pericarp browning of harvested litchi (Pang *et al*., 2001), the BI increased in C_2H_4 treated 'Huaizhi' fruit during storage at 28 – 30 ºC for 6 days. Respiration in the pericarp also increased after a dip in ethephon (50 g L^{-1}) in 'Guiwei' fruit, but this increase was not observed in the aril and did not induce cyanide-insensitive respiration, one of the features of non-climacteric fruit (Lin *et al*., 1990). According to Qu *et al*. (2006), C_2H_4 treated fruit showed lower energy charge while untreated control revealed higher energy charge. However, the 1-MCP treatment reduced the charge in energy charge, maintaining normal metabolism.

4. CONCLUSION

1-Methylcyclopropene at the lower concentration (300 nL L^{-1}) is effective with MAP to prevent pericarp browning and senescence related mechanisms and the loss of fruit quality due to senescence of the aril. The release of 1-MCP through polypropylene film is negligible (Hotchkiss and Watkins, 2007). Although, we assume there is no significant change in 1-MCP concentration within the bioriented polypropylene bag based on Hotchkiss and Watkins (2007), this needs to be investigated in the future. The colour retention and absence of pericarp browning in 'McLean's Red' reveals that this cultivar is better suited for integrated treatments. However, we consider the use of non-uniform coloured, late-seasonal fruit and the time delay between harvesting and packing operations to be the limiting factors for this treatment.

Table 2. Pearson's correlation coefficients between 1-MCP concentrations and Hunter colour values and browning related parameters in 'Mauritius' and 'McLean's Red' at 2 ºC for 14 and 21 days.

Parameter	Mauritius		McLean's Red	
	14 Day	21 Day	14 Day	21 Day
Hunter colour values				
L	-0.501*	-0.654*	-0.597	-0.697*
a	-0.668*	-0.674*	-0.677	-0.702*
b	-0.680*	-0.780*	-0.640	-0.680*
Browning related parameters				
Browning Index	0.757*	0.803*	ns	0.698*
PPO	0.701*	0.772*	0.567	0.739*
POD	0.624*	0.725*	0.634	0.649*
Anthocyanin content	-0.695*	-0.740*	-0.490	-0.627*
Membrane leakage	0.729*	0.727*	0.621	0.657*

*Significant at P < 0.01. ns- non significant.

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CHAPTER 6

EFFECT OF INTEGRATED MODIFIED AMTOPSHERE PACKAGING AND POSTHARVEST TREATMENTS ON LITCHI MICROBIAL QUALITY AND SAFETY

ABSTRACT

This study was conducted to determine the effect of integrated postharvest treatments; 1) modified atmosphere packaging and chitosan and; 2) integrated (MAP and 1-MCP) and (MAP and chitosan coating) on foodborne bacterial pathogens, *Escherichia coli* O157:H7 and *Staphylococcus aureus*, inoculated on the litchi fruit surface, and to reduce decay caused by *Penicillium* spp on litchi fruit. Litchi 'McLean's Red' fruit were treated with: 1) 0.1 g L^{-1} chitosan; 2) 1.0 g L^{-1} chitosan; 3) 300 nL L^{-1} 1-MCP and; 4) 1000 nL L^{-1} 1-MCP and packed into bioriented polypropylene bags. Samples were stored at 2 ºC for 21 days and then at 20 ºC for two days. The total microbial population of the litchi fruit surface (fructoplane) was determined. Results showed integrated MAP and chitosan (0.1 g L^{-1} and 1.0 g L^{-1}) treatments significantly reduced high and low inoculum loads of *E. coli* O157:H7 and *S. aureus* on litchi fruit after 21 days of cold storage. Integrated MAP and 1000 nL L^{-1} 1-MCP had a negative effect on *Penicillium* decay, thereby, promoting disease severity. Integrated MAP and chitosan (0.1 g L^{-1} and 1.0 g L^{-1}) treatments showed effective decay control. The total microbial populations of the litchi fruit surface were also determined. Integrated MAP and 1.0 g L^{-1} significantly reduced the total microbial flora after 21 days of cold storage.

1. INTRODUCTION

Fresh fruit retain a microbial residual population composed mainly of epiphytes that are not of plant or human health significance and are generally considered safe to eat by consumers (Zagory, 1999). However, sporadic contamination of produce with low levels of human pathogens may occur directly or indirectly via animals, soil, water, dirty equipment or human handling (Beuchat, 1996). Good agricultural and management practices common to growing, harvesting, washing, sorting, packing and transporting, together with improved safe food handling practices, may help ensure the safety of most unprocessed or minimally processed fruits and vegetables (De Roewer, 1998). However, this isn't always practised and in recent years a higher frequency of outbreaks associated with foodborne illnesses (e.g. salmonellosis and listeriosis) have been reported with fresh fruits and vegetables (Beuchat, 1996; De Roewer, 1998). Up to date, no foodborne disease outbreak has been reported with regard to litchi fruit.

More frequent handling of fresh fruit increases the likelihood of introducing foodborne pathogens into the supply chain. Foodborne pathogens that may be of concern with respect to litchi are *Escherichia coli* O157:H7 and *Staphylococcus aureus*. The presence of *E. coli* O157:H7 and *S. aureus* on fruit are indicators of faecal contamination and poor hygiene, respectively. Fruit stored at low temperatures may limit the range of pathogens that can grow on fruit surfaces. Fruit kept at temperatures below 7 – 8°C will allow for the survival of the foodborne pathogens. The growth of salmonellae, shigellae and enterohaemorrhagic *E. coli* will be inhibited at these low temperatures (De Roewer, 1998). Moreover, incorrect handling of fruit, such mechanical damage, will cause breaks in the protective epidermal barrier, which limits shelf-life of the fruit and provide more entry points for postharvest- and foodborne pathogens (King and Bolin, 1989). Consumption patterns of litchi fruit are usually associated with cracking the hard peel using fingernails or teeth thereby exposing the consumer to the fructoplane microorganisms. The potential safety aspects associated with these customary behaviour patterns have not been shown to represent a potential risk

In the past few years, non-sulphured litchis have become very popular and consumers now demand fruit to be fresh and unsulphured. This demand has also contributed to the drive in developing an alternative postharvest treatment that is environmentally friendlier and healthier. The application of modified atmosphere packaging (MAP) technology as a partial alternative has shown good litchi quality retention (Sivakumar and Korsten, 2006). However, this technology on its own, do not provide sufficient protection against decay- or foodborne pathogens. The integration of antimicrobial agents and MAP can create an environment inside the package that may delay or prevent the growth of microorganisms on the product's surface and thereby extend its shelf-life. Therefore, further investigation with regard to best treatments from previous studies (Chapter 4 and 5), i.e. integrated

MAP and 1-MCP (300 and 1000 µL L^{-1}) treatment and integrated MAP and chitosan coating (0.1 and 1.0 g L^{-1}) treatment, and the best performing cultivar, 'McLean's Red' were used to determine the impact of these treatments on microbial dynamics on the fructoplane. In addition, the impact of the best postharvest treatments on challenge inoculated litchi fruit using foodborne pathogens, *Escherichia coli* O157:H7 and *Staphylococcus aureus*, and postharvest pathogens *Penicillium chrysogenum, P. crustosum, P. expansum, P. glabrum* and *P. solitum* were also studied.

2. MATERIALS AND METHODS

2.1. CULTURES

2.1.1. *Bacterial Pathogens*

American Type Culture Collection (ATCC, Manassas, USA) cultures namely *Escherichia coli* O157:H7 (ATCC 35150) and *Staphylococcus aureus* subsp. aureus (ATCC 1200) strains were used in this study. Strains were maintained lyophilised at -75 °C. Each strain was streaked onto selective media, Eosin Methylene Blue and Baird-Parker media (Biolab, Johannesburg) and incubated at 37 ºC for 24 hrs. A single colony was inoculated in Tryptone Soy Broth (TSB) (Biolab, Johannesburg) and shake-incubated at 37 ºC for 18 hrs to obtain an inoculum level of 10^8 colony forming units per millilitre (CFU mL^{-1}). Two consecutive 1:10 dilutions for *E. coli* O157:H7 and *S. aureus* were made in peptone buffered water (0.01 %) to obtain an estimated final inoculum level of 10^6 CFU mL^{-1}. A further two consecutive 1:10 dilutions were made for each pathogen to obtain an inoculum level of 10^4 CFU mL^{-1}.

2.1.2. *Penicillium Isolates*

Five *Penicillium* isolates, *P. chrysogenum, P. crustosum*, *P. expansum*, *P. glabrum* and *P. solitum* were obtained from the culture collection at the Plant Pathology Laboratories, University of Pretoria, South Africa. The purity of *Penicillium expansum*, *P. crustosum*, *P. chrysogenum*, *P. glabrum* and *P. solitum* were determined by aseptically transferring isolates onto MEA (Merck, Johannesburg) (amended with 10 mg g^{-1} chloramphenicol per litre MEA) and plates which were incubated for 5 - 7 days at 25 ºC.

2.2. FRUIT TREATMENT

Litchi fruit (*Litchi chinensis* Sonn.) 'McLean's Red' was harvested at commercial maturity and selected for uniformity of size and colour, and any apparent injuries or infections were removed. After being superficially disinfected by immersion for 1 min in diluted bleach (0.05 % sodium hypochlorite), the samples were rinsed with sterile water and allowed to air dry at room temperature

(25 °C). Fruit were divided into five lots each of 42 Kg, and each lot was given one of five treatments; 1) untreated (control); 2) 0.1 g L^{-1} chitosan dip treatment; 3) 1.0 g L^{-1} chitosan dip treatment; 4) 300 nL L^{-1} 1-methylcyclopropene (1-MCP) and; 5) 1000 nL L^{-1} 1-MCP. The chitosan solutions were prepared as previously described in Chapter 4 and the 1-MCP treatments were prepared as previously described in Chapter 5. Each lot was further divided in to three groups; A) nine kilograms of fruit (for total viable counts); B) 15 Kg of fruit (for *Penicillium* spp. inoculation) and; C) 18 Kg of treated fruit (for foodborne pathogen challenge inoculation). All fruit allocated to each group was further divided into one kilogram bioriented polypropylene (BOPP) bags (Sivakumar and Korsten, 2006). Samples were kept at 2 ºC for 21 days, followed by 2 days at room temperature (20 °C).

2.3. FRUIT INOCULATION

2.3.1. *Foodborne Pathogens*

Nine sample units were inoculated with 100 µL of 10^6 cfu/mL *E. coli* O157:H7 and 100 µL of 10^6 cfu/mL *S. aureus* (representing a high inoculum load for foodborne pathogens). The remaining nine 1-Kg BOPP units were inoculated with 100 µL of 10^4 cfu/mL *E. coli* O157:H7 and 100 µL of 10^4 cfu/mL *S. aureus* (representing a lower inoculum load for the pathogens). Samples were sealed using a heat sealer (multivac C200, Multivac, Heidelberg, South Africa). Three samples per treatment for each inoculum load were immediately (day 0) processed. Samples were stored at 2 °C for 21 days. After 21 days, three replicates were removed to be processed and the remaining three samples were analysed after storage at room temperature (20 °C) for 48 hrs.

2.3.2. *Penicillium*

Penicillium expansum, *P. crustosum*, *P. chrysogenum*, *P. glabrum* and *P. solitum* were grown on MEA (amended with 10 mg g^{-1} chloramphenicol per litre MEA) and plates which were incubated for 5 - 7 days at 25 °C. Conidial suspensions were prepared by flooding 7-day-old sporulating cultures with sterile distilled water. The conidial suspensions were re-suspended and transferred to 9 mL of quarter strength Ringer's Solution (Merck, Johannesburg). Spore concentrations were determined using a hemocytometer and further diluted with sterile distilled water to obtain a 1 x 10^6 spore suspension.

Fifteen sample-units were divided into five subgroups (one of the *Penicillium* spp. inoculums was allocated to each of the subgroups) with three sample-units each. Fifty fruit in each subgroup were wounded (2-mm-diameter and 2-mm-deep) on the equatorial region of the fruit using a sterile popped rivet. Two hours later 20 µL of *P. expansum*, *P. glabrum*, *P .solitum*, *P. chrysogenum* and *P. crustosum* were inoculated into each wound. After 4h, the litchis were packed into BOPP bags and sealed. Samples were stored at 2 °C for 21 days. The percentage of infected fruit and their lesion diameters were examined every seven days up to 21 days.

2.4. MICROBIAL GROWTH AND ANALYSIS

2.4.1. *Microbial Populations*

The microbial quality of treated litchi fruit were determined by randomly selecting three sample units from Group A. Fruit were suspended in 500 mL quarter strength Ringer's solution amended with 0.02% (v/v) Tween 80 (Associated Chemical Enterprises (Pty) Ltd, Johannesburg) and washed in a ultrasonic bath (Ultrasonic Manufacturing Company (Pty) Ltd., Johannesburg), at 25 ºC for 30 s. The liquid solution was filtered through a 0.45 µm membrane filter in a vacuum assembly (Sartorius, Goettingen, Germany). Filter discs were cut aseptically and transferred to 9 mL of Ringer's solution and subjected to a serial dilution. The enumeration of particular microbial groups (bacterial, fungal and yeast) was performed by plating 100 uL aliquots of diluted sample onto the following media: Standard 1 Nutrient Agar (STD1) (Biolab) (amended with 10 mL 0.1% cyclohexamide per litre STD1), incubated at 25 ºC for 24 hrs; Malt Extract Agar (Biolab) (amended with 10 mg g^{-1} chloramphenicol per litre MEA), incubated at 25 °C for 96 hrs. Total surface bacterial, yeast and fugal populations were reported as log colony forming units per centimetres square (CFU cm^{-2}) (De Jager, 1999).

2.4.2. *Foodborne pathogen survival*

The survival of high and low inoculum loads of each pathogen were determined by randomly selecting three samples from Group B; 1) immediately after inoculation; 2) 21 days at 2 °C and; 3) 21 days at 2 °C plus two days at room temperature. Samples were processed by suspending one kilogram of fruit in 500 mL Ringer's solution amended with 0.02% Tween 80. Samples were sonicated for 30 s in an ultrasonic bath, followed by filtering the liquid solution through a 0.45 µm membrane filter. Filter discs were suspended in nine millilitres of Ringer's solution, followed by serial dilution. Enumeration of *E. coli* O157:H7 and *S. aureus* cell were performed by plating aliquots of 100 uL onto selective agar media, Eosin Methylene Blue and Baird-Parker, respectively. Plates were incubated for 24 hrs at 37 ºC. Bacterial counts were reported as log CFU cm^{-2} (De Jager, 1999).

2.5. STATISTICAL ANALYSIS

Experiments were done twice adopting a complete randomised design. Data were subjected to analysis of variance (ANOVA) using the statistical program SAS 9.2 (2002). Fisher's protected *t*-test, least significant difference (LSD) at the 5% level of significance, was performed.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF INTEGRATED MODIFIED ATMOSPHERE PACKAGING AND POSTHARVEST TREATEMENTS ON MICROBIAL POPULATIONS OF LITCHI FRUIT SURFACES

The total microbial population on the fruit surfaces of the control treatment and integrated treatment (MAP and 300 nL L^{-1}) did not show significant population increase when fruit were removed from cold storage and placed at 20 ºC for two days (Table 1). The integrated treatment with MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) had higher total microbial populations than the control treatment after 21 days at cold storage. After two days at 20 ºC, a significant ($P<0.05$) decrease in total yeast populations was observed in the integrated treatment MAP and 1000 nL L^{-1} 1-MCP. Reports on the effect of 1-MCP on total microbial populations are limited. Ku et al. (1999) found that the application of 1-MCP concentrations greater than 15 nL L^{-1} on strawberries increased decay of the fruit. Similarly, Jiang *et al*. (2001) also observed that an increase in disease with high 1-MCP concentrations on strawberries was due to decreased phenylalanine ammonia lyase (PAL) activity and lower phenolic content. With respect to integrated (MAP and 1.0 g L^{-1} chitosan) treatments, the total microbial population was significantly ($P< 0.05$) lower compared to the other treatments maintained at both temperatures. The integrated treatment (MAP and 1.0 g L^{-1} chitosan) significantly ($P< 0.05$) reduced the total yeast population after two days at 20 ºC. Studies by Roller *et al*. (2002) suggest that yeasts tend to be more sensitive to the antimicrobial action of chitosan than bacteria, which correlate with our observations. However, the total yeast and fungal population in integrated treatment (MAP and 0.1 g L^{-1}chitosan) was observed to increase significantly ($p< 0.05$) when fruit were kept at 20 ºC. The antimicrobial activity of chitosan is dependent on concentration (Liu *et al*., 2006), thus the appropriate rate should be applied.

3.2. EFFECT OF INTEGRATED MODIFIED ATMOSPHERE PACKAGING AND POSTHARVEST TREATMENTS ON *ESCHERICHIA COLI* O157:H7 AND *STAPHYLOCOCCUS AUREUS*

Enumeration of viable cells showed a significant decrease in both high and low inoculum loads of *E. coli* O157:H7 and *S. aureus* in all five treatments after 21 days of cold storage (Table 2). The antimicrobial activity of chitosan treatment significantly ($p<0.05$) reduced the high *E. coli* O157:H7 and *Staphylococcus aureus* loads within two hours before processing commenced. The level of high inoculum load was significantly reduced by both chitosan treatments. Chitosan 0.1 g L^{-1} reduced *E. coli* O157:H7 loads to very low levels after 21 days of cold storage. However, *E. coli* O157:H7 survived and proliferated and increased by 0.75 logs after fruit were kept at room temperature for two

Table 1. Microbial counts (log CFU cm^{-2}) of litchi integrated (MAP and chitosan) and (MAP and 1-MCP) after cold storage.

Treatment	Day	Total Bacterial Count	Total Fungal Count	Total Yeast Count
Control	Immediate	2.98 ± 0.54 [a]	2.63 ± 0.07 [a]	2.80 ± 0.21 [dc]
	21 days at 2 °C	2.64 ± 0.31 [a]	1.98 ± 0.35 [b]	2.59 ± 0.05 [dce]
	2 days at 20 °C	3.35 ± 0.59 [a]	1.99 ± 0.09 [ab]	2.66 ± 0.07 [dc]
MAP + 0.1 g L^{-1} chitosan	Immediate	2.98 ± 0.54 [a]	2.63 ± 0.07 [a]	2.80 ± 0.21 [dc]
	21 days at 2 °C	3.60 ± 0.09 [a]	1.51 ± 0.28 [c]	2.42 ± 0.58 [de]
	2 days at 20 °C	3.61 ± 0.24 [a]	1.99 ± 0.56 [b]	3.83 ± 0.02 [a]
MAP + 1.0 g L^{-1} chitosan	Immediate	2.98 ± 0.54 [a]	2.63 ± 0.07 [a]	2.80 ± 0.21 [dc]
	21 days at 2 °C	1.66 ± 0.87 [b]	0.62 ± 0.45 [d]	2.08 ± 0.82 [e]
	2 days at 20 °C	1.12 ± 0.37 [b]	0.92 ± 0.21 [d]	1.00 ± 0.31 [f]
MAP + 300 nL L^{-1} 1-MCP	Immediate	2.98 ± 0.54 [a]	2.63 ± 0.07 [a]	2.80 ± 0.21 [dc]
	21 days at 2 °C	3.33 ± 0.44 [ac]	2.24 ± 0.08 [ab]	3.02 ± 0.29 [bc]
	2 days at 20 °C	3.81 ± 0.05 [c]	2.27 ± 0.22 [ab]	2.938 ± 0.22 [dc]
MAP + 1000 nL L^{-1} 1-MCP	Immediate	2.98 ± 0.54 [a]	2.63 ± 0.07 [a]	2.80 ± 0.21 [dc]
	21 days at 2 °C	3.44 ± 0.33 [ac]	2.59 ± 0.51 [a]	3.54 ± 0.09 [ab]
	2 days at 20 °C	3.79 ± 0.07 [c]	2.52 ± 0.38 [a]	2.95 ± 0.48 [c]

Means followed by the same letter do not differ significantly at the 0.05% level of the Fisher's Protected Least Significant Test.

days. Chitosan 1.0 g L^{-1} also significantly ($P<0.05$) decreased *E. coli* O157:H7 counts with 2.5 logs. A significantly lower ($p<0.05$) E. coli O157:H7 counts were observed in the 1.0 g L^{-1} chitosan treatment compared to the 0.1 g L^{-1} chitosan treatment after 21 days of cold storage. After fruit were removed from cold storage, fruit were kept at room temperature which did not promote any significantly growth or inhibition.

Low inoculum loads of *E. coli* O157:H7 and *S. aureus* were used in this study to simulate a more realistic scenario that may potentially occur during supply chain operations. Foodborne pathogen counts significantly decreased in all treatments when fruit were kept at 2 °C for 21 days. However, by removing fruit from cold storage resulted in increased pathogenic bacterial counts. However, fruit treated with chitosan 0.1 g L^{-1} significantly reduced *E. coli* counts to the extent that no bacterial colonies were found. Chitosan 1.0 g L^{-1} immediately reduced low inoculum loads of *E. coli* O157:H7 counts (1.5 log). Following cold storage, very few cells were enumerated and higher counts were observed when fruit were kept at 20 °C. Counts in both chitosan 0.1 g L^{-1} and 1.0 g L^{-1} treatments were significantly lower than those observed in the control and integrated MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) treatments. Similar observations regarding treatment effects on the survival of *S. aureus*, were made. Chitosan treatment significantly ($P<0.05$) inhibited *S. aureus*, and after two days at room temperature, lower bacterial counts were obtained. Thus, chitosan 1.0 g L^{-1} had the best inhibitive effect on *E. coli* O157:H7 and *S. aureus*.

Different theories on the antimicrobial mechanism of chitosan have been proposed: 1) positively charged chitosan molecules could interfere with the negatively charged residues on the bacterial surface, which thereby interacted with the membrane of the bacteria to alter cell permeability (Helander *et al*., 2001); and 2) dissociated chitosan molecules in solution could bind with DNA and inhibit synthesis of mRNA through penetration toward the nucleic of the microorganisms and interfere with the synthesis of mRNA and proteins (Rabea *et al*., 2003). Recently, Xing et al. (2009a) investigated the antimicrobial mode of oleoyl-chitosan nanoparticle (OCNP) against *Escherichia coli* and *Staphylococcus aureus*. This novel antibacterial dispersion system was based on a combination of approaches, such as measurement of the effect of lecithin and phosphate groups, the conformation of membrane protein, internalisation of fluorescein isothiocyanate (FITC)-labeled oleyol-chitosan nanoparticles observed under fluorescence microscopy and DNA/RNA assay. Results showed that 1) the OCNP influenced the structure of bacterial membranes by changing the conformations of the cell membrane proteins; 2) the OCNP bound to the cytoplasmic membrane phospholipids of *S. aureus*, and that phosphate groups might be an extracellular target contributing to the interaction between OCNP and the cell wall surface; 3) *E. coli* and *S. aureus* had different internalisation pathways which can be attributed to the different cell wall compositions of Gram-negative and –positive bacteria; and 5) the OCNP were capable of binding to intracellular targets such as DNA and RNA (Xing *et al*., 2009). Thus, chitosan exerts its antibacterial activity against bacteria by damaging the structures of the cell.

Table 2. Effect of integrated postharvest treatments on foodborne pathogen surivival (log CFU cm^{-2}) on litchi fruit surfaces.

	Control	MAP + chitosan 0.1 g L^{-1}	MAP + chitosan 1.0 g L^{-1}	MAP + MCP 300 nL L^{-1}	MAP + 1-MCP 1000 nL L^{-1}
Escherichia coli **O157:H7 (high inoculumload)**					
Immediate	4.30 ± 0.31 [a]	1.81 ± 0.44 [bcd]	0.50 ± 0.12 [fg]	4.06 ± 0.36 [a]	4.38 ± 0.13 [a]
21 days at 2 °C	0.95 ± 0.56 [ef]	1.53 ± 0.24 [cd]	0.31 ± 0.28 [g]	1.73 ± 0.03 [bcd]	1.30 ± 0.38 [de]
2 days at 20 °C	1.90 ± 0.19 [bc]	0.09 ± 0.00 [g]	0.01 ± 0.00 [g]	2.24 ± 0.15 [b]	2.05 ± 0.34 [bc]
Escherichia coli **O157:H7 (low inoculum load)**					
Immediate	2.33 ± 0.22 [a]	1.90 ± 0.03 [bc]	0.092 ± 0.00 [h]	2.24 ± 0.27 [ab]	2.24 ± 0.29 [ab]
21 days at 2 °C	0.28 ± 0.19 [gh]	0.01 ± 0.00 [h]	0.01 ± 0.00 [h]	0.83 ± 0.14 [ef]	0.62 ± 0.35 [fg]
2 days at 20 °C	1.47 ± 0.23 [cd]	0.34 ± 0.00 [gh]	0.08 ± 0.02 [h]	1.53 ± 0.43 [cd]	1.24 ± 0.39 [de]
Staphylococcus aureus **(high inoculum load)**					
Immediate	4.24 ± 0.10 [a]	3.47 ± 0.23 [bc]	3.19 ± 0.47 [c]	3.85 ± 0.13 [ab]	4.24 ± 0.10 [a]
21 days at 2 °C	2.34 ± 0.14 [d]	1.09 ± 0.56 [fg]	0.01 ± 0.00 [g]	2.32 ± 0.26 [d]	2.12 ± 0.38 [de]
2 days at 20 °C	1.60 ± 0.52 [ef]	0.86 ± 0.6 [g]	0.67 ± 0.28 [g]	2.99 ± 0.29 [c]	1.63 ± 0.21 [ef]
Staphylococcus aureus **(low inoculum load)**					
Immediate	2.06 ± 0.20 [a]	2.16 ± 0.49 [a]	0.27 ± 0.37 [d]	2.05 ± 0.29 [a]	1.75 ± 0.55 [ab]
21 days at 2 °C	0.39 ± 0.08 [cd]	0.01 ± 0.12 [d]	0.01 ± 0.00 [d]	0.37 ± 0.12 [d]	0.27 ± 0.12 [d]
2 days at 20 °C	1.38 ± 0.20 [b]	0.01 ± 0.05 [d]	0.30 ± 0.00 [d]	1.61 ± 0.05 [b]	0.79 ± 0.71 [c]

Means followed by the same letter do not differ significantly at the 0.05% level of the Fisher's Protected Least Significant Test.
High inoculation load (1 x 10^6 cfu mL^{-1})
Low inoculation load (1 x 10^4 cfu mL^{-1})

membrane, which results in the leakge of intracellular contents (Xing *et al*., 2009b), as well as binding to intracellular targets such as DNA and RNA, thereby disrupting vital activity (Xing *et al*., 2009a)

3.3. EFFICACY OF INTEGRATED MODIFIED ATMOSPHERE PACKAGING AND POSTHARVEST TREATMENTS ON *PENICILLIUM* SPP.

The effect of integrated modified atmosphere packaging and postharvest treatments on *Penicillium chrysogenum, P. crustosum, P. expansum, P. glabrum* and *P. solitum* decay are given in Table 3. It was observed that fruit inoculated with *P. chrysogenum* had a very high disease incidence (86.67 – 100%) compared with control (MAP) fruit and the integrated treatment of MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}). No disease were recorded on fruit treated with MAP and chitosan (0.1 g L^{-1} and 1.0 g L^{-1}) treatments. A high incidence (93.33 – 100%) of *P. crustosum* was observed in MAP and integrated 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) treatments. Significantly ($P<0.05$) larger lesions were observed on fruit treated with integrated MAP and 1000 nL L^{-1} 1-MCP. Fruit in integrated MAP and 0.1 g L^{-1} chitosan had a lower disease incidence. No decay was observed on fruit that were treated with MAP and 1.0 g L^{-1} chitosan. All fruit (100%) in the treatment control and integrated MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) treatments inoculated with *P. expansum* showed disease after 21 days of storage. Integrated treatment MAP and 1000 nL L^{-1} 1-MCP had a significantly ($P<0.05$) higher decay severity compared to the treatment control. The disease incidences low on fruit treated with the MAP and 0.1 g L^{-1} chitosan were lower than on the other treatments and no disease was observed on fruit treated with MAP and 1.0 g L^{-1} chitosan. Disease incidences due to challenge inoculation of *P. glabrum* were high in the control and the integrated treatment MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) (76.7 – 90%). Decaying lesions observed on fruit treated with MAP and 1000 nL L^{-1} 1-MCP was significantly higher compared to those in the control and the integrated MAP and 300 nL L^{-1} 1-MCP. No disease development was recorded for fruit in integrated MAP and chitosan treatments. All fruit (100%) in the control and integrated MAP and 1-MCP treatments showed *Penicillium* decay. Fruit in the integrated MAP and 1-MCP (300 nL L^{-1} and 1000 nL L^{-1}) had significantly ($P<0.05$) larger decay lesions. Disease incidences in the integrated MAP and 0.1 g L^{-1} chitosan were very low (3.33%); no disease incidences were recorded in the integrated MAP and 1.0 g L^{-1} chitosan.

The higher disease severity observed for all *Penicillium* spp. in the MAP and 1000 nL L^{-1} 1-MCP treatment is attributed to the negative effect of a too high 1-MCP concentration on fruit. High levels of 1-MCP decrease the phenylalanine ammonia lyase (PAL) activity and the total phenolic content. The decrease in PAL activity has been reported to be due to the lowering of the host's defense mechanism (Wilson *et al*., 1994). Ku *et al.* (1999) and Jiang *et al.* (2001) observed increased decay when higher 1-MCP concentrations applied to strawberry fruit.

Table 3. Effect of integrated modified atmosphere packaging and postharvest treatment on *Penicillium spp*. decay after 21 days at 2 °C

Treatment	Disease Incidence (%)	Lesion Diameter (mm)
Penicillium chrysogenum		
Control	86.67	23.17 ± 2.01 [a]
MAP and 300 nL L^{-1} 1-MCP	93.33	23.57 ± 1.78 [a]
MAP and 1000 nL L^{-1} 1-MCP	100	23.98 ± 2.85 [a]
MAP and 0.1 g L^{-1} chitosan	0	0 ± 0 [b]
MAP and 1.0 g L^{-1} chitosan	0	0 ± 0 [b]
P. crustosum		
Control	100	23.014 ± 2.60 [a]
MAP and 300 nL L^{-1} 1-MCP	96.67	21.732 ± 4.62 [a]
MAP and 1000 nL L^{-1} 1-MCP	93.33	33.392 ± 1.83 [b]
MAP and 0.1 g L^{-1} chitosan	3.33	1.75 ± 3.03 [c]
MAP and 1.0 g L^{-1} chitosan	0	0 ± 0 [c]
P. expansum		
Control	100	29.22 ± 2.27 [a]
MAP and 300 nL L^{-1} 1-MCP	100	32.983 ± 1.42 [ab]
MAP and 1000 nL L^{-1} 1-MCP	100	30.65 ± 1.91 [b]
MAP and 0.1 g L^{-1} chitosan	3.33	0.58 ± 1.01 [c]
MAP and 1.0 g L^{-1} chitosan	0	0 ± 0 [c]
P. glabrum		
Control	76.67	22.53 ± 2.50 [a]
MAP and 300 nL L^{-1} 1-MCP	76.67	19.18 ± 5.78 [a]
MAP and 1000 nL L^{-1} 1-MCP	90	29.1 ± 4.06 [b]
MAP and 0.1 g L^{-1} chitosan	0	0 ± 0 [c]
MAP and 1.0 g L^{-1} chitosan	0	0 ± 0 [c]
P. solitum		
Control	100	22.53 ± 2.50 [a]
MAP and 300 nL L^{-1} 1-MCP	100	29.65 ± 2.87 [b]
MAP and 1000 nL L^{-1} 1-MCP	100	27.72 ± 1.53 [b]
MAP and 0.1 g L^{-1} chitosan	3.33	0.33 ± 0.58 [c]
MAP and 1.0 g L^{-1} chitosan	0	0 ±0 [c]

Means followed by the same letter do not differ significantly at the 0.05% level of the Fisher's Protected Least Significant Test.

The direct fungal activity of chitosan against *Penicillium* spp. is dependent on its concentration. Similar direct antifungal activity of chitosan was also reported on bell pepper, apple, lemon and peach fruits against *Botrytis cinerea*, *P. expansum*, *P digitatum* and *Monilinia fructicola*, respectively (El Ghaouth *et al*, 1997, 2000, Li and Yu, 2001). Chitosan elicit two biological activities: 1) inhibition of fungal pathogens by reducing the growth of the pathogen, or by inducing marked morphological changes, structural alterations and molecular disorganization of the fungal cells; and 2) the induction host defence reponses such as accumulation of chitinases, β-1,3-glucanases and phenolic compounds, induction of lignifications, synthesis of phytoalexinx and inhibition of host tissue maceration enyzumes, which help the host to be more resistant to subsequent pathogen attack (Rabea *et al*., 2003; Bautista-Banos *et al*., 2006).

4. CONCLUSION

In spite of the existing recommendations for safe food production, biological contamination is still high at the point of sale in various countries of the world. The application of the integrated MAP and chitosan dip treatments provide an alternative control of decaying- and foodborne pathogens. As means to ensure safe quality fruit, an integrated approach are needed to successfully adopt integrated MAP and postharvest technology.

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CHAPTER 7

GENERAL DISCUSSION

A part from the undesirable health and safety effects associated with sulphur dioxide fumigation, the treatment is still most commonly practiced in industry to control litchi pericarp browning and decay. Sulphur dioxide fumigation is also cost effective since large volumes of fruit can be treated. The introduction of an alternative postharvest treatment for the litchi industry requires that the new technology should provide equal or better quality retention, low implementation costs and practical handling of large volumes of fruit. A number of alternative postharvest technologies have shown limited prevention of pericarp browning and/or decay control, thereby preventing their successful implementation into the commercial production chain (Ilangantileke *et al*., 1993; Korsten *et al*., 1993; Lichter *et al*., 2000). However, one technology that has shown considerable potential is modified atmosphere packaging (MAP) which retains moisture within the packaging environment and reduced water loss thereby retaining quality (Kader, 1994; Pesis *et al*., 2002). Modified atmosphere packaging technology is low in cost and easy to implement at the commercial level and should therefore be considered as a partial alternative to SO_2 fumigation.

Modified atmosphere packaging is based on specific permeation properties of polymer films to O_2 and CO_2 to generate a suitable gas atmosphere around fruit (Pesis *et al*., 2002). The design of MAP with regard to 1) product mass, 2) respiratory activity, 3) required gas composition in the atmosphere, 4) film permeability to gases and its dependence on temperature, and 5) the rate of respiration of the commodity as affected by the gas composition and temperature, are all critical factors to be considered for the successful implementation of this technology (Talasila *et al*., 1995; Artes and Martinez 1998; Beaudry 2007). Film permeability, provided by micro- or macro-perforations, and the respiration rate of the fruit, determines the final gas composition within the package. Inside-package gas compositions evolve due to fruit respiration (consumption of O_2 and production of CO_2) (Kader and Watkins, 2000), and gas exchange through the semi-permeable film. The degree of permeability (i.e. number of perforations) has a direct influence on the final gas composition, since gas components diffuse and reach equilibrium more rapidly in film with a higher permeability. With respect to both litchi cultivars, 'Mauritius' and 'McLean's Red', fruit in packages with increased perforations showed higher browning indices, oxidation enzyme activities and lower anthocyanin content due to higher O_2 availability. Gas composition levels with respect to CO_2 and O_2 at equilibrium also plays an important role in fruit quality retention. Different gas compositions with respect to litchi 'Mauritius' and 'McLean's Red' were related to different cultivar fruit respiration rates. In addition, it is important that film permeability should allow sufficient transmission of O_2 and CO_2 in order to maintain a suitable atmosphere for aerobic respiration. The use of inappropriate films may lead to anoxic conditions and result in anaerobic respiration, production of high CO_2 levels (CO_2 injury) and fermentation.

Advances in the development of integrated MAP and other postharvest treatments have opened the opportunity to improve MAP technology. The application of chitosan coatings on litchi fruit in

combination with MAP reduced fungal decay and pericarp browning. This was achieved by the antimicrobial property of chitosan, as well as the coating on the litchi fruit surface which provided a protective barrier that reduced respiration and transpiration rates through the litchi pericarp. Chitosan film-coating modifies endogenous CO_2 and O_2 concentrations of the fruit (Zhang and Quantick 1997). However, an increased chitosan concentration resulted in a lower respiration rate and a higher O_2 composition in the package. These higher O_2 levels present a favoured increased oxidation enzyme activity, which resulted in lower anthocyanin content and higher browning index. In addition, high RH maintained in MAP prevent desiccation of the pericarp. Chitosan coating also reduced the relative leakage rate by preventing the loss of membrane integrity, thereby reducing browning of the litchi pericarp. In terms of cultivar performance, 'Mauritius' had a higher BI and respiration rate compared to 'McLean's Red'. Thus, the application of integrated MAP and chitosan provides for a more suitable treatment for 'McLean's Red'. Integrated MAP and chitosan showed reduced pericarp browning, extended storability and 'protection' against postharvest decay. In addition, effective cold chain management practices contribute to retaining quality and preventing decay and browning. One negative aspect of chitosan treatments is the required use of a weak organic acid which could be hazardous to workers and difficult to implement practically.

Integrated application of MAP + 1-methylcyclopropene at a lower concentration (300 nL L^{-1}) effectively prevents pericarp browning and -senescence of the aril. 1-MCP binds to ethylene receptors, thereby reducing the rate of respiration and reducing the polyphenol oxidase (PPO) and peroxidase (POD) activity. It also retains membrane integrity, anthocyanin content and prevented the decline of pericarp colour during cold storage. However, the use of higher concentrations of 1-MCP showed a negative effect on membrane integrity, pericarp browning, PPO and POD activity in both cultivars. The application of 1-MCP in combination with the use of MAP can extend the storage life of 'McLean's Red' up to 21 days. However, large-scale application of 1-MCP would be more practical by fumigating fruit. It is important to note that fruit used in such treatments must be damage- and disease free, since 1-MCP is an inhibitor of PAL activity and affected fruit will therefore respond negatively with the addition of 1-MCP.

The successful implementation of MAP technologies depends on cultivar type, fruit maturity (in terms of early and late harvested fruit), absence of decay and strict adherence to correct cold chain regimes (Pesis *et al*., 2002; Lichter *et al*., 2000; Sivakumar *et al*., 2005). Good agricultural practices and good hygiene management systems are vital to assure product quality and safety. Postharvest cold chain management plays a vital role in maintaining fruit quality since temperature fluctuations during transportation of fruit to destination markets and its distribution will have a negative impact on fruit quality, resulting in increased respiration, transpiration and fruit decay. Thus, successful implementation of MAP as a partial alternative treatment requires all role players in the litchi

postharvest production chain, from the field to the consumer, to contribute to fruit quality assurance and customer satisfaction.

RECOMMENDATIONS FOR MODIFIED ATMOSPHERE PACKAGING TECHNOLOGY:

- Fruit maturity in terms of late or early harvest is a critical factor when considering the successful use of MAP.
- The selection of suitable films with specific permeability to create a desirable atmosphere around the fruit is required in order to maintain superior overall quality.
- Readily access to cold storage is essential to retain litchi quality.
- A negative impact on fruit quality can be encountered when the fruit in MAP is subjected to temperature fluctuations during shipping, handling or at retail display. Maintenance of an adequate temperature at 14 °C is essential on the marketing shelf to retain the overall quality of litchi packed in MAP. The storage temperature varies among cultivars.
- In MAP packaging it is essential that the fruits must be almost 100% disease free since the pre-sorting of fruits before sale is not practicable in the large scale marketing chain. The presence of postharvest pathogens can act as an inoculum source and contaminate surrounding fruits within the packaging.
- The use of a protectant such as a biocontrol agent (*Bacillus subtilis*) or fruit coatings (chitosan) can be used to control decay within the modified atmosphere environment.

The adoption of these technologies will be suitable for air freight as well as sea shipment. In the case of transporting fruit via air freight, passive or active modified atmosphere packaging for cv. ‘Mauritius’ will provide fruit shelf-life up to 1 to 4 days, and up to 14 days for cv. ‘McLean’s Red’, would be ideal. The integrated application of MAP and 1-MCP technology will be most suitable for the use of exporting litchi ‘McLean’s Red’ by sea shipment, since it provide an extended shelf-life of up 21 days. However, large volume shipment must be taken into consideration and the MAP technology would not be feasible. Therefore, shipping of fruit should rather be done under controlled atmosphere storage conditions, followed by further re-packing at the retailer end into MAP, which would be ideal for the consumers’ need. Future research should however focus to run a semi-commercial trial of exporting fruit to the United Kingdom. This will allow an ample opportunity to determine the success of this technology as a alternative to the commercial sulphur dioxide fumigation practice applied in many litchi exporting countries.

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