THE POTENTIAL USE OF UVASYS SULFUR DIOXIDE SHEETS AND PACKAGING MATERIALS TO RETAIN 'MAURITIUS' LITCHI (*LITCHI CHENENSIS* SONN.) FRUIT RED PERICARP COLOUR

BY

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DECLARATION

I, Harold Kgetja Malahlela declare that this mini-dissertation, which I hereby submit at the University of Limpopo for the degree Master of Science in horticulture is my own work and expressed in my own words. I further declare that this mini-dissertation has not been previously submitted for a degree or diploma in any university.

…… ……

Malahlela HK Date

DEDICATION

This study is dedicated to my son, Tumisho Marabi.

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ABSTRACT

After harvesting litchi fruit, the red pericarp colour is rapidly lost resulting in discolouration and browning during storage and marketing. To mitigate this challenge, the South African litchi industry uses sulfur dioxide fumigation to retain litchi fruit red pericarp colour during extended storage and shelf-life. However, there are health concerns regarding the commercially used (SO_2) fumigation for litchi pericarp colour retention due to high levels of SO_2 residues in fruit aril. Therefore, this study aimed to explore the possibility of Uvasys slow release SO_2 sheets to retain 'Mauritius' litchi fruit red pericarp colour when packaged in plastic-punnets and bags. Treatment factors were two packaging materials (plastic-punnets and bags), six SO_2 treatments (control; SO_2 fumigation and four SO_2 sheets *viz*. Uva-Uno-29% $Na_2S_2O_5$; Dual-Release-Blue-35.85% $Na_2S_2O_5$; Slow-Release-36.5% $Na_2S_2O_5$ and Dual-Release-Green-37.55% $Na_2S_2O_5$) and four shelf-life periods (day 0, 1, 3 and 5). 'Mauritius' fruit were assessed for pericarp Browning Index (BI), Hue angle (h^o), Chroma (C*) and Lightness (L*). In this study, an interactive significant effect ($P < 0.05$) between packaging type and SO_2 treatments was observed on 'Mauritius' fruit pericarp L*, C* and h^o during shelf-life. Fruit stored in plastic-bags and treated with SO_2 fumigation showed higher pericarp C* and L*, while SO_2 fumigated fruit in plastic-punnets had higher pericarp h^o. Lower pericarp BI was observed in SO_2 fumigated fruit stored in plastic-bags, which showed less pericarp browning than fruit in other treatments. In general, commercial SO_2 fumigation resulted in lower pericarp BI, and higher pericarp L*, C* and h^o throughout the storage and shelf-life. Our correlation analyses results further showed that litchi fruit red pericarp colour was better preserved as SO_2 treatment levels increased, especially in plastic-bags. In retaining 'Mauritius' litchi fruit red pericarp colour, Uvasys SO_2 sheets were not effective when compared with commercial SO_2 fumigation. However, commercially SO_2 fumigated fruit were bleached throughout the storage and shelf-life. Furthermore, fruit from all treatments were spoiled due to decay and mould growth after day 5 of shelf-life. Inclusion of pathogen protectants is important in future research to demonstrate whether Uvasys SO_2 sheet-packaging technology can retain 'Mauritius' litchi fruit pericarp colour.

Keywords: *'Mauritius litchi', Packaging, Pericarp browning, Pericarp colour, Sulfur dioxide (SO_2)*

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Litchi (*Litchi chinensis* Sonn.) is a tropical to subtropical fruit belonging to the Sapindaceae family (Huang *et al*., 2005). In South Africa, 'Mauritius' and 'McLean's Red' are the major produced litchi cultivars at 89.8 and 6.4%, respectively. 'Mauritius' litchi cultivar dominates the export market due to their aesthetic natural red colour, high nutritional value, and sour-sweet taste (Kumar *et al*., 2016). This litchi cultivar is mostly distributed in the tropics and subtropics regions (Cronje, 2008). In South Africa, an estimated 70% litchi fruit are produced at Nelspruit, Malalane and Hazyview in Mpumalanga province. This is followed by the Levubu and Tzaneen areas in Limpopo province which produces roughly 25% of the crop, while the remaining 5% is produced in KwaZulu-Natal province (Begemann, 2014). Economically, 56.4% of the litchi crop is exported, 36.7% sold locally, and the remaining 6.9% processed to juice (Begemann, 2014). Despite the recent severe droughts in most parts of South Africa, export volumes have increased from 1.47 million cartons in 2015 to approximately 2.15 million in 2016 (South African Litchi Growers Association, 2017).

Litchi fruit market growth constraints includes quality loss due to pericarp browning, fungal decay, desiccation and quarantine barriers imposed by highly profitable markets such as the United States (Mathaba *et al*., 2015). Among the identified market, limitations of litchi fruit pericarp browning is the primary cause of post-harvest economic loss in the industry (Jiang *et al*., 2006). Moreover, the success of the litchi market growth is dependent upon the resolution of horticultural and post-harvest problems that strongly influence cosmetic eye-appeal of the fruit (Kaiser, 1996). In order to attain higher market prices, preservation of litchi red colour is important for many exporting countries.

Commercially, gaseous sulfur dioxide (SO_2) fumigation has been adopted by the South African litchi industry to mitigate against pericarp browning and loss of fruit cosmetic appeal. However, this treatment resulted in undesirable residues which constitutes a potential health risk for SO_2 asthmatics (Kremer-Köhne, 1993). Furthermore, SO_2 residues result in altered fruit taste due to higher aril titratable acidity

and it is ineffective against some post-harvest fungi where resistance has appeared (Holcroft *et al*., 2005). In addition, surplus SO_2 can bleach the red fruit pericarp colour to pale yellow-green which leads to poor litchi appeal (Rattanachai, 1997). Acid treatments *viz*. hydrochloric acid dips have been applied on SO_2 fumigated litchi fruit to convert back the pale-yellow pericarp to a red-pink colour by decreasing the pericarp pH. Nevertheless, this treatment confers an unnatural pliable red colouration of the pericarp which is unpleasant.

Health hazards and undesirable effects constituted by SO_2 fumigation have led to post-harvest research towards the development of alternative strategies to mitigate litchi fruit pericarp browning. These include post-harvest dip treatments with ethylenediaminetetraacetic acid, calcium disodium salt hydrate; phosphoric-acid, and 4-hexylresorcinol (Sivakumar and Korsten, 2006a; Kumar *et al*., 2012). In addition, antioxidants and salicylic acid (Jiang and Fu, 1999; Kumar *et al*., 2013), controlled atmosphere storage (Reichel *et al*., 2017), and modified atmosphere packaging (Somboonkaew and Terry, 2011) have also been used to retain litchi fruit red pericarp colour. Of the above mentioned alternative treatments, modified atmosphere with various polymer packaging has an advantage of application ease at commercial level (Flores *et al*., 2004). Furthermore, packaging material maintains high humidity around the fruit, and can reduce the oxygen concentration inside the package necessary for retarding oxidation activities related to browning (Fishman *et al*., 1996). However, excessive high in-package relative humidity may promote microbial spoilage (Hussein *et al*., 2015).

Recently, SO_2 packaging sheets are one technology that has shown promising results in delaying loss of litchi red pericarp colour (Schutte *et al*., 1990). This technology is an active packaging technology involving incorporation of antimicrobial agents and/or antioxidant releasing systems such as sodium metabisulfite ($Na_2S_2O_5$) salt. Packaging fresh produce with SO_2 sheets has been successful in the table grapes for eliminating storage fungi during transportation (Zoffoli *et al*., 2009). In recent years, few studies investigated the effect of SO_2 packaging sheets on litchi fruit pericarp colour (Schoeman *et al*., 2007; Wermund *et al*., 2014).

1.2 Problem statement

The currently used post-harvest SO_2 fumigation for pericarp colour retention of litchi fruit leads to economic losses due to growing concern over food safety and environmental pollution (Rattanachai, 1997). Thus, European markets have set a maximum residue limit (MRL) of < 10 ppm of SO_2 residues in the edible aril of SO_2 fumigated litchi fruit (Ducamp-Collin, 2001). Consequently, litchi fruit consignment from South African must be inspected by the Perishable Products Export Control Board (PPECB) in order to ensure that fruit destined for export conform to the European Food Safety Standards (Begemann, 2014). Moreover, litchi fruit stored in SO_2 packaging sheets always have SO_2 residues below the set MRL, and are safe for consumption. However, there is meagre information about the potential of SO_2 packaging sheets to delay loss of litchi fruit pericarp colour. Therefore, the efficacy of SO_2 sheets to retain red skin colour of 'Mauritius' litchi fruit would be investigated in this study.

1.3 Rationale of the study

The introduction of an alternative method to preserve litchi fruit pericarp colour entails that the new technology should provide equal or better-quality retention, low implementation costs and practical handling of large volumes than the currently used SO_2 fumigation (De Reuck, 2010). Sulfur dioxide (SO_2) packaging sheet technology provides an added advantage over the current used SO_2 fumigation treatment since it results in SO_2 levels far below the MRL. Sulfur dioxide (SO_2) sheet-packaging is a low-cost technology, easy to implement at the commercial level and also environmentally safe for consumers and packhouse workers. Furthermore, SO_2 sheets have a lifespan of 2 years in storage when properly used. Therefore, this technology should be investigated as a potential alternative treatment to SO_2 fumigation for litchi fruit red pericarp colour preservation.

1.4 Aim and objective

1.4.1 Aim

The aim of this study was to determine the potential of SO_2 packaging sheets in retaining 'Mauritius' litchi fruit red pericarp colour.

1.4.2 Objective

To investigate whether SO_2 sheets would retain red pericarp colour of ‘Mauritius’ litchi fruit packaged in perforated plastic-punnets and bags.

1.5 Outline of the mini-dissertation

Under Chapter 1 (General Introduction), the background, and the South African litchi industry is briefly covered. Furthermore, constraints in litchi industry, post-harvest treatments used as well as the problem statement, rationale of the study and research aim, and objective are outlined. Chapter 2 covers literature on the research problem, focusing on pericarp colour and browning and treatments used to control browning of litchi. While, Chapter 3 deals with research methodology and Chapter 4 deals with results and discussion of assessing the efficacy of SO_2 sheets to retain red pericarp colour of ‘Mauritius’ litchi fruit stored in perforated plastic-punnets and bags. Lastly, summary, conclusions and recommended future research are provided in Chapter 5.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In litchi (*Litchi chinensis* Sonn.) fruit, pericarp colour is one of the characteristic maturity index used to quantify commercial quality (Bryant, 2012). Red fruit pericarp colour preservation throughout the cold-chain has been a major focus of post-harvest litchi research. Thus, this literature review is focused on litchi pericarp colour and browning. The ensuing literature review would be focused on sulfur dioxide (SO_2) treatments and packaging materials as commercial methods used to mitigate litchi pericarp browning. Lastly, this chapter will identify the existing gaps on the research problem and explanation of how the gaps could be addressed.

2.2 Litchi pericarp colour

Litchi fruit red pericarp colour is conferred by chlorophyll degradation; and concomitant anthocyanin biosynthesis (Lee and Wicker, 1991). Anthocyanin pigments are responsible for the pink-red litchi pericarp colour (Valero and Serrano, 2010). In terms of grouping, anthocyanins fall under secondary plant metabolites and belong to the flavonoids phenolic compounds (Castañeda-Ovando *et al*., 2009). Anthocyanidins (aglycons) are the basic structures of the anthocyanins and consist of an aromatic ring (A) bonded to a heterocyclic ring (C) with an oxygen bonded by a carbon-carbon bond to a third aromatic ring (B) (Figure 2.1) (Konczak and Zhang, 2004). Anthocyanidins bonded to a sugar moiety (glycoside form) are known as anthocyanins.

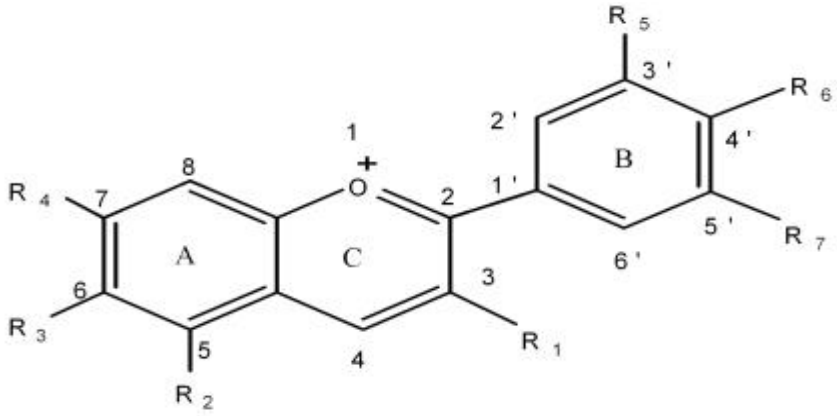

Figure 2.1 General anthocyanidin structure. R1-R7 are substitution patterns from Table 2.1, which are later glycosylated to form anthocyanins

Table 2.1 Structural identification of major anthocyanidins (aglycons) (Castañeda-Ovando *et al*., 2009).

Aglycons	Abbreviations	Substitution pattern							Colour
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	
Cyanidin	Cy	OH	OH	H	OH	OH	OH	H	Orange-red
Delphinidin	Dp	OH	OH	H	OH	OH	OH	OH	Blue-red
Malvidin	Mv	OH	OH	H	OH	OMe	OH	OMe	Blue-red
Pelargonidin	Pg	OH	OH	H	OH	H	OH	H	N.R*
Peonidin	Pn	OH	OH	H	OH	OMe	OH	H	Orange-red
Petunidin	Pt	OH	OH	H	OH	OMe	OH	OH	Blue-red

N.R*, not reported

Lee and Wicker (1991) identified cyanidin-3-*O*-rutinoside, malvidin-3-*O*-acetylglucoside and cyanidin-3-*O*-glucoside as three major anthocyanins in 'Brewster' litchi pericarp. Whereas, Somboomkaew and Terry (2010) found cyaniding-3-glucoside to be the major anthocyanin in 'Mauritius' litchi fruit pericarp. Zhang *et al*. (2000) found malvidin-3-*O*-glucoside to be the major anthocyanin in 'Huaizhi' litchi fruit. However, 67 to >95% of total anthocyanins in other different litchi cultivars ('Meiguili', 'Baila', 'Baitangying', 'Guiwei' 'Nuomici' and 'Guinuo') from China are constituted by cynidin-3-*O*-rutinoside (Zhang *et al*., 2005). Therefore, pericarp anthocyanin concentrations vary amongst litchi fruit cultivars (Somboonkaew and Terry, 2010).

'McLean's Red' litchi fruit showed higher anthocyanin concentration when compared with 'Mauritius' fruit after 14 and 21 days storage (De Reuck *et al*., 2009). In addition to the cultivar influence, anthocyanin concentration in fruit pericarp may vary with respect to growing locations, pre-harvest treatments and climatic conditions (Singh *et al*., 2014). Pre-harvest 150 and 300 mgL^{-1} abscisic acid application resulted in higher 'Culcuttia' litchi pericarp total anthocyanin accumulation compared to the control

(Singh *et al*., 2014). According to Shiukhy *et al*. (2014), 'Sangiro Moro' navel orange rind showed higher total anthocyanin content in south canopy position compared with other positions. Jing *et al*. (2007) reported that different growing locations affected total anthocyanin contents of 'Jubilee' purple corncob. Lastly, high temperature (25/30°C) growing conditions increased anthocyanin content in unknown strawberry fruit cultivar (Wang and Zheng, 2001). Therefore, pre-harvest and climatic factors influence anthocyanin concentrations of fresh produce at postharvest period.

2.3 Litchi pericarp browning

Once litchi fruit are harvested, the red pericarp colour is lost within 48 hours and completely turns brown after 72 hours (Neog and Saikia, 2010). The browning reaction is reported to be triggered by moisture loss and desiccation, temperature stress and micro-cracking of the pericarp, which cause anthocyanin degradation (Sivakumar *et al*., 2010). In this section, post-harvest factors that influence litchi red pericarp colour loss are reviewed.

2.3.1 Moisture loss and desiccation

Pericarp browning reduces litchi fruit appeal and marketability (Kumar *et al*., 2016). According to Scott *et al*. (1982), litchi fruit red colour loss is related to pericarp moisture or desiccation. However, there is contradicting literature about moisture loss amount required to result in litchi pericarp browning. Underhill and Critchely (1994) reported that litchi fruit browning can occur when about 2% of the pericarp moisture is lost after harvest. Brown (1986) postulated that browning may commence when 3-5% litchi fruit moisture is lost. According to Liang *et al*. (1998) research work, pericarp browning of 'Tai So' litchi commenced when 7.6% of fruit moisture was lost. Moreover, these authors found that fruit were completely brown after 18% moisture was lost. Furthermore, Wu *et al*. (1997) suggested that 9% fruit moisture loss was sufficient to trigger litchi pericarp browning. These conflicting findings may be attributed to various factors such as moisture assessment methods, cultivars, moisture contents at harvest and post-harvest treatments prior to moisture content measurements (Bryant, 2012).

According to Bryant (2004), browning caused by moisture loss occurs due to water potential gradient that draws water vapour into the surrounding air. Moreover, Bryant (2012) found that air current had a significant effect on 'Kwa May Pink' and 'Wai Chee'

litchi fruit moisture loss rate during 6 to 27.5 hours storage at room temperature. Similarly, 'Oliver' brussels sprouts stored in 0.1 $m.s^{-1}$ air flow lost 18 times higher moisture than those protected from air current (Van den Berg, 1987). However, moisture loss rate may vary for different fruit cultivars and vegetables due to differences in skin cuticle physiological properties. 'Britewell' blueberry with cuticular wax removed showed higher moisture loss and poor pericarp colour compared with unremoved culticular wax control fruit (Chu *et al*., 2018). Huang *et al*. (2004) reported that 'Huaizhi' litchi fruit showed less moisture loss and browning when compared with 'Nuomici' litchi fruit. Moreover, resultant physical cell structure changes due to moisture stress caused enzymatic pericarp browning of 'Guiwei' litchi fruit during 9 days storage at 25°C and 85-95% relative humidity (Zhang *et al*., 2015).

2.3.2 Temperature stress

Bhushan *et al*. (2015) reported that browning of 'Shahi' litchi induced by temperature stress was distinguished from moisture loss by a distinctive dark and water-soaked areas on the pericarp surface. Temperature stress can result from either heat or chilling injury. With regards to chilling injury, pericarp tissue necrosis and browning occurs when litchi fruit are stored at temperatures below 0°C or less than 2°C (Kaiser, 1996). Consequently, De Reuck (2010) suggested that 'Mauritius' and 'McLean's Red', South African litchi cultivars should be stored at 2-5°C to avoid pericarp chilling damage; and therefore, browning. Furthermore, peels of 'Kluai Khai' (Musa AA Group) and 'Kluai Hom Thong' (Musa AAA Group) banana fruit stored at 6 and 10°C were brown due to chilling injury (Nguyen *et al*., 2003). However, peel browning was highly significant at 6°C cold storage. This suggest that pericarp/peel browning severity as a result of chilling injury is temperature and cold storage duration dependent.

Heat treatments are being actively used for post-harvest quality retention of fresh produce. However, heat treatments can cause tissue damage and pericarp browning when inappropriately used. Therefore, it is challenging to find a time-temperature regime that will produce the desired effect on fruit quality retention (Valero and Serrano, 2010). Bagshaw *et al*. (1991) reported that storing 'Kwai May Pink' litchi fruit at temperatures above 50°C will cause pericarp browning. According to Underhill and Critchley (1993), hot water dip for 10 minutes at 60°C resulted in 'Kwai May Pink' litchi pericarp browning. Fan *et al*. (2011) found that hot air treatment for 8 and 12 hours at

46°C caused peel browning of ‘Jonagold’ and ‘Cortland’ apple fruit. Moreover, it is reported that the basic principle behind browning mechanism due to temperature stress is similar to that of moisture loss and desiccation (Sivakumar *et al*., 2010).

2.3.3 Micro-cracking

Pericarp micro-cracking also results in litchi browning (Huang *et al*., 2004). During litchi fruit development, micro-cracks (20 to 100 μm in width) occur on the pericarp surface due to aril expansion (Huang *et al*., 2004). Litchi pericarp micro-cracking occurs when fruit aril exerts an increased pressure against the pre-grown pericarp (Underhill and Simons, 1993). Wet and dry periods fluctuation during the later fruit development stage also accelerate micro-cracking (Sivakumar *et al*., 2010). Furthermore, fruit dropping during post-harvest cold chain operations can result in micro-cracking (Figure 2.2).

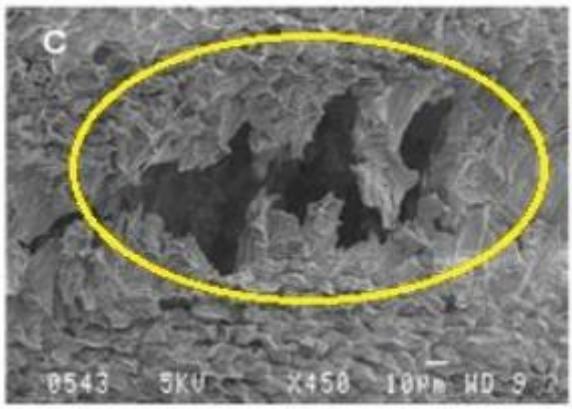

Figure 2.2 Micro-cracking during post-harvest cold chain operations when the fruit drops from 15 cm height (Sivakumar *et al*., 2007).

Underhill and Simons (1993) suggested that micro-cracking could result from moisture loss and desiccation. Nevertheless, it is likely that micro-cracks promote pericarp browning by constituting to a further desiccation on the pericarp tissues. Micro-cracks would also be likely to encourage tissue browning through increased cellular breakdown, and exposure of underlying pericarp tissues to air and/or oxygen for phenolics oxidation (Bryant, 2004).

2.3.4 Physiological changes

Litchi fruit pericarp browning can occur enzymatically through hydrolysis of anthocyanin pigments by anthocyanase, forming an anthocyanidin (Zhang *et al*., 2005). Anthocyanidins may be oxidised by polyphenol oxidase (PPO) and peroxidase

(POD). In turn, oxidative products of phenolics (4-methylcatechol) resulting from PPO and POD actions then accelerate anthocyanidin degradation leading to polymeric brown pigments (Figure 2.3) (Jiang *et al*., 2004).

i) Polyphenol oxidases

Pericarp browning of fruits results from phenolic compound oxidation. The enzymes responsible for this oxidation are polyphenol oxidase (PPO) namely, laccase and catechol oxidase (Zhang *et al*., 2000). According to Jiang *et al*. (2004), enzymatic litchi pericarp browning is mainly due to phenolic oxidation and anthocyanin degradation by PPO. In addition, PPO activities in litchi pericarp are pH dependent and their pH optima is 6.5 (Jiang and Fu, 1999). Mizobutsi *et al*. (2010) found PPO activities in 'Bengal' litchi pericarp higher between pH 6.5 and 7.0 and no enzyme activity were detected at pH 2.5. Jiang *et al*. (1997) found PPO activity of unknown litchi fruit cultivar to increase at higher pH (7-7.4), whereas no PPO enzyme activity was observed below pH 4.2. However, these findings contradicted with those reported by Underhill and Critchley (1995) who showed that 'Kwai May Pink' litchi pericarp PPO contents were high at pH > 4.

Liu *et al*. (2010) reported that (-)-epicatechin was the major endogenous substrate of PPO enzyme in 'Feizixiao' litchi pericarp tissue. They sated that (-)-epicatechin oxidative products catalyse anthocyanin degradation, thereby, leading to pericarp browning. Furthermore, Reichel *et al*. (2017) proposed a mechanism for litchi browning: (1) PPO-mediated oxidation of abundant (-)-epicatechin, leading to dark brown pigments and (2) micro-cracks induce formation of brown pericarp surface scurf, possibly with additional action by peroxidase (POD).

ii) Peroxidase

Peroxidase (POD) is also an oxidative enzyme like the PPO present in litchi pericarp tissue (Zhang *et al*., 2005). According to Jang and Moon (2011), POD is an indicator of various biodegradation reactions, which implies that it is also relevant in fruits enzymatic browning. Lin *et al*. (1988) and Underhill and Critchley (1994) found POD enzyme activities to increase during 'Huaizhi' litchi fruit pericarp browning. Moreover, involvement of POD in 'Caffra' marula (Mdluli, 2005) and 'Gala and Fuji' apple fruits (Valderrama and Clemente, 2004) skin browning has been documented. Gong and

Tian (2002) reported that POD purified partially from ‘Heiye’ litchi pericarp can rapidly oxidise 4-methylcatechol in hydrogen peroxide presence (H_2O_2). This suggests that POD is involved in litchi enzymatic browning.

iii) Anthocyanase

Anthocyanase (anthocyanin-β-glucosidase) also play a vital role in enzymatic litchi browning by removing the sugar moiety, which result in degradation of an anthocyanin (Huang, 1955). Zhang *et al*. (2001) found high activity of anthocyanase in ‘Huaizhi’ litchi pericarp turning brown and postulated that litchi browning may involve: (1) co-oxidation of phenolics and anthocyanins by browning promoting enzymes (PPO and POD) with the formation of *o*-quinones, and (2) the hydrolysis of anthocyanins by anthocyanase, leading to the production of anthocyanidin, which hasten enzymatic degradation of the anthocyanins.

In summary, pericarp browning of litchi is a result of enzymatic oxidation, which involves loss of enzymes and substrates compartmentalization facilitated by moisture loss, temperature stress and micro-cracking. When litchi fruit is picked at about 80% maturation, metabolism reactions in the pericarp tissue still continue and cells are able to maintain their membrane integrity, which ensures separation of enzymes and substrates in the cytosol and vacuole, respectively (Bhushan *et al*., 2015). However, after 2-3 days at ambient temperatures, high activities of PPO and POD increases oxidation, leading to browning (Jiang and Chen, 1995). Nonetheless, the distinction of PPO and POD enzymes actions in litchi pericarp browning is a complex physiological process to elucidate (Bhushan *et al*., 2015). Nevertheless, these enzymes function complementary to result in litchi pericarp browning (Zhang *et al*., 2005).

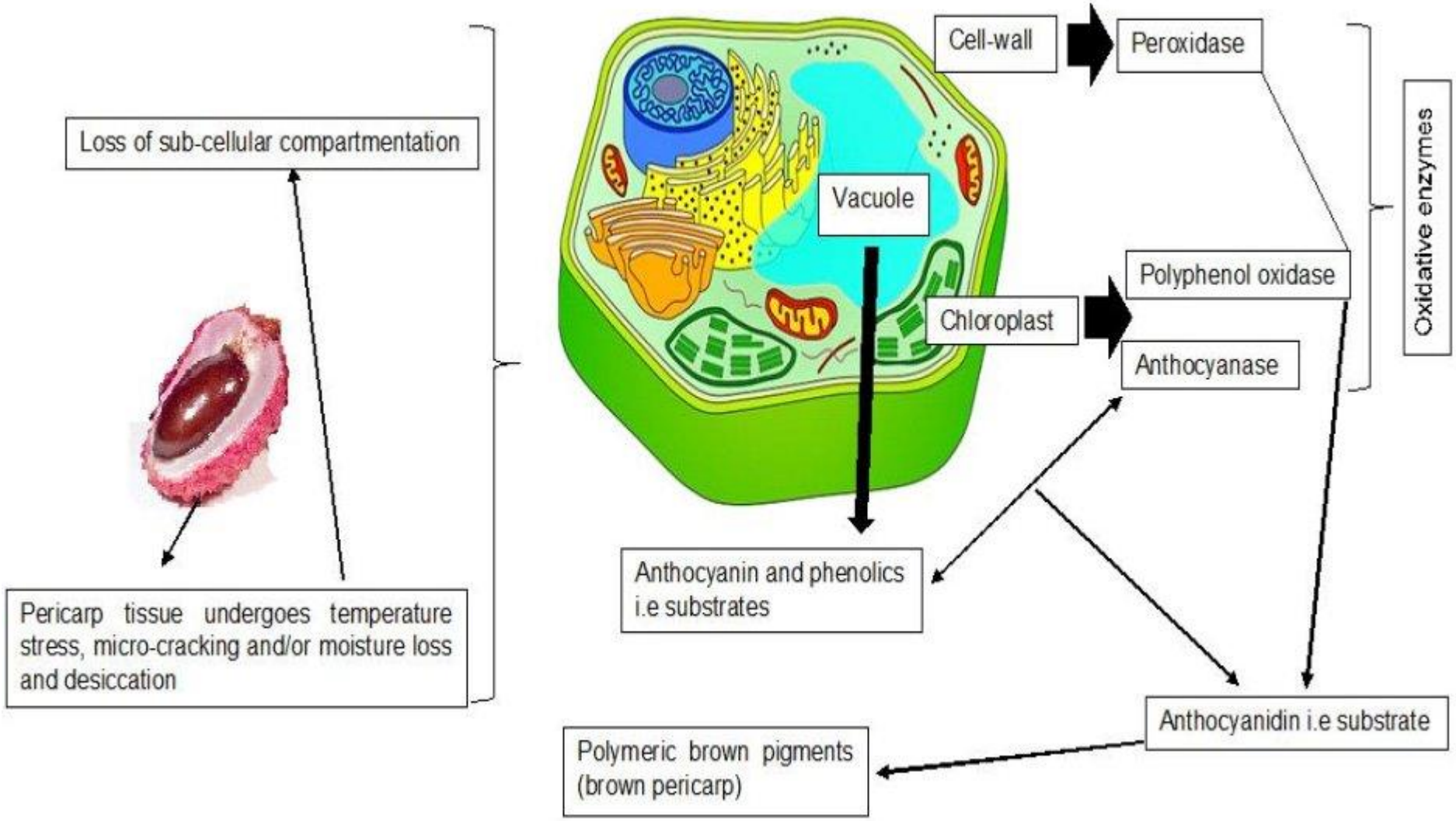

Figure 2.3 Proposed schematic presentation for enzymatic browning in litchi fruit pericarp (Jiang *et al*., 2004)

2.4 Mitigation of litchi pericarp browning

2.4.1 Sulfur dioxide (SO_2) treatments

Post-harvest pericarp browning mitigation of litchi is achieved by interfering with browning biochemical processes. Post-harvest SO_2 treatments are one of the methods used for interfering with biochemical processes that lead to pericarp browning. The principal mechanism involved in inhibition of browning by SO_2 is due to SO_2 reducing oxygen; and thus, making it unavailable for oxidizing polyphenols, or reacting with quinones or other intermediates (Kaiser, 1996). Furthermore, SO_2 reacts with anthocyanins and results in litchi pericarp bleaching, thereby, stabilizing the pigments against degradation.

Sulfite bleaching is an ionic reaction involving a nucleophilic attack by a negative ion of sulfurous acid on the flavylium cation to form chromen-4 (or-2) sulfonic acid. In addition, Holcroft and Mitcham (1996) reported that SO_2 and anthocyanins form an anthocyanin-SO_3H complex that is more stable, and effects of SO_2 in controlling browning may be the results of this complexing rather than PPO and POD inhibition. The proceeding sections of the literature will review findings on SO_2 treatments effect on litchi pericarp colour parameters.

i) Pericarp browning index

Litchi fruit pericarp browning increases rapidly after harvest, with peak activity occurring after 48 hours (Zauberman *et al*., 1991). Kumar *et al*. (2013) found browning index of untreated ‘Rose Scented’ litchi fruit to rapidly increase during 6 days storage at ambient conditions. However, SO_2 fumigation treatment resulted in lower ‘McLean’s Red’ litchi browning index, thereby reducing pericarp browning when compared with untreated control (Sivakumar *et al*., 2008). In another study, sodium metabisulfite ($Na_2S_2O_5$) dip treatment resulted in lower ‘Feizixiao’ litchi pericarp browning index when compared with control (Liang *et al*., 2012). Moreover, browning index of SO_2 fumigation treatment was significantly higher than 0.5% salicylic acid and 0.1% N-acetyl cysteine treatments in ‘Taiso’ litchi fruit (Kumar *et al*., 2013).

ii) Pericarp hue angle

According to Sivakumar *et al*. (2007), pericarp bleaching is one of the drawbacks on litchi colour associated with current commercial SO_2 fumigation. Pericarp bleaching occurs when excess sulfur is used, leading to fruit pericarp turning yellow to pale green. Sivakumar and Korsten (2010) reported that hue angle (*h*°) of SO_2 fumigated 'McLean's Red' litchi fruit was higher, indicating a pinkish-yellow bleached pericarp colour. Furthermore, SO_2 fumigation resulted in higher 'Mauritius' fruit pericarp hue angle when compared with freshly harvested control fruit after 34 days at 2 and 14°C for 2 days (Sivakumar and Korsten, 2006a). Somboonkaew and Terry (2011) also found pericarp hue angle of 'Mauritius' litchi fruit stored in 5 and 13°C to be higher in commercial SO_2 fumigation treatment than in control fruit for 11 days storage.

iii) Pericarp chroma

Litchi fruit red pericarp colour loss is related to lower pericarp chroma. Sivakumar and Korsten (2006a) investigated the influence of modified atmosphere packaging and post-harvest dip treatments on quality retention of 'Mauritius' litchi fruit. They found that SO_2 fumigated 'Mauritius' fruit had higher pericarp chroma than control and fruit treated with ethylenediaminetetraacetic acid, calcium disodium salt hydrate (EDTA). Commercial SO_2 fumigation 'Mauritius' litchi fruit showed higher pericarp chroma when compared with non-adulterated control fruit (Somboonkaew and Terry, 2011). Furthermore, SO_2 fumigation treatment resulted in higher 'Taiso' litchi fruit pericarp chroma, ranging from yellow to pink-red in colour for 5 weeks storage at 2°C (Ramma, 2014).

iv) Pericarp lightness

Pericarp lightness is also one of the most important colour parameter which represents the degree of darkness (0) or lightness (100) of a fruit surface. Pericarp lightness of SO_2 fumigated 'Mauritius' litchi fruit stored at 5 and 13°C for 6 days were significantly higher than control (Somboonkaew and Terry, 2011). In addition, Sivakumar and Korsten (2006a) found 'Mauritius' litchi fruit pericarp to have a higher lightness when compared with freshly harvested untreated fruit. Mahajan *et al*. (2003) found visual appearance of untreated control 'Culcuttia' litchi fruit to be dark-brown (lower pericarp lightness) when compared with reddish (higher pericarp lightness) SO_2 fumigated fruit

with stalked bunch. Generally, SO_2 fumigation results in fruit with higher pericarp lightness than untreated fruit.

2.4.2 Packaging materials

Post-harvest litchi fruit browning can also be mitigated by reducing pericarp desiccation and moisture loss stress. These stress factors lead to loss of cellular compartmentalisation, subsequently resulting in substrates mixing and browning enzymes (Siracusa, 2012). According to Hussein *et al*. (2015), packaging plays a crucial role in delaying pericarp moisture loss of fresh produce by maintaining desirable humidity in the package headspace. Generally, low oxygen permeability packaging materials extend shelf-life of fruit since in-package oxygen pressure drops, thereby reducing oxidation activities (Siracusa, 2012). However, resulting water vapour condensation during fluctuating temperature conditions may facilitate development of microbial growth and fruit decay (Fonseca *et al*., 2000). Furthermore, enzymes derived from fungi are thought to lead to pigment degradation and browning (Lee and Wicker, 1991). The following section of the literature will review effect of packaging on litchi fruit colour parameters.

i) Pericarp browning index

Approximately 90% of the materials used in modified atmosphere packaging of fresh fruit are plastic packaging films (Mangaraj *et al*., 2009). These materials provide a wide range of permeability to gases and water vapour, which is vital for reducing litchi pericarp browning (Hussein *et al*., 2015). According to Jitareerat *et al*. (2013), polyethylene terephthalate (PET) trays covered with active bags significantly reduced ‘Chakkaphat’ litchi fruit pericarp browning when compared with control during 28 days cold storage at 4°C. Sivakumar *et al*. (2008) found that sole biorientated polypropylene (BOPP) packaging showed higher ‘McLean’s Red’ litchi fruit pericarp browning index in contrast when used with EDTA treatment. Furthermore, packing in perforated plastic-punnets delayed ‘Mauritius and McLean’s Red’ litchi fruit (De Reuck *et al*., 2009), and ‘Wild’ strawberry fruit (Almenar *et al*., 2007) skin browning than non-perforated plastic-punnets.

ii) Pericarp hue angle

Hue angle (h°), defined as the angle between the hypotenuse and 0° on the a* axis (red: green colour ratio) can also be used to measure litchi skin colour. Generally, litchi fruit red pericarp colour ranges between hue angles of 30 to 40°, and higher hue angle values designate a fruit becoming brown. The combination of polyvinyl chloride (PVC) and biorientated polypropylene (BOPP) modified atmosphere packaging resulted in lower 'Shahi' litchi fruit pericarp hue angle when compared with unpacked control fruit (Mangaraj *et al*., 2012). Chaiprasart (2004) reported that PVC film produced lower pericarp hue angle of unknown litchi fruit cultivar than polyethylene (PE) film during 12 days storage at 5°C. While, wrapping with PropaFresh™ PFAM film resulted in lower 'Mauritius' litchi fruit pericarp hue angle at 5 and 13°C storage for 11 days when compared with unwrapped control fruit (Somboonkaew and Terry, 2011). Furthermore, De Reuck *et al*. (2009) found 'Mauritius' and 'McLean's Red' litchi fruit pericarp hue angle to be lower under non-perforated plastic-punnets than in perforated-plastic punnets stored at 2ºC and 90% RH for 14 and 21 days.

iii) Pericarp chroma

According to Farina *et al*. (2017), lower litchi fruit pericarp chroma correspond with commencement of pericarp browning. The effect of packaging materials and temperature on 'Mauritius' litchi fruit pericarp chroma has been documented (Somboonkaew and Terry, 2011). PropaFresh™ PFAM film wrapping resulted in higher 'Mauritius' litchi fruit pericarp chroma than unwrapped control fruit at 5 and 13°C, respectively. Perforated (4 holes, 0.6 mm diameter) plastic-punnets packaging showed higher 'Mauritius' and 'McLean's Red' litchi fruit pericarp chroma than 10 holes (0.6 mm diameter) perforated plastic-punnets (De Reuck *et al*., 2009). 'Himbo Top' raspberry fruit placed in polyethylene terephthalate trays wrapped with biodegradable and compatible film showed higher chroma than unwrapped control after 48 hours following 18±1°C storage (Giuggioli *et al*., 2015).

iv) Pericarp lightness

A decrease in litchi fruit pericarp lightness (L*) value (L*=0 represents black; L*=100 represents white) reflects fruit becoming brown, possibly due to commencement of senescence (Giuggioli *et al*., 2015). Pericarp lightness of 'Napoleon' cherry fruit was found to be not significantly affected when stored in polypropylene (PP) and polyvinyl chloride-polyethylene (PVC-PE) trays covered with biaxially oriented polypropylene film (BOPP) for 42 days storage at ambient temperatures (Esturk *et al*., 2012). However, Chaiprasart (2004) found differences in pericarp lightness of unknown litchi fruit cultivar stored in PET trays and wrapped with PE and PVC film for 12 days storage. 'Shan-i-Punjab' peach packed in corrugated trays under shrink film wrapping showed a lightness value of 62.42, which was higher than 60.60 of unwrapped control (Pongener *et al*., 2011). Furthermore, 'Mauritius' litchi fruit stored in plastic punnets had a higher pericarp lightness under Cellophane^{TM} WS film wrapping when compared with unwrapped fruit (Somboonkaew and Terry, 2011).

2.5 The existing gap/s on research problem

The South African litchi industry needs safe and environmentally friendly post-harvest treatments other than SO_2 fumigation for prevention of pericarp browning. In the Table Grape Industry, SO_2 releasing sheets are used commercially for post-harvest refrigerated storage and transport of grapes. Schutte *et al*. (1990) have investigated the use of SO_2 sheets on pericarp browning inhibition for 'Mauritius' litchi fruit stored in polyethylene bags (without perforations). The study indicated that Slow-Release SO_2 sheets inside polyethylene bags resulted in 2% pericarp browning of 'Mauritius' fruit. In recent years, only a few studies have investigated using SO_2 sheets to preserve red pericarp colour of litchi fruit (Schoeman *et al*., 2007; Wermund *et al*., 2014). Moreover, there is little information about the effect of SO_2 packaging sheets on red pericarp colour of 'Mauritius' fruit, and as a potential alternative post-harvest treatment to retain litchi pericarp colour.

2.6 How the identified existing gap/s would be addressed

To close the existing gap/s, this study would explore the possibility of SO_2 sheets to preserve red pericarp colour of 'Mauritius' litchi fruit stored in plastic-punnets and bags. SO_2 sheets contain sodium metabisulfite ($Na_2S_2O_5$) compound which gradually

generate gaseous SO_2 in the produce headspace (Liang *et al*., 2012). Furthermore, SO_2 sheets result in safe fruit for consumption after treatment since they produce less than 10 ppm of residual levels in the edible portion of litchi. Moreover, to demonstrate whether SO_2 sheets could be recommended as a safe post-harvest treatment to reduce browning of litchi fruit, the performance of SO_2 sheet treated fruit would be compared with that of SO_2 fumigation and untreated fruit.

CHAPTER 3
MATERIALS AND METHODS

3.1 Fruit material and description of experimental sites

Fumigated and non-fumigated (SO_2) 'Mauritius' litchi fruit were collected from Halls and Sons packhouse, in Mpumalanga province, South Africa (25°27'34.5"S 30°56'43.4"E). All fruit were then transported to the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory in Nelspruit, Mpumalanga (25°27'06.7"S; 30°58'10.9"E) where the experiment was conducted.

3.2 Sulfur dioxide (SO_2) treatments

On arrival at the postharvest laboratory, fruit were sorted for uniform size, absence of defects and diseases symptoms. Fumigated (SO_2) 'Mauritius' fruit, which served as a positive control were obtained by burning 99% pure sulfur powder under a tarpaulin (1 kg sulfur powder/1600 kg of fruit) for 20-30 minutes in ventilated crates. This fumigation procedure was commercially carried out by packhouse workers where fruit were collected. Untreated 'Mauritius' fruit were included in this trial as a negative control. Four different 356 x 260 mm Uvasys SO_2 sheets (Figure 3.1) were obtained from Tessara (Pty) Ltd in Cape Town. These sheets contain > 98% pure $Na_2S_2O_5$ salt encapsulated in wax matrix between a thin polyester film (top layer) and inert non-woven layer (bottom layer). Uvasys SO_2 sheets generate SO_2 gas by reacting with moisture produced by the fruit during cold storage as shown in Figure 3.2 (Wermund *et al*., 2014).

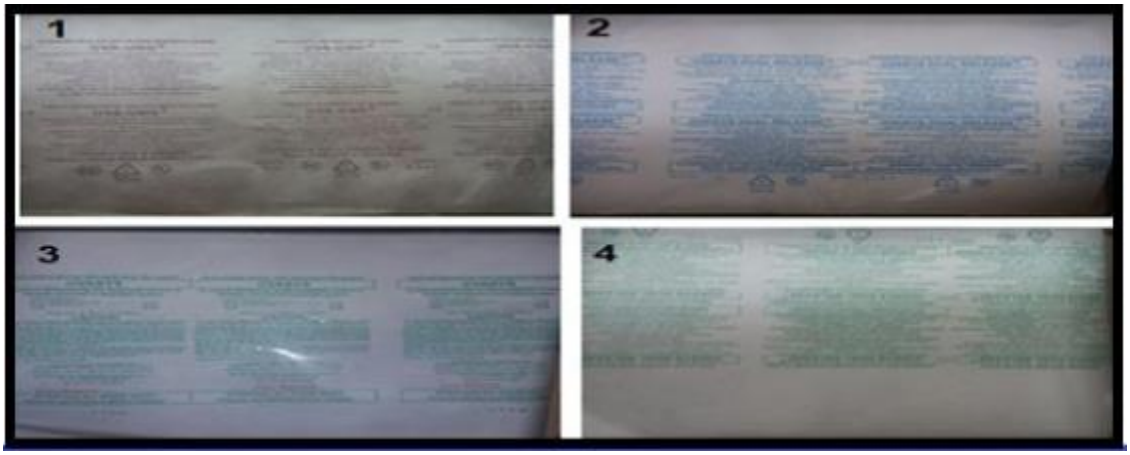

Figure 3.1 Different Uvasys sulfur dioxide (SO_2) sheets: (**1**) Uva-Uno (29% $Na_2S_2O_5$); (**2**) Dual-Release-Blue (35.85% $Na_2S_2O_5$); (**3**) Slow-Release (36.5% $Na_2S_2O_5$) and (**4**) Dual-Release-Green (37.55% $Na_2S_2O_5$)

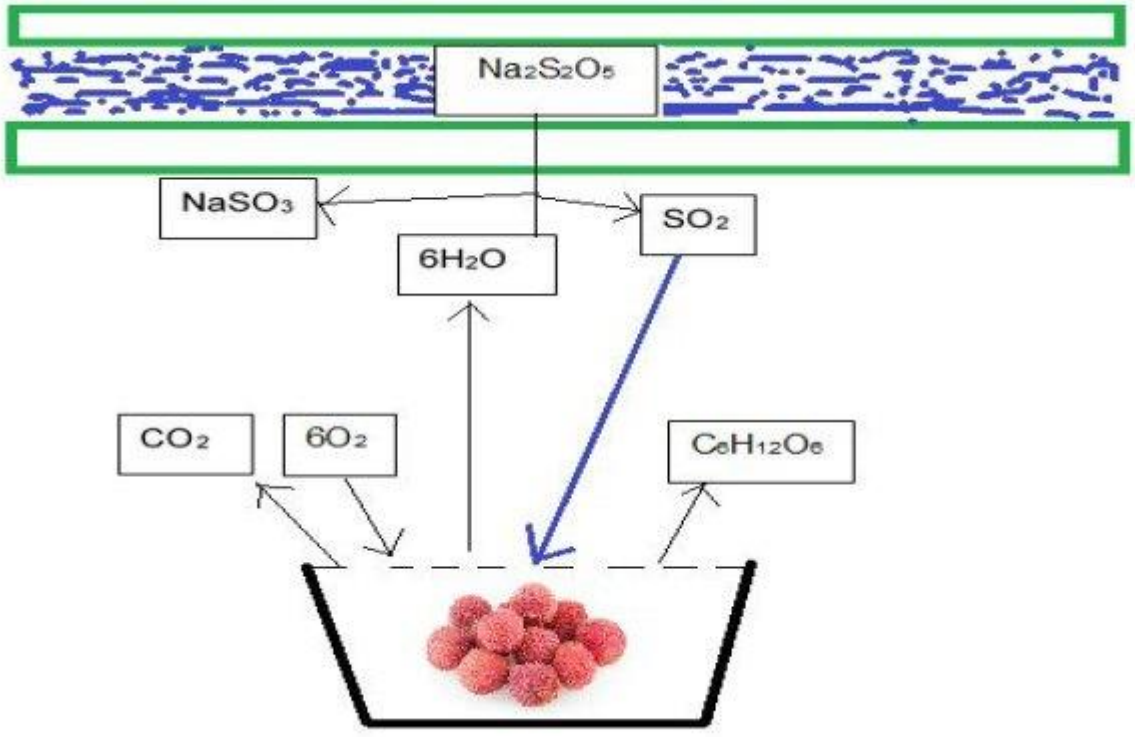

Figure 3.2 Schematic presentation of how Uvasys SO_2 packaging sheets work

3.3 Packaging procedure

Approximately 624 untreated control and SO_2 fumigated ‘Mauritius’ fruit were equally packed in 24 plastic punnets (140 x 115 mm size) and plastic bags (500g size bag) inside corrugated boxes, respectively. Whereas, approximately 1248 untreated ‘Mauritius’ fruit were equally packed in 48 plastic punnets and bags, thereafter; each of the four Uvasys SO_2 sheets was placed on top of each packaging material (Figure 3.3). Treatments (Control, SO_2 fumigation and 4 different Uvasys SO_2 sheets) had 3 replicates in plastic punnets and bags. ‘Mauritius’ fruit from all treatments were then cold stored at 4 °C for 10 days. After withdrawal from cold storage, pericarp colour parameters were evaluated at day 1, 3 and 5 of fruit shelf-life. Ten litchi fruit per replicate were evaluated for pericarp colour parameters (n=30), while the remaining fruit used for observation and sampling.

Figure 3.3 Uvasys SO_2 sheet-packaging procedure for litchi fruit

3.4 Fruit pericarp colour evaluation

Pericarp colour was measured objectively using a chromameter (Konika Minolta CR-400 model: DFM50) by averaging three measurements taken around ‘Mauritius’ fruit equator. Data was expressed as pericarp lightness (L*=lightness ranging from 0-100), chroma (C*) and hue angle (h^o). From the chlorophyll a* (-greenness to +redness) and b* (-blueness to +yellowness) values obtained, pericarp hue angle was calculated using the following formulae:

$$h° = \tan^{-1}\left(\frac{b*}{a*}\right)$$

For a 100% saturated red, the hue angle is 30°. In this study, a hue angle of ≥40° would indicate considerable pericarp browning. Furthermore, pericarp chroma, which represent pericarp colour saturation was calculated using the following formulae:

$$C^* = \sqrt{(a*^2 + b*^2)}$$

Severity of pericarp browning per replicate was examined as pericarp browning index (BI) calculated using the following formula (Maskan, 2001).

$$BI = \frac{(X - 0.31) \times 100}{0.17}$$

Where $X = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)}$

3.5 Experimental design and data analysis

Three-factor analysis of variance in a completely randomised design was used to extract information about the effect of main three treatment factors (packaging materials, SO_2 treatments and shelf-life periods) and their second and third order interactions on triplicates pericarp colour parameters of 'Mauritius' fruit. Analysis of variance was performed on the data using Statistical Analysis System (SAS) for windows 9.4. Treatment means were separated using Duncan Multiple Range test at 5% level of significance. Pearson's correlation coefficients were also calculated to determine the relationship between pericarp colour parameters and SO_2 treatment levels separately per packaging materials and per day of storage and shelf-life.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results

The influence of packaging materials, sulfur dioxide (SO_2) treatments, storage periods and their interactions on 'Mauritius' fruit pericarp colour parameters are presented in Table 4.1. Highly significant differences for SO_2 treatments ($P < 0.001$) were observed in fruit pericarp browning index (BI), chroma (C*) and (L*). However, SO_2 treatments had no significant effect ($P = 0.21$) on fruit pericarp hue angle (h°). Packaging materials showed highly significant differences ($P < 0.01$) with respect to pericarp L*. Furthermore, significant differences ($P = 0.02$) for packaging materials were also observed for pericarp BI. Moreover, storage periods showed highly significant differences ($P < 0.01$) in all fruit pericarp colour parameters.

Packaging materials and SO_2 treatments interaction had no significant effect on fruit pericarp BI. However, significant ($P \leq 0.05$) and highly significant ($P < 0.01$) differences were observed on fruit pericarp L*, C* and h°, respectively. An interaction between SO_2 treatments and storage periods showed significant differences ($P \leq 0.01$) on all fruit pericarp colour parameters, except for pericarp BI. Highly significant differences ($P < 0.01$) for packaging materials and storage periods interaction were noted on all fruit pericarp colour parameters, except on pericarp C*. Nonetheless, packaging materials and storage periods interaction had a significant effect ($P \leq 0.05$) on fruit pericarp h°. In addition, an interaction of packaging materials, SO_2 treatments and storage periods had no significant effect ($P > 0.05$) on all fruit pericarp colour parameters.

Table 4.1 Analysis of variance (ANOVA) for the influence of packaging materials, SO_2 treatments, storage periods and their interactions on 'Mauritius' fruit pericarp colour parameters

Pericarp colour parameters	**A**	**B**	**C**	**A X B**	**A X C**	**B X C**	**A X B X C**
Browning index (BI)	0.00**	0.02*	0.00**	**0.10^{ns}**	**0.26^{ns}**	0.00**	**0.33^{ns}**
Hue angle (h°)	**0.21^{ns}**	**0.09^{ns}**	0.00**	0.01*	0.00**	0.05*	**0.16^{ns}**
Chroma (C*)	0.00**	**0.91^{ns}**	0.00**	0.00**	0.01*	**0.26^{ns}**	**0.29^{ns}**
Lightness (L*)	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	**0.96^{ns}**

A = SO_2 treatments, **B** = Packaging materials, **C** = Storage periods

**Highly significant at P < 0.01, *Significant at P ≤ 0.05, ^{ns} not significant at P < 0.05

Only significant means of packaging materials, SO_2 treatments, storage periods and their second order interactions were separated and explained. The mean values of main treatment effects on fruit pericarp colour parameters and their second order interactions are presented in Table 4.2 and Figure 4.1 to Figure 4.4, respectively. It must be noted that fruit pericarp colour parameters were not evaluated on the fifth day of shelf-life period in all treatments under plastic-bags due to spoilage. Whereas; in plastic-punnets, pericarp colour parameters were evaluated only in commercially fumigated (SO_2) fruit during day 5 shelf-life period. Therefore, all results of pericarp colour parameters after day 5 of shelf-life include commercial SO_2 fumigated fruit packed in plastic-punnets.

4.1.1 Pericarp browning Index

'Mauritius' litchi fruit pericarp browning index (BI) was lower in commercial SO_2 fumigation than in control and all Uvasys SO_2 sheets (Table 4.2). On average, fruit pericarp BI was found to be higher for fruit packed in plastic-bags when compared with plastic-punnets. Furthermore, fruit pericarp BI was higher after day 1 of shelf-life, thereafter, decreased significantly during day 3 and 5 of shelf-life.

4.1.2 Pericarp hue angle

Commercial SO_2 fumigation showed higher fruit pericarp hue angle (h°) than control and all Uvasys SO_2 sheets throughout the storage and following 5 days of shelf-life (Table 4.2). There were no significant differences between mean values of packaging materials on fruit pericarp h°. Moreover, fruit pericarp h° was higher after day 1 of shelf-life, which significantly decreased as shelf-life increased.

Table 4.2 Mean values of packaging materials, SO_2 treatments and storage periods on pericarp colour parameters of 'Mauritius' fruit stored at 4 °C for 10 days and followed by 5 days of shelf-life

Pericarp colour Parameters	Browning index (BI)	hue angle (h°)	Chroma (C*)	lightness (L*)
Treatments				
Control	113.82±3.50^{a}	38.67±1.16^{ab}	31.16±1.13^{b}	42.82±1.25^{b}
SO_2 fumigation	95.13±3.4^{b}	40.33±2.06^{a}	35.92±1.55^{a}	56.75±1.52^{a}
Uva-Uno	110.65±2.9^{a}	37.93±1.3^{ab}	30.63±1.0^{b}	42.73±0.7^{b}
Dual Release Blue	113.28±3.2^{a}	38.22±1.14^{ab}	30.14±0.9^{b}	41.76±0.6^{b}
Slow Release	112.47±2.9^{a}	37.34±0.9^{b}	30.98±0.8^{b}	42.93±0.7^{b}
Dual Release Green	112.97±3.7^{a}	38.49± 0.9^{ab}	30.43±0.8^{b}	42.06±0.8^{b}
LSD	**6.25**	**3.69**	**2.00**	**1.87**
Packaging				
Plastic-punnets	107.45±1.6^{b}	39.12±0.7^{a}	31.69±0.5^{a}	45.88±0.8^{a}
Plastic-bags	111.13±2.5^{a}	37.93±0.8^{a}	31.64±0.8^{a}	44.40±1.0^{b}
LSD	**3.60**	**2.14**	**1.15**	**1.08**
Shelf-life periods (days)				
0	109.02±2.0^{b}	39.13±0.8^{ab}	34.63±0.4^{a}	49.30±1.3^{a}
1	122.39±2.5^{a}	40.29±0.8^{a}	33.82±0.9^{ab}	43.92±1.2^{b}
3	97.23±1.08^{c}	36.45±1.04^{b}	26.58±0.5^{c}	41.98±0.7^{c}
5	98.16±2.8^{c}	35.59±0.7^{b}	31.24±0.3^{b}	48.79±1.5^{a}
LSD	**4.42**	**2.62**	**1.42**	**1.33**

Different letters denote significant differences at $P < 0.05$.
Values are means± Standard error of triplicates

4.1.3 Pericarp chroma

'Mauritius' litchi fruit treated with commercial SO_2 showed higher pericarp chroma (C*) than control and all Uvasys SO_2 impregnated sheets (Table 4.2). However, fruit pericarp C* values were not significantly different in both packaging materials.

Furthermore, fruit pericarp C* decreased from 0 to 3 days of shelf-life, thereafter, increasing after day 5 of shelf-life.

4.1.4 Pericarp lightness

'Mauritius' fruit fumigated with standard SO_2 showed higher pericarp lightness (L*) compared with control and all Uvasys SO_2 sheets during storage and shelf-life (Table 4.2). Higher fruit pericarp L* was recorded in fruit packed in plastic-punnets, when compared with plastic-bags. Furthermore, fruit pericarp L* decreased from 0 to 3 days of shelf-life, subsequently, increasing after 5 days of shelf-life.

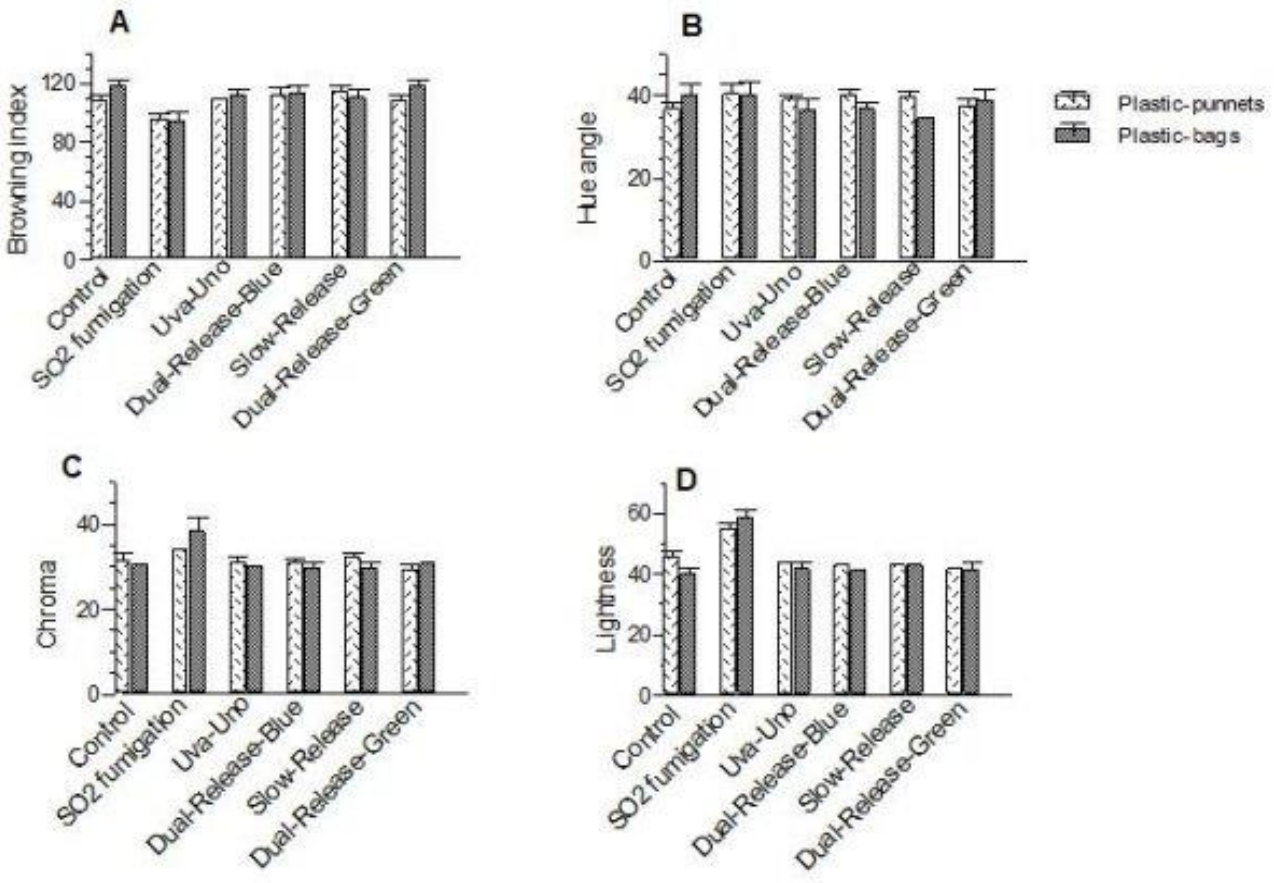

Figure 4.1 An interactive effect of SO_2 treatments and packaging materials on pericarp (**A**) browning index, (**B**) hue angle, (**C**) chroma and (**D**) lightness of 'Mauritius' fruit stored at 4 °C for 10 days and followed by 5 days of shelf-life. Values are means± Standard error of triplicates

Commercial SO_2 fumigation in both packaging materials resulted in lower fruit pericarp BI than control and all Uvasys SO_2 sheets (Figure 4.1A). Additionally, higher fruit pericarp h°, C* and L* in both packaging materials was found in SO_2 fumigation

treatment when compared with control and all Uvasys SO_2 sheets (Figure 4.1B-D). Overall results show that fruit packed in plastic-punnets had higher pericarp L*, C* and h° when compared with plastic-bags (Figure 4.1B-D).

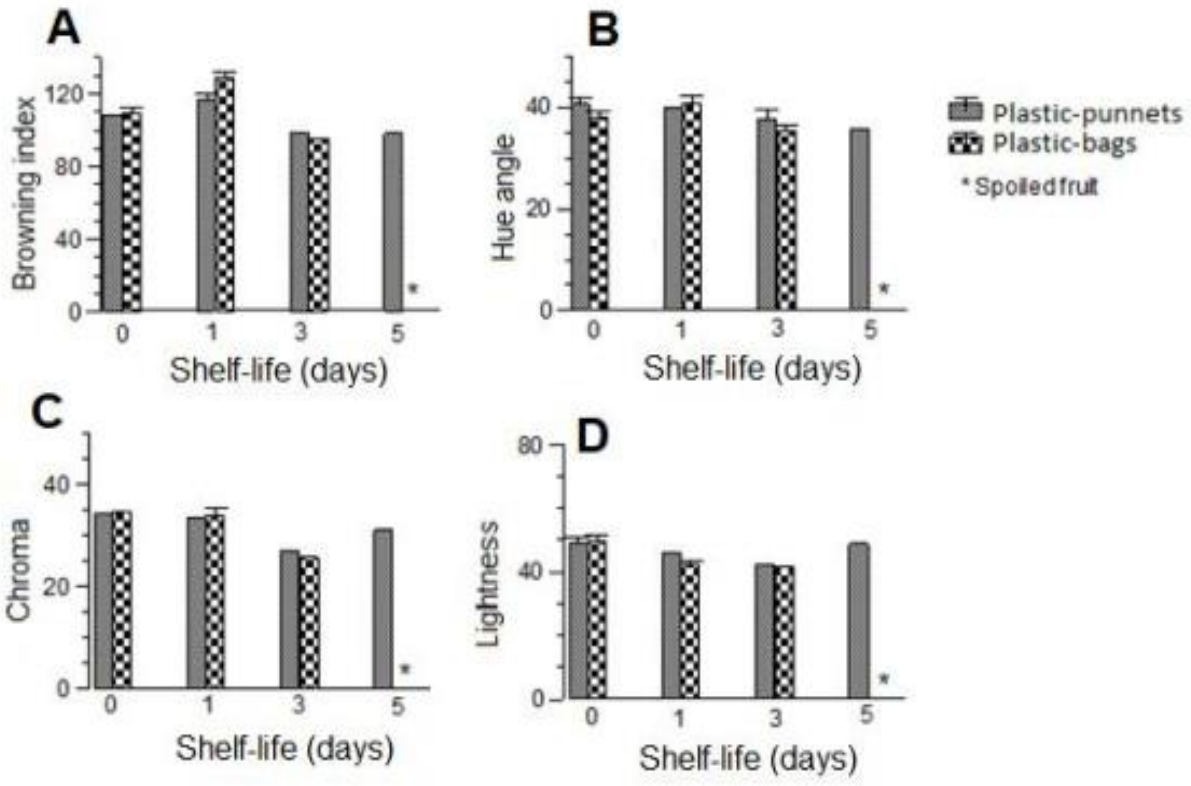

Figure 4.2 An interactive effect of packaging materials and storage periods on pericarp (**A**) browning index, (**B**) hue angle, **(C**) chroma and (**D**) lightness of 'Mauritius' fruit stored at 4 °C for 10 days and followed by 5 days of shelf-life. Values are means± Standard error of triplicates

Higher pericarp BI, h° and C* was observed in fruit stored in plastic-bags when compared with plastic-punnets after day 1 of shelf-life (Figure 4.2A-C). However, plastic-punnets resulted in higher fruit pericarp BI, h° and C* than plastic-bags at day 3 of shelf-life. Meanwhile, higher fruit pericarp L* was found in plastic-punnet packed fruit when compared with plastic-bags after 1 and 3 days of shelf-life (Figure 4.2D).

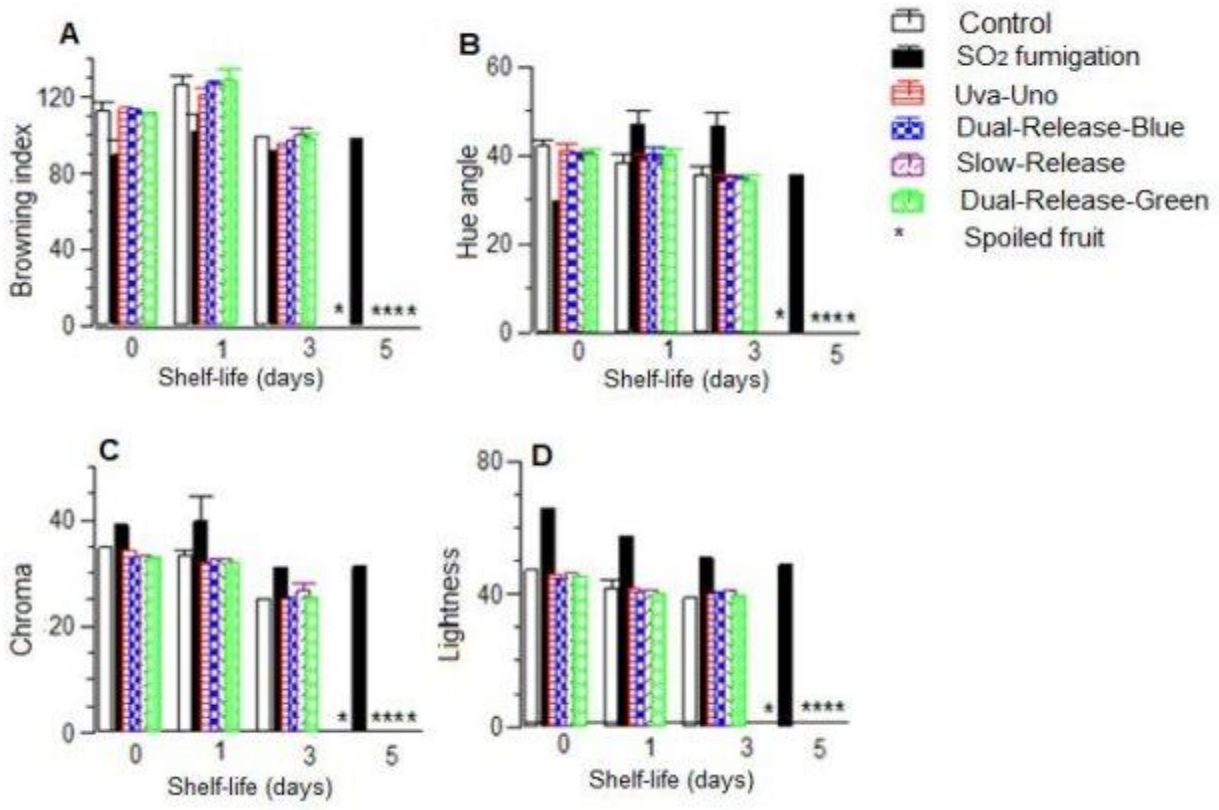

Figure 4.3 An interactive effect of SO_2 treatments and storage periods on pericarp (**A**) browning index, (**B**) hue angle, (**C**) chroma and (**D**) lightness of 'Mauritius' fruit stored at 4 °C for 10 days and followed 5 days of shelf-life. Values are means± Standard error of triplicates

Commercial SO_2 fumigation resulted in lower fruit pericarp BI than control and all Uvasys SO_2 sheets throughout the storage and 5 days shelf-life period (Figure 4.3A). However, Uva-Uno SO_2 treatment resulted in lower fruit pericarp BI than control fruit after 1 and 3 days of shelf-life. Furthermore, Figure 4.3B-D show that SO_2 fumigated fruit had higher pericarp h°, C* and L* when compared with control and all Uvasys SO_2 sheets. In general, 'Mauritius' fruit pericarp C* decreased with increasing shelf-life, irrespective of treatments (Figure 4.3C). In addition, 'Mauritius' fruit showed a trend of decreasing pericarp L* as shelf-life increased.

Table 4.3 Pearson's correlation coefficients between postharvest SO_2 treatments and pericarp colour parameters in 'Mauritius' fruit stored at 4°C for 10 days and followed by 5 days of shelf-life

Pericarp colour Parameters	**Plastic-punnets**			**Plastic-bags**		
	1 d	3 d	5 d	1 d	3 d	5 d
Browning index (BI)	-0.430	-0.272	-	-0.313	-0.175	-
Hue angle (h°)	0.518*	0.573*	-	0.390	0.329	-
Chroma (C*)	-0.099	0.399	-	0.447	0.718**	-
Lightness (L*)	0.393	0.529*	-	0.661**	0.777**	-

* Correlation is significant at $P < 0.05$

** Correlation is significant at $P < 0.01$

- Correlation could not be computed because at least one of the variable was constant due to fruit spoilage

4.1.5 Pearson's correlation analysis

The correlation analysis data obtained for 5 days of shelf-life showed weak negative relationship between postharvest SO_2 treatments and fruit pericarp BI in both packaging materials after day 1 and 3 of shelf-life (Table 4.3). Moderate and weak positive correlations were observed for fruit pericarp h° in plastic-punnets and plastic-bags during shelf-life, respectively. However, correlation between postharvest SO_2 treatments and fruit pericarp hue angle was only significant in plastic-punnets, in contrast with plastic-bags. Furthermore, SO_2 treatments and fruit pericarp C* in plastic-punnets showed weak negative and positive correlation after day 1 and 3 of shelf-life, respectively. Whereas, in plastic-bags, highly significant and strong correlation was observed between SO_2 treatments and pericarp C* after 3 days of shelf-life. 'Mauritius' fruit pericarp L* showed positive correlation with postharvest SO_2 treatments in both packaging materials after day 1 and 3 of shelf-life period. Nevertheless, these correlations were significant in plastic-punnets at day 3 of shelf-life and highly significant after day 1 and 3 of shelf-life in plastic-bags.

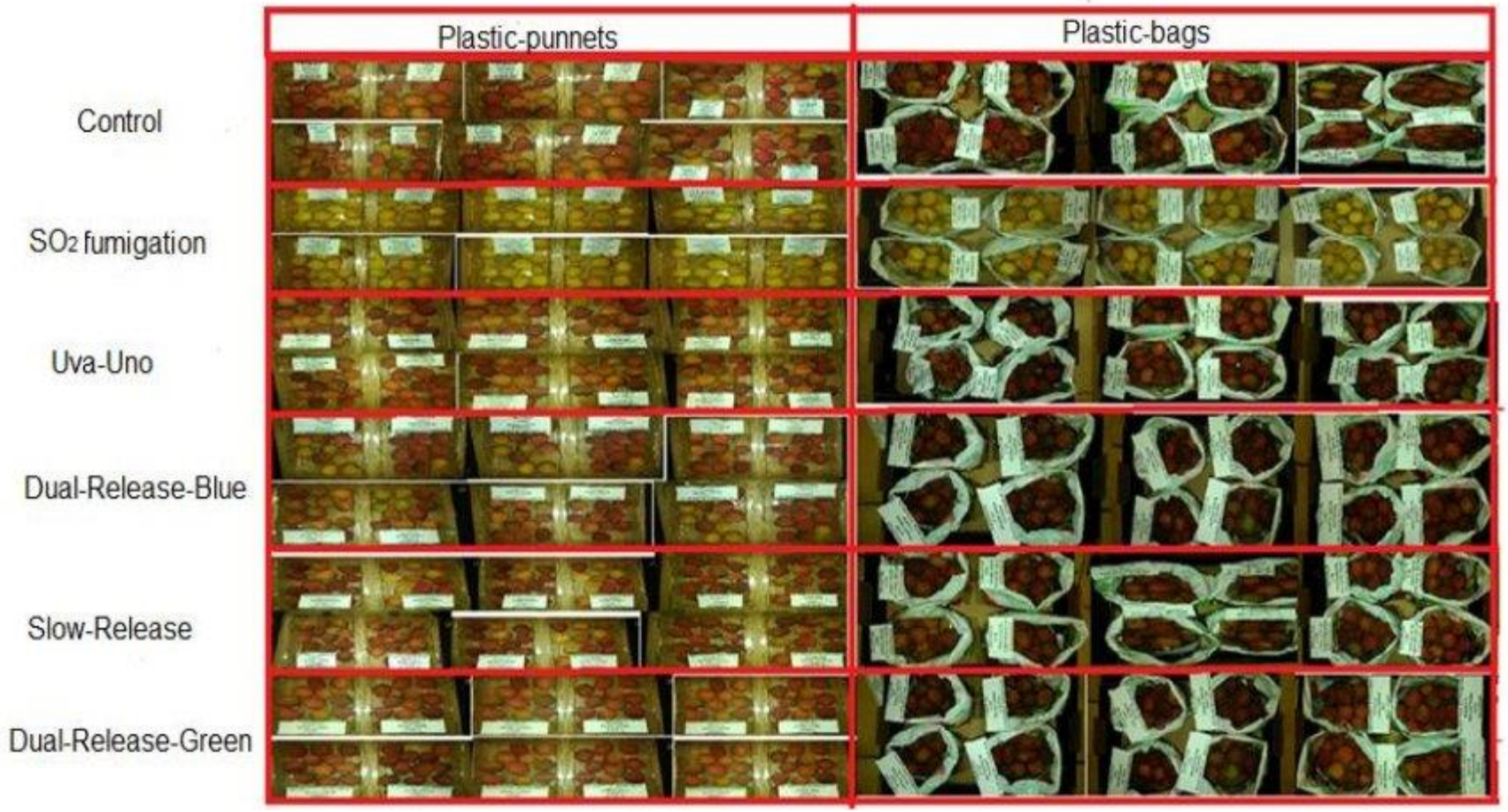

Figure 4.4 ‘Mauritius’ litchi fruit packed in plastic-punnets and bags during day 0 of shelf-life

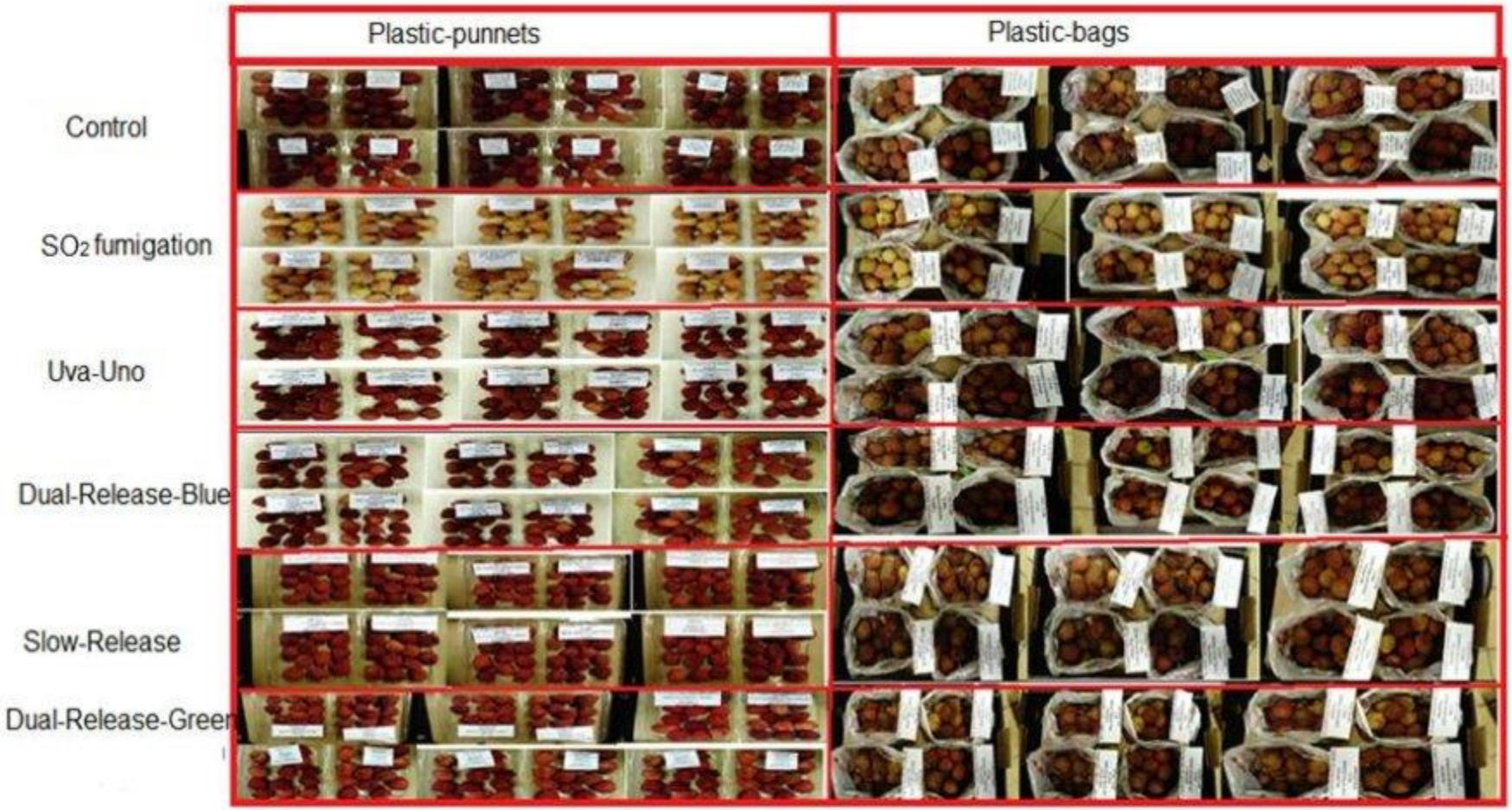

Figure 4.5 ‘Mauritius’ litchi fruit packed in plastic-punnets and bags after day 1 of shelf-life

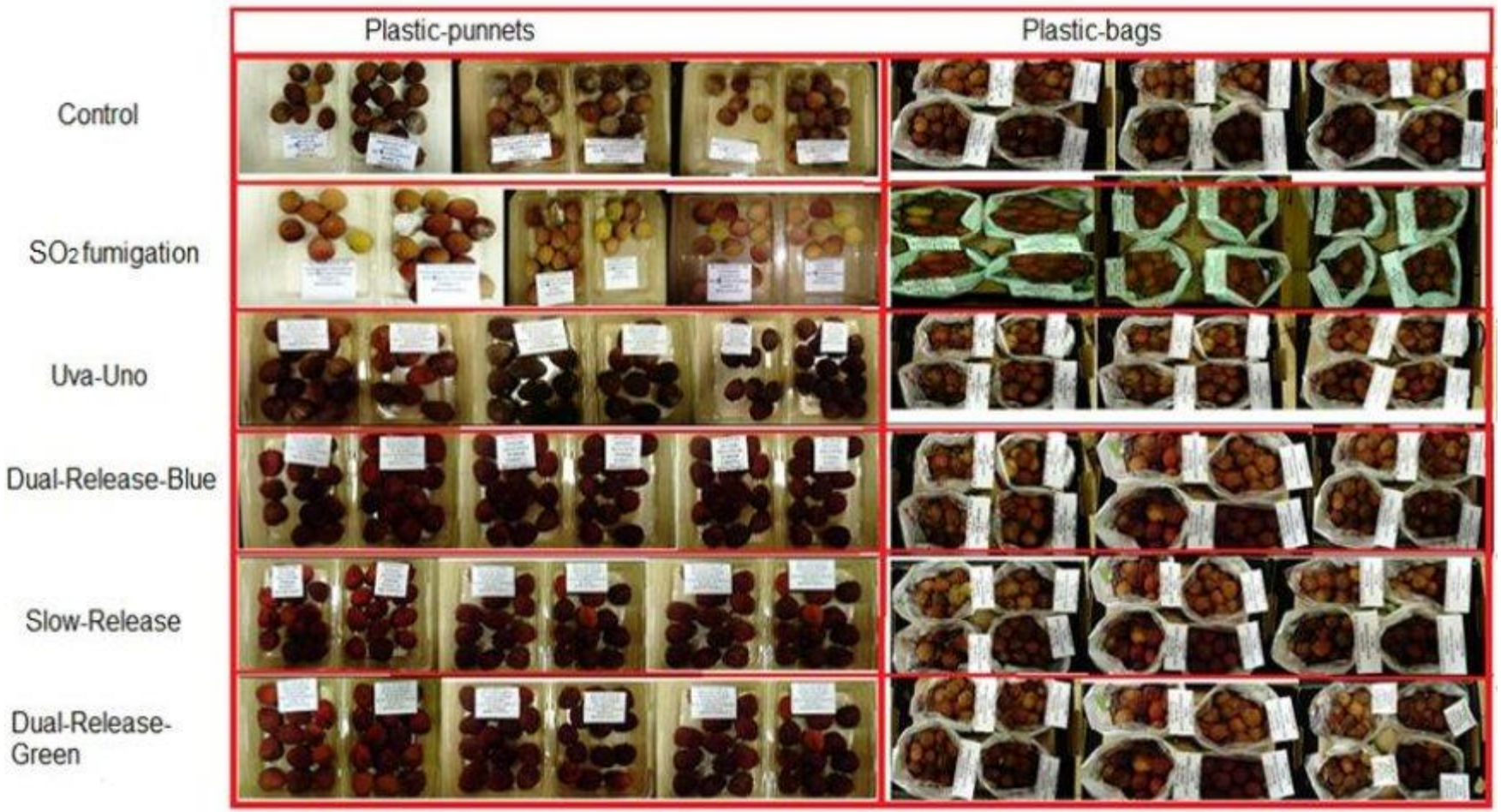

Figure 4.6 ‘Mauritius’ litchi fruit packed in plastic-punnets and bags after day 3 of shelf-lie

4.2 Discussion

Results indicated that changes on fruit pericarp BI, L* and C* were mainly influenced by SO_2 treatments, which agreed with the findings documented on ‘Shahi and China’ litchi fruit (Kumar *et al*., 2013), and ‘Mauritius’ litchi fruit (Somboonkaew and Terry, 2011). According to Joas *et al*. (2005), SO_2 treatments altered pericarp pH and stability, co-pigmentation and spectra of the anthocyanins of stored ‘Mauritius’ fruit.

Packaging type influenced pericarp BI and L*; whereas, pericarp h° and C* were not differently affected by type of packaging. This was consistent with the results reported on ‘Érdi jubileum and Érdi bőtermő’ sweet cherry fruit (Davarynejad *et al*., 2014) and ‘Bombay’ litchi fruit (Molla *et al*., 2017). In this study, a significant decrease in all fruit pericarp colour parameters in relation to shelf-life periods was observed, which was also reported on ‘Shahi’ litchi fruit (Mangaraj *et al*., 2012), and ‘Gola’ litchi fruit (Ali *et al*., 2016). A decrease in all pericarp colour parameters indicated that ‘Mauritius’ fruit became less red (lower C*, L*) and darker (higher BI, h°) as shelf-life period advance.

In this present work, fruit pericarp BI was not significantly affected by second and third order interactions of the three main factors. However, SO_2 treatments and packaging materials interaction had a distinct effect in pericarp L*, C* and h°, which was in agreement with Sivakumar and Korsten (2006a) on ‘Mauritius’ litchi fruit. According to Mgaya-Kilima *et al*. (2015), ‘Dodo’ mango fruit peel colour intensity was not affected by packaging materials and storage periods interaction which supports our findings. Gas permeability of plastic-punnets and bags could have maintained similar in-package conditions throughout the storage times (Jitareerat *et al*., 2013).

In this study, main treatment factors and their second order interactions played a significant role in elucidating influence of packaging materials, SO_2 treatments and shelf-life periods in fruit pericarp colour parameters. Thus, third order interaction of packaging materials, SO_2 treatments and storage time did not affect all fruit pericarp colour parameters.

4.2.1 Pericarp browning index

Polyphenol oxidase (PPO) and peroxidase (POD) are terminal oxidase present in litchi fruit pericarp, which are involved in degradation of anthocyanins red pigments (Zhang and Quantick, 1997). Thus, different SO_2 treatments under different packaging materials were used in this study to delay loss of anthocyanins and red pericarp colour of 'Mauritius' fruit quantified as browning index. Uva-Uno effectively reduced pericarp browning (Lower pericarp BI) than control and other Uvasys SO_2 sheets, but not effective as commercial SO_2 fumigation, irrespective of shelf-life periods and packaging materials. This observation suggested that SO_2 fumigation inhibited activities of browning promoting enzymes (PPO and POD) effectively than other treatments (Kumar *et al*., 2012). However, commercially fumigated (SO_2) fruit were bleached in both packaging materials (Figure 4.4-4.6) which was also observed on 'Mauritius' fruit (Sivakumar and Korsten, 2006b).

According to Zoffoli *et al*. (2009), SO_2 fumigation is used in table grape industry as a preservative agent and inhibitor of PPO and POD activities. This may also explain the role of SO_2 fumigation in this study to preserve fruit pericarp colour (Fuchs *et al*., 1993). Higher loss of red pericarp colour in control fruit was due to anthocyanin pigments degradation by condensing with quinones formed from endogenous phenolics due to the action of PPO and POD. These results support the findings by Liang *et al*. (2012), who found that 60 gL^{-1} $Na_2S_2O_5$ + 1.1M HCL dipping was effective in reducing pericarp browning of 'Feizixiao' litchi fruit compared with control and 30 gL^{-1} $Na_2S_2O_5$ + 1.1M HCL dipping.

In 'Taiso' (Ramma, 2014), 'Feizixiao' (Liang *et al*., 2012), and 'Rose Scented' (Kumar *et al*., 2013) litchi fruit, pericarp tended to become brown as storage time progressed. However, these findings contradicted our results as fruit pericarp BI significantly decreased after day 3 of shelf-life (Figure 4.2A and 4.3A). This observation implies that relative humidity (RH) around 'Mauritius' fruit stored in plastic-punnets and bags could have reduced or prevented weight loss and desiccation associated with pericarp browning (Pesis *et al*., 2002; Sivakumar *et al*., 2008). Therefore, desiccation related pericarp browning was not evident in this study, but due to decay as reported by Sivakumar *et al*. (2008). Nevertheless, reduced browning in fruit 'Mauritius' litchi fruit was noted in plastic-punnets than in bags. Thus, the assumption is that plastic-punnets

may have maintained sufficient optimum RH and reduced the supply of oxygen for enzymatic oxidation of phenolics than plastic-bags (Zhang and Quantick, 1997). Furthermore, 'Mauritius' fruit packed in plastic-bags were terminated after day 5 of shelf-life due to decay caused by condensation of water vapour in the package (Molla *et al*., 2017). Although there are health concerns regarding the use of SO_2 fumigation, 'Mauritius' fruit pericarp browning was well delayed by combination of commercial SO_2 fumigation + plastic-punnet packaging.

4.2.2 Pericarp hue angle

Litchi pericarp colour can be measured as hue angle (h°), with a hue angle of 30-40° indicating red fruit; while a hue angle above 40° indicating a brown fruit, and a further increasing hue angle indicating a fruit becoming yellow (Archibald and Bower, 2008). Generally, yellow coloured litchi fruit can result from bleaching effect caused by commercial SO_2 fumigation (Sivakumar *et al*., 2008), which affirmed our findings since SO_2 fumigation treatment bleached 'Mauritius' fruit pericarp (Figure 4.4-4.6), thereby producing higher pericarp h° than other treatments. Somboonkaew and Terry (2011) also observed bleached SO_2 fumigated 'Mauritius' fruit stored in different packaging materials. Pericarp bleaching is caused by sulfites through nucleophilic ion reactions, whereby a negative ion of sulfuric acid attacks the flavylium cation, forming a colourless anthocyanin; chromenol-4-sulfonic acid complex in the pericarp (Neog and Saika, 2010). However, Slow-Release and Uva-Uno in plastic-bags produced unbleached fruit with reddish-pink pericarp during storage (Figure 4.4-4.5).

A general decrease in pericarp h° with advancement of shelf-life was observed in all treatments except for commercial SO_2 fumigation, irrespective of packaging materials (Figure 4.3B). Similarly, results of pericarp h° are consistent with those reported by Chaiprasart (2004), who also found 'Hong Huay' litchi fruit pericarp h° to decrease with storage. Nevertheless, pericarp h° of ethylenediaminetetraacetic acid, calcium disodium salt hydrate (EDTA) treated 'Shahi' litchi fruit stored in different polymer films increased concomitantly with storage (Mangaraj *et al*., 2012), which supports our results with respect to commercial SO_2 fumigated 'Mauritius' fruit. A combination of Slow-Release and plastic-bags showed acceptable pericarp h° (≤40°) indicating reddish-pink 'Mauritius' fruit (Figure 4.1B). However, these fruit did not reach day 5 of shelf-life following fungal growth and spoilage (Data not shown).

4.2.3 Pericarp chroma

High C* value indicates a high colour saturation and/or purity, while a decrease in C* value indicates loss of colour saturation and/or loss of red colour (De Reuck, 2010). Pericarp C* mean values represented in Table 4.2 and Figure 4.1C to 4.3C indicate that SO_2 fumigated 'Mauritius' fruit had higher colour saturation (Higher pericarp C*) when compared with control and all Uvasys SO_2 sheets. However, this saturation was dominated by yellowish-pink pericarp colour as a result of bleaching. Similar effect was also reported on SO_2 fumigated 'Mauritius' fruit (Sivakumar and Korsten, 2006a; Somboonkaew and Terry, 2011). On average, 'Mauritius' fruit pericarp C* was slightly higher in plastic-punnets (31.69±0.5) than in plastic-bags (31.64±0.8), which were not significantly different. This suggested that plastic-punnets could have maintained slightly higher relative humidity around 'Mauritius' fruit than plastic-bags, thereby, reducing loss of red pericarp colour (De Reuck *et al*., 2009).

'Mauritius' fruit pericarp colour intensity was reduced as shelf-life progressed, irrespective of SO_2 treatments and control (Figure 4.3C), which corresponded with the results reported on SO_2 fumigated and SO_2 free 'Mauritius' fruit (De Reuck *et al*., 2009; Sombookaew and Terry, 2011). However, an interaction of packaging materials and shelf-life times (Figure 4.2C) showed that 'Mauritius' fruit pericarp C* decreased from 0 to 3 days of shelf-life, and significantly increased after day 5 in fruit stored in plastic-punnets. This is because only SO_2 fumigated 'Mauritius' fruit in plastic-punnets were quantified for pericarp C* since fruit in other treatments were discarded due to spoilage. Nonetheless, red pericarp C* loss was retarded effectively by commercial SO_2 fumigation + plastic-bag packaging.

4.2.4 Pericarp lightness

In this present work, 'Mauritius' fruit pericarp browning was also estimated as a measure of pericarp lightness (L*), because brown fruit showed lower pericarp L* compared with reddish-pink fruit. Thus, reddish-yellow (Higher pericarp L*) 'Mauritius' fruit pericarp was observed in SO_2 fumigation when compared with other treatments which was consistent with Somboonkaew and Terry (2011) on 'Mauritius' litchi fruit. Furthermore, plastic-punnets (High gas permeability) showed reddish-pink 'Mauritius' fruit when compared with plastic-bags (Low gas permeability) fruit during shelf-life. This is because low permeability packaging material (plastic-bags) could result in high

CO_2 accumulation, thereby, producing red-brown dark litchi pericarp (De Reuck *et al*., 2009). Similar results were reported on wild strawberries packed in cups with and without micro-perforations (Almenar *et al*., 2007).

Davarynejad *et al*. (2014) found peels of 'Érdi jubileum and Érdi bőtermő' sweet cherry fruit packed in polyethylene covers to become darker with increasing storage time. In our findings, 'Mauritius' fruit pericarp also became darker, irrespective of treatments and packaging materials from 0 to 3 days of shelf-life. However, pericarp L* increased after 5 days of shelf-life since only SO_2 fumigated 'Mauritius' fruit in plastic-punnets were sampled; meanwhile, fruit in plastic-bags were spoiled and discarded.

A study by Molla *et al*. (2017) also showed that sodium hypochlorite solution and chitosan coating dipping resulted in 'Bombay' litchi fruit shelf-life termination after 3 and 6 days under polyethylene bags in ambient conditions. Although, SO_2 fumigation + plastic-bag packing showed higher 'Mauritius' fruit pericarp L* without any browning, SO_2 fumigation persistently maintained yellow to pale fruit due to bleaching. In both packaging materials, Slow-Release produced reddish-brown fruit without bleaching after 3 days of shelf-life. Therefore, Slow-Release + either packaging effectively delayed loss of 'Mauritius' fruit pericarp L*.

4.2.5 Pearson's correlation analysis

Sulfur dioxide has long been known as an inhibitor of browning promoting enzymes PPO and POD (Fuchs *et al*., 1993). However, little information is available about the relationship between SO_2 treatment levels as an antioxidant, and pericarp browning expressed as BI, L*, C* and h°. Our correlation analysis in Table 4.3 showed that 'Mauritius' fruit pericarp browning was minimally reduced by commercial SO_2 treatments, as SO_2 levels showed a negative but weak correlation with pericarp BI in both packaging materials after 1 and 3 days of shelf-life.

'Mauritius' fruit became brown-yellow (increasing pericarp h°) as SO_2 levels increased. Nevertheless, this effect was moderate in both packaging materials. 'Mauritius' fruit pericarp colour (yellowish-pink) intensity was more pronounced in plastic-bags after 3 days of shelf-life, following a strong positive linear relationship (R=0.718) with SO_2 levels. This indicated that better colour saturation was maintained in 'Mauritius' fruit treated with higher SO_2 levels (commercial SO_2 fumigation). 'Mauritius' fruit became reddish-yellow as SO_2 levels increased during storage and following 5 days of shelf-

life period. This was evident in plastic-bags, whereby, SO_2 levels showed strong positive correlations (R=0.66 and 0.77, respectively) with pericarp L* after 1 and 3 days of shelf-life. Therefore, loss of ‘Mauritius’ fruit red pericarp colour was better delayed as SO_2 levels increased, predominantly in plastic-bags.

CHAPTER 5
SUMMARY, CONCLUSIONS AND RECOMMENDED FUTURE RESEARCH

5.1 Summary

To expand the South African Litchi Industry and attract new profitable markets, considerable attention must be given on red pericarp colour retention and reduction of browning. Consequently, the study aimed at investigating the potential of Uvasys SO_2 sheets in delaying pericarp browning of 'Mauritius' litchi fruit during storage and shelf-life. At present, litchi exporting countries sulfur fumigate (SO_2) their fruit to preserve red litchi skin colour. However, sulfur dioxide (SO_2) fumigated litchi fruit are yellow to pale green; have low consumer appeal; constitute a health hazard for asthmatics, has led to a 10 $mg.kg^{-1}$ limit of SO_2 residues in fresh pulp being set for some European markets. Thus, SO_2 fumigated 'Mauritius' litchi fruit were included in this work for comparison since there is a need to replace SO_2 fumigation treatment. Moreover, Uvasys SO_2 sheets have been used successfully as packaging sheets in table grapes, therefore, the objective of this study was to assess their effectiveness in preserving red colour of 'Mauritius' litchi fruit packaged in perforated plastic-punnets and bags.

Our results demonstrated 'Mauritius' fruit performance with respect to different Uvasys SO_2 sheets in plastic-punnets and bags. Overall, 'Mauritius' fruit retained better pericarp colour when stored in plastic-bags. Uva-Uno (29% $Na_2S_2O_5$) and Slow-Release (36.5% $Na_2S_2O_5$) plus plastic-bag packaging resulted in lower 'Mauritius' fruit pericarp BI and h°, and higher pericarp C* and L* than control. However, the current used commercial SO_2 fumigation treatment in plastic-bag maintained lower 'Mauritius' fruit pericarp BI and higher h°, C* and L* than other treatments. Moreover, fruit decayed in all treatments after day 3 of shelf-life which is speculated to be the major cause of 'Mauritius' fruit browning observed in this study.

5.2 Conclusions

Uvasys SO_2 sheets with plastic-bags showed a potential to delay pericarp browning of 'Mauritius' litchi fruit. Based on our correlation analysis results, it was observed that 'Mauritius' fruit red pericarp colour was better preserved as SO_2 treatment levels increase. Future research should however focus to run the same trial under controlled in-package atmosphere conditions to gases, water vapour and control over spoilage

micro-organisms. This will allow ample opportunity to determine the success of Uvasys SO_2 sheet-packaging technology in controlling browning on 'Mauritius' fruit pericarp, and potentially replacing commercial SO_2 fumigation as the dominant treatment for litchi exporting countries.

5.3 Recommended future research

In this present work, it appeared that choice of packaging material to use for Uvasys SO_2 sheets; mould growth and uncontrolled storage conditions limited the success to demonstrate whether Uvasys SO_2 sheet-packaging technology can be used to retain 'Mauritius' fruit red pericarp colour. Therefore, the following were identified as future research areas:

- Barrier properties to gases (CO_2, O_2) and water vapour of packaging materials used in this study are unknown. Consequently, further research is needed for selection of suitable packaging materials with specific permeability to create a desirable atmosphere around the fruit in Uvasys SO_2 sheet-packaging. Gases and water vapour properties of packaging materials vary according to cultivar (Hussein *et al*., 2015).

- In this research project, fungal decay was the major cause of red pericarp colour loss on 'Mauritius' litchi fruit. Thus, the use of protectants such as a biocontrol agent (*Bacillus subtilis*) or fruit coatings (chitosan) can be used to control decay within the Uvasys SO_2 sheet-packaging.

- A negative impact on fruit quality can be encountered when the fruit in Uvasys SO_2 sheet-packaging is subjected to temperature fluctuations during shipping, handling or at retail display. Therefore, storage at low refrigerated temperatures is necessary to be investigated in combination with Uvasys SO_2 sheet-packaging for litchi storage. Storage temperature may vary according to cultivar.

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APPENDICES

Appendix 1 ANOVA table for effect of packaging materials, SO_2 treatments and storage periods on ‘Mauritius’ fruit pericarp browning index

Source of variation	DF	SS	Mean Square	F Value	Pr > F
(A) SO_2 treatment	5	5247.53395	1049.50679	14.16	<.0001
(B) Packaging	1	376.49105	376.49105	5.08	0.0272
A X B	5	696.07004	139.21401	1.88	0.1084
(C) Storage periods	3	11794.56903	3931.52301	53.04	<.0001
A X C	10	945.21518	94.52152	1.28	0.2602
B X C	2	1080.55447	540.27724	7.29	0.0013
A X B X C	10	854.56527	85.45653	1.15	0.3362

Appendix 2 ANOVA table for effect of packaging materials, SO_2 treatments and storage periods on ‘Mauritius’ fruit pericarp hue angle

Source of variation	DF	SS	Mean Square	F Value	Pr > F
(A) SO_2 treatment	5	102.193659	20.438732	1.47	0.2108
(B) Packaging	1	39.769911	39.769911	2.86	0.0953
A X B	5	218.405924	43.681185	3.14	0.0127
(C) Storage periods	3	305.350861	101.783620	7.31	0.0002
A X C	10	1670.092197	167.009220	11.99	<.0001
B X C	2	85.565020	42.782510	3.07	0.0523
A X B X C	10	206.137690	20.613769	1.48	0.1641

Appendix 3 ANOVA table for effect of packaging materials, SO_2 treatments and storage periods on ‘Mauritius’ fruit pericarp chroma

Source of variation	DF	SS	Mean Square	F Value	Pr > F
(A) SO_2 treatment	5	480.716073	96.143215	11.09	<.0001
(B) Packaging	1	0.094820	0.094820	0.01	0.9170
A X B	5	149.046219	29.809244	3.44	0.0076
(C) Storage periods	3	1414.228687	471.409562	54.36	<.0001
A X C	10	107.383768	10.738377	1.24	0.2816
B X C	2	23.186229	11.593115	1.34	0.2690
A X B X C	10	105.667183	10.566718	1.22	0.2936

Appendix 4 ANOVA table for effect of packaging materials, SO_2 treatments and storage periods on ‘Mauritius’ fruit pericarp lightness

Source of variation	DF	SS	Mean Square	F Value	Pr > F
(A) SO_2 treatment	5	3495.096919	699.019384	178.57	<.0001
(B) Packaging	1	61.488135	61.488135	15.71	0.0002
A X B	5	178.544543	35.708909	9.12	<.0001
(C) Storage periods	3	1076.725136	358.908379	91.69	<.0001
A X C	10	430.510036	43.051004	11.00	<.0001
B X C	2	72.494563	36.247281	9.26	0.0003
A X B X C	10	13.613191	1.361319	0.35	0.9643