EVALUATING THE EFFECTS OF PRE-PACKAGING, PACKAGING AND VARYING STORAGE ENVIRONMENT TREATMENTS ON THE QUALITY OF AVOCADOS (*PERSEA AMERICANA* MILL.)

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As the candidate's Supervisor(s), I (we) agree/do not agree to the submission of this thesis.

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

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ABSTRACT

Avocados are characterized as climacteric fruit and are vulnerable to rapid physiological deterioration. Maintaining avocado fruit quality requires proper integrated postharvest technologies. Thus, the primary aim of this study was to evaluate the effects of combined postharvest treatments and storage conditions on the physical, chemical and subjective sensory quality parameters of the 'Hass' avocado. The study was divided into two experiments. Experiment I investigated the combined effects of postharvest handling treatments and fixed temperature (5°C or 10°C) and relative humidity (85% or 90%) on the quality of avocados for a storage period of 12 days. Experiment II focused on the combined effects of postharvest treatments and temperature-varying storage conditions (5.5°C ± 0.01°C for two days, 5°C ± 0.01°C for six days and 4.5°C ± 0.01°C for 20 days and 95% relative humidity), by simulating a realistic avocado cold chain for 28 days. A Randomised Complete Block Design with pre-packaging (hot water and Avoshine® wax coating), packaging (low density polyethylene (LDPE) and corn starch biodegradable films) and storage conditions (as mentioned above and ambient) with three replications was used in both experiments. The quality parameters that were evaluated included physiological weight loss (PWL), respiration rate, marketability, skin colour, firmness, puree colour, puree viscosity, moisture content (MC), dry matter (DM), pH, total soluble solids (TSS) and total titratable acidity (TTA). The storage conditions and the storage period significantly ($P \leq 0.001$) affected the quality parameters that were evaluated in both experiments. Low temperature storage offered the greatest benefit in maintaining high marketability, reduced PWL and delayed the peak in respiration, compared to ambient conditions in both experiments. Control samples exhibited increased rates of ripening, which was evident in increased PWL, reduced firmness, darkened skin colour, rapid decline in pH as well as increased TTA and TSS. The Avoshine® coating, combined with LDPE packaging, was favourable in maintaining a lower PWL, higher marketability, higher MC and lower DM indicative of delayed ripening. Hot water treatment promoted the darkening of the skin, decreased pulp firmness and lowered the marketability. The findings show that cold storage, combined with Avoshine® and LDPE packaging improved the shelf life by two weeks and preserving the quality of avocados during short and extended storage durations, compared to control samples.

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1. INTRODUCTION

The avocado (*Persea americana* Mill.) is described as being a climacteric fruit (Starrett and Laties, 1991; Jeong *et al*., 2002; Yahia, 2002; Jeong *et al*., 2003; Workneh and Osthoff, 2010; Wu *et al*., 2011), in which the ripening process is largely governed by ethylene, a plant hormone, inducing physico-chemical changes. This characteristic contributes to avocados being highly perishable, with a limited shelf life (Perez *et al*., 2004; Maftoonazad and Ramaswamy, 2008). Avocados form one of the major fruits that are produced in, and exported from South Africa, particularly to Europe (Vorster, 2005; DAFF, 2010; Nelson, 2010; Ntombela, 2012). The production areas in South Africa are concentrated in the warm subtropical eastern regions of Limpopo, Mpumalanga and KwaZulu-Natal. DAFF (2010) reports that the main cultivars produced in South Africa are 'Fuerte' (42%) and 'Hass' (33%), with international markets particularly inclined to 'Hass'. The leading exporters of avocados to Europe include Israel, supplying 29% of imports, followed by South Africa, contributing 21% (Van Zyl and Ferreira, 1995). Export volumes from South Africa in the last decade were the lowest in 2004, 2006, 2007 and 2009 and at an all-time high in 2005 (DAFF, 2010). Nelson (2010) reports that in 2009, South African exported avocados were considered to be of an inferior quality, which corresponds to the decrease in export volumes as previously mentioned by DAFF (2010) for 2009. The year 2011 also proved to be challenging, with a 40% reduction in exportation (Ntombela, 2012). This volatility in the volume of exported avocados is undesirable, because it not only threatens the revenue gained, but creates a negative perception on the quality of avocado fruit produced in South Africa. The quality of avocados at their final market destination is a major concern, both for export and local markets. Thus, the development of integrated and sustainable postharvest technologies could improve the quality and consequently extend the shelf life of avocados locally and when exported to distant markets.

Avocado ripening is an irreversible process, during which the fruit undergoes a complex series of biochemical processes involving changes in colour, firmness, texture, aroma and flavour, making it acceptable for consumption (Eaks, 1978; Chernys and Zeevaart, 2000). The final quality can be described as the degree of excellence of a product or the suitability of the product for a particular use (Abbott, 1999). Ripening and quality are important factors to consider in the avocado industry, as excessive ripening is likely to manifest itself in reduced fruit quality, which is undesirable to producers and consumers. Avocados continue respiring after harvest, which initiates the ripening process almost immediately, due to their climacteric characteristic of a high respiration rate. The duration of complete avocado ripening can take five to seven days at 25°C (Villa-Rodriguez *et al.*, 2011). Numerous studies were conducted to exhibit the effect of pre-packaging treatments, packaging materials and storage conditions on avocados and other produce (Meir *et al*., 1997; Hofman *et al*., 2003; Woolf *et al*., 2003; Perez *et al*., 2004; Workneh and Osthoff, 2010; Workneh *et al*., 2011; Wu *et al*., 2011), but not in a complete integrative manner. Pre-packaging methods, such as hot and cold treatments, waxes and the application of 1-methylcyclopropene to the avocado surface were shown to reduce chilling injury and improve the overall avocado quality. The application of polyethylene and biodegradable packaging films have been shown to extend the shelf life of avocados (Bhaskaran *et al*., 2002; Aguilar-Mendez *et al*., 2008). Studies by Tefera *et al*. (2007) demonstrated positive effects on the quality of mangos in Ethiopia by integrating suitable pre-packaging, packaging and storage conditions. Such studies can be applied to avocados in South Africa to create efficient postharvest handling conditions.

A vast number of studies have identified that optimal temperature and relative humidity conditions were fundamentally beneficial in maintaining high quality avocados by delaying the onset of ripening and ultimate senescence (Hopkirk *et al*., 1994; Zauberman and Jobin-Decor, 1995; Paull, 1999; Yahia, 2002; Perez *et al*., 2004; Mashau *et al*., 2012). Avocados stored at temperatures between 5-13°C demonstrated a shelf life of up to four weeks (Perez *et al*., 2004). Lutge *et al*. (2012) have emphasised the importance of adequate cold chain management in maintaining the quality of avocado fruit during extended transport periods. Temperature-varying conditions from high to low, known as a step-down temperature regime, have also been adopted to improve avocado quality, by reducing the onset of physiological disorders (Mans *et al*., 1995). However, there is a limit of such studies that deal with the effect of varying temperature conditions on the quality of avocados. Temperature and relative humidity are of importance during the storage of perishable items. Temperatures that are too low and/or relative humidity that is too high can result in chilling injury or the proliferation of micro-organisms (Maftoonazad and Ramaswamy, 2008). Alternatively, temperatures that are too high and/or relative humidity that is too low can promote excessive water loss, reduced firmness and an undesirable shrivelled appearance (Paull, 1999). Consequently, by combining effective pre-packaging treatments with suitable packaging and optimal storage conditions, it could be possible to mitigate postharvest losses in avocado fruit and improve the South African export conditions.

A limited number of peer-reviewed studies pertain to the integrated effect of postharvest handling conditions, including pre-packaging, packaging and storage conditions, on the quality of avocados, specifically in Africa and South Africa (Milne, 1998; Ouma, 2001). This deficiency creates a research opportunity necessitating the need for additional work to be undertaken in South Africa. The questions that may arise are:

(a) what are the effects of different combinations of pre-packaging and packaging treatments on the physical, chemical and subjective sensory quality of avocado fruit?

(b) what are the effects of different temperature and relative humidity storage conditions on physical, chemical and subjective sensory quality of avocado fruit? and,

(c) what are the optimum postharvest handling techniques, which target the quality preservation of the avocado fruit?

This study has been divided into two experiments. Experiment I, detailed in Chapter 3, evaluated the integrated effect of different pre-packaging, packaging and storage conditions, involving fixed temperature and fixed relative humidity regimes on the physical, chemical and subjective sensory quality parameters of the avocado. Experiment II in Chapter 4, evaluated the combined effect of different pre-packaging and packaging techniques during the simulation of a realistic avocado cold chain (step-down temperature regime) on the physical, chemical and subjective sensory quality parameters of the avocado. This is then followed by an overall conclusion and recommendations section presented in Chapter 5.

The aim of this study is to investigate the combined effect of postharvest treatments on the quality of avocados and to provide practical recommendations during avocado postharvest handling in South Africa. The specific objectives formulated for this study are: (a) to investigate the combined effect of pre-packaging treatments, packaging films and multiple fixed temperature and relative humidity storage conditions on the physical, chemical and subjective sensory quality of avocados, and (b) to investigate the combined effect of pre-packaging treatments, packaging films and temperature-varying conditions during storage on the physical, subjective sensory and chemical quality of avocados. By improving the combination of pre-packaging, packaging and the storage conditions, it is anticipated that these techniques may be adopted by emerging farmers and even the fresh produce industries, in order to maintain and enhance the quality of avocados. The potential improvement in the quality may, in turn, have other positive consequences, such as allowing South Africa to be at the forefront of international fresh produce markets, expand avocado production and create value adding facilities throughout South Africa.

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2. A REVIEW OF LITERATURE PERTAINING TO PRE- AND POST HARVEST FACTORS AFFECTING THE QUALITY OF AVOCADOS

2.1 Introduction

This chapter provides a review of the literature pertinent to pre- and postharvest handling conditions that have been adopted to enhance the quality of avocados and to prolong their shelf life. An outline of the significant physical, sensory and chemical quality parameters associated with evaluating avocado maturity is mentioned. Attention has also been focused on pre-harvest conditions, such as the exposure of avocados to ambient temperature and water stress as these factors have been proven to affect the postharvest behaviour of avocados. The effects of pre-packaging treatments, packaging materials and storage conditions on the quality parameters of avocados are then presented and discussed. Finally, the avocado cold chain, regulations, guidelines and recommendations during postharvest handling are identified.

2.2 Changes in Avocado Quality Parameters after Harvest

Colour, texture, flavour and aroma are essential avocado quality parameters and the main characteristics to which consumers refer during purchase (Lee e*t al*., 1983; Forero, 2007). An outline of the avocado quality-related attributes is presented in this section.

2.2.1 Physical properties

Physical properties are primarily related to the appearance and aesthetic appeal of avocados, which consumers are initially exposed to and, which influences their decision to purchase. Some of the physical quality parameters of avocados include skin colour, firmness, texture and physiological disorders of whole avocados, and the viscosity and colour of avocado purees.

2.2.1.1 Skin colour

To both industry and consumers, avocado skin colour is an important indication of the stage of ripening (Cox *et al*., 2004; Arzate-Vazquez *et al*., 2011). Skin colour can be measured either objectively, commonly using a chroma meter or colorimeter, or alternatively, using subjective means by experienced sensory panellists by means of eye colour rating. Skin colour has been found to vary among different avocado cultivars. The 'Hass' cultivar, for example, is characteristic of a colour change from green to purple and eventually black (Cox *et al*., 2004; Forero, 2007; Arzate-Vazquez *et al*., 2011). However, Chen *et al.* (2009) revealed that the skin colour of the 'Sharwil' variety does not darken with maturity, therefore, other methods must be utilised to distinguish the various stages of maturity. According to Maftoonazad and Ramaswamy (2008), the parameters relating to colour measurement are:

L = Lightness or brightness,

a* = redness or greenness, and

b* = yellowness or blueness.

From these parameters, the chroma (C) and hue angle can be determined as follows (Maftoonazad and Ramaswamy, 2008):

$$C = \sqrt{a^2 + b^2} \qquad (2.1)$$

$$\text{hue angle} = \tan^{-1}\left(\frac{a}{b}\right) \qquad (2.2)$$

The colour parameters L, a* and b* can be represented on the CIELAB (Commission Internationale de l'Eclairage L, a*, b*) space coordinate system, as illustrated in Figure 2.1.

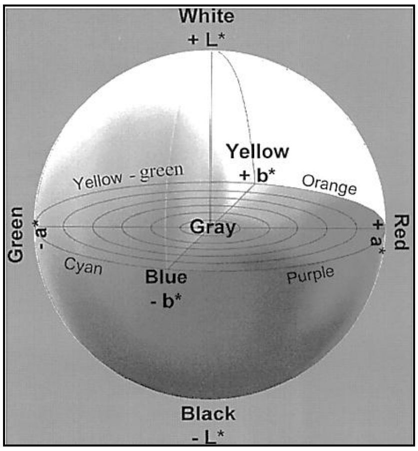

Figure 2.1 CIE L*a*b* colour coordinate system (Abbott, 1999)

Cox *et al*. (2004) found that despite the fruit being considered as ripe, storage of ‘Hass’ at 15°C did not exhibit a fully black colouration, compared to storage temperatures of 20°C and 25°C. This demonstrated the dependence of colour change on the storage temperature. The change in colour influenced by the ripening process in ‘Hass’ was attributed to a decrease in chlorophyll, L, C and hue of the skin and an increase in cyanidin 3-*O*-glucoside. Ashton *et al.* (2006) found comparable results by observing a decline in the chlorophyll of the skin with time.

2.2.1.2 Firmness

The firmness of avocados is a vital determinant in assessing the degree of ripening (Mizrach and Flitsanov, 1999; Flitsanov *et al*., 2000; Arzate-Vazquez *et al*., 2011). Firmness can be described as the resistance to penetration (Mizrach and Flitsanov, 1999) that can be determined by employing (a) invasive methods, such as hand tactile methods, (b) destructive methods, such as the Magness-Taylor puncture test (M-T), or (c) non-destructive methods, such as impulse response and ultrasonic methods. Destructive techniques do not allow for continuity in monitoring on a commercial basis but are, rather, well-suited for laboratory analysis. Mizrach and Flitsanov (1999), Flitsanov *et al*. (2000) and Mizrach *et al*. (2000) employed ultrasonic techniques to evaluate the firmness in a non-destructive manner, which rendered comparable results to those of the destructive methods. Gomez *et al*. (2005) found that impulse response techniques were more sensitive to firmness changes in pear fruit, compared to the M-T tests and have the potential to replace destructive testing methods in determining fruit firmness and shelf life. A stiffness coefficient to determine the firmness of spherical fruit can be calculated using Equation 2.3 (Gomez *et al*., 2005).

$$S = f^2\, m^{2/3} \tag{2.3}$$

where:

S = stiffness coefficient [$kg^{2/3}.\,s^{-2}$],

f = dominant frequency where response magnitude is the greatest [Hz], and

m = fruit mass [g].

Studies by Lewis (1978; cited by Mizrach *et al.*, 2000), demonstrated a strong correlation between fruit firmness, maturity stage and expected storage time. Storage temperature is fundamental in the reduction of firmness, as avocados subjected to low temperatures exhibited reduced rates of softening (Paull, 1999; Flitsanov *et al*., 2000; Mizrach *et al.*, 2000). Villa-Rodriguez *et al*. (2011) discovered that when avocados were stored at 15°C, the firmness diminished from approximately 130.51 N to 54.62 N, 19.92 N and 7.37 N on storage Day 0, 4, 8 and 12, respectively. Arzate-Vazquez *et al*. (2011) also observed a reduction in the firmness from 75.43 N to 2.63 N over a period of 12 days at 20°C and 75% relative humidity.

2.2.1.3 Texture

Texture can be quantified as the resistance to an applied force experienced by the produce (Maftoonazad and Ramaswamy, 2008), and is a significant indicator of avocado quality and is of concern to the consumer (Maftoonazad and Ramaswamy, 2008; Toivonen and Brummell, 2008; Landahl *et al.*, 2009). Avocados undergo drastic changes in texture (Landahl *et al*., 2009; Li *et al*., 2010). Chen *et al.* (2009) states that the oil content is a key component in the texture of avocados and, which was identified by Hofman *et al*. (2002a) as contributing to the ‘smoothness’. Despite the relationship between texture and oil content, it was discovered by Chen *et al.* (2009) that an increase in the oil content over the harvest period did not manifest itself in any change in the texture. Storage temperature, oxygen and carbon dioxide concentrations and wounding have a direct effect on the texture (Maftoonazad and Ramaswamy, 2008). The relationship between texture and firmness can be extended to the avocados ability to withstand loading during storage. It was found that, as the avocados ripened, the texture, firmness and strength reduced.

2.2.1.4 Physiological disorders

Every biological system operates optimally within specific limits. If these limits, such as temperature are significantly altered, physiological disorders are likely to ensue. Low temperature storage is frequently adopted to extend the shelf life of fresh commodities, but can result in chilling injury (Eaks, 1976; Florissen *et al*., 1996; Yahia and Gonzalez-Aguilar, 1998; Woolf *et al*., 2003; Hershkovitz *et al*., 2005; Woolf *et al*., 2005; Adams and Brown, 2007). The main symptoms associated with chilling injury are black spots on the peel or grey or dark-brown discolouration in the mesocarp (Pesis *et al*., 1994; Meir *et al*., 1995; Florissen *et al*., 1996; Pesis *et al*., 2002; Hershkovitz *et al*., 2005). Florissen *et al*. (1996), Woolf and Ferguson (2000), Hofman *et al*. (2002b) and Hofman *et al*. (2003) found that hot water treatments were effective in diminishing the effects of chilling injury. In addition to hot water treatments, exposing the avocados to extremely low temperatures, just above those temperatures at which chilling injury is likely to occur, have also proven to alleviate the effects of chilling injury (Woolf *et al*., 2003). The optimum temperature to reduce chilling injury was found by Woolf *et al*. (2003), to be between 6ºC and 8ºC for three to five days. However, Sanxter *et al*. (1994) and Woolf *et al*. (2003) revealed 4°C to be adequate. Hopkirk *et al*. (1994) found that postharvest disorders in 'Hass' increased, with increased storage time and temperature.

2.2.1.5 Viscosity and colour of avocado puree

Rheological characteristics are essential in determining the yield stress, which is significant in the sensory perception, as well as to determine the end-use performance of avocados (Tabilo-Minizaga *et al*., 2005). However, much of the previous work is related to already processed avocado purees, with additives such as water or sodium hydroxide ready for direct consumption (Lopez *et al*., 1998). The combined effect of pre-packaging, packaging, temperature and relative humidity storage conditions on the viscosity and colour of avocado purees has not been previously investigated, therefore, creating a research opportunity.

2.2.2 Sensory properties

Sensory evaluation involves flavour, hand-feel and mouth-feel of the product (Abbott, 1999). In this section, the flavour of avocados will be discussed.

2.2.2.1 Flavour

One of the main sensory properties of avocados is flavour, which encompasses both aroma and taste and forms an important component of the eating quality of the fruit. Paull (1999), Workneh and Osthoff (2010) and Paull and Duarte (2011) define flavour as the ratio of sugar to acid influenced by temperature. This can be observed in the case of grapefruit, held at 8ºC, resulting in a greater decline in the acidity, compared to those stored at 12ºC (Paull, 1999). The concentration of volatiles contained in a horticultural commodity increases during the progression of the ripening process (Abbott, 1999). Premature harvesting can lead to an undesirable taste (Brown, 1972; Perez *et al*., 2004; Wu *et al*., 2011) or lack of flavour

(Gamble *et al*., 2010; Osuna-Garcia *et al*., 2010). The off-flavour can be ascribed to increased levels of ethanol and acetaldehyde (Thompson, 2010; Paull and Duarte, 2011). The treatment of avocados with 0.25% oxygen and 80% carbon dioxide causes an increase in ethanol and acetaldehyde (Ke *et al*., 1995). Burdon *et al*. (2007) observed that the exposure of 'Hass' to oxygen and carbon dioxide concentrations of less than 0.5% and up to 20%, respectively, resulted in increased levels of acetaldehyde and ethanol, which is in accordance with the findings of Ke *et al*. (1995).. An oil content ranging between 10-30% contributed to avocados of acceptable flavour and a soft and even consistency, depending on the cultivar.

2.2.3 Chemical properties

The identification of horticultural maturity is often difficult to determine in avocados, as changes in external appearance are sometimes not easily distinguishable (Lee *et al*., 1983). Other maturity determination techniques that employ chemical properties are, therefore, required. The chemical properties of avocados discussed within this section are pH, total titratable acidity, moisture content, oil content, dry matter content and total soluble sugars.

2.2.3.1 pH

Maftoonazad and Ramaswamy (2008) observed a decrease in the pH, with time, during storage. Avocados treated with pectin-based coatings illustrated a slower rate of decrease in pH values, compared to untreated fruit and those exposed to higher temperatures. Subjecting avocados to low oxygen and/or high carbon dioxide levels were used for short periods, as a pre-treatment to alleviate physiological disorders (Ke *et al*., 1995) and to enhance storage atmospheres (Meir *et al*., 1995). These conditions also led to a decrease in the intracellular pH, which altered the various pH-dependant physiological processes (Ke *et al*., 1995). Ke *et al*. (1995) exposed avocados to 0.25% oxygen or 20% oxygen and 80% carbon dioxide or 0.25% oxygen and 80% carbon dioxide and observed a reduction in the pH from 6.9 to 6.7, 6.3, and 6.3, respectively, at 20°C. Similarly, Lange and Kader (1997) stored avocados at 20°C in atmospheres of varying concentrations of oxygen and carbon dioxide. Both studies show that the lowest concentration of oxygen and the highest concentration of carbon dioxide results in a decline of pH to form an acidic medium.

2.2.3.2 Total titratable acidity

Acidity is associated with both the sweetness and sourness of fruit. The method used to measure acidity is titratable acidity (Lobit *et al.*, 2002). Lobit *et al*. (2002) explains that the pH is representative of the free hydrogen ion activity bond, whereas titratable acidity is the quantity of weakly-bound hydrogen ions. Maftoonazad and Ramaswamy (2008) observed that in both pectin-based coated and non-coated avocados, an increase in the titratable acidity was more apparent at higher storage temperatures. Holcroft and Kader (1999) showed that strawberries exposed to higher concentrations of carbon dioxide at 5°C exhibited increased pH and decreased levels of titratable acidity. In the case of mangoes that were subjected to postharvest treatments, packaged and stored for 28 days, a decrease in the titratable acidity from 3.42% to 0.2% was observed (Tefera *et al*., 2007).

2.2.3.3 Moisture content

Moisture content is the preferred indicator of maturity in South Africa, with the recommended moisture content in the range of 69-75%, depending on the cultivar (Mans *et al*., 1995). The export of early season 'Fuerte' commences once the moisture content has reached 78-80%, which is equivalent to an oil content of 9-11% (Dodd *et al*., 2008). The current standard of moisture determination in South Africa, as stated within the Agricultural Product Standards Act (1990), is considered to be inaccurate (Retief, 2012). Work is ongoing to determine more accurate means of moisture determination. This creates a research gap for the refinement of moisture determination of avocados in South Africa, which is, however, not within the scope of this study. According to the Quality and Food Safety Standards in the Agricultural Product Standards Act (1990), the stipulated maximum moisture content of various avocado cultivars can be viewed in Table 2.1.

Table 2.1 Maximum moisture content of avocados

Cultivar	**Fuerte**	**Pinkerton**	**Ryan**	**Hass**	**Lamb Hass**	**Maluma Hass**	**Nature's Hass**	**Other**
Moisture Content (%)	80	80	80	77	73	78	77	75

2.2.3.4 Oil content

Avocado is considered to be an important oil fruit and oil content serves as a significant indicator of fruit maturity (Hofman *et al*., 2002a; Ozdemir and Topuz, 2004; Gamble *et al*., 2010; Blakey, 2011). As the fruit matures, the concentration of oil within the mesocarp increases, as described by Hofman *et al*. (2002a), Ozdemir and Topuz (2004), Chen *et al*. (2009) and Blakey (2011). The increase in oil results in a reduction in water by the same amount within the fruit, implying that the combined content of water and oil generally remains constant throughout the avocado life (Hofman *et al*., 2002a; Ozdemir and Topuz, 2004). Lee *et al*. (1983) and Chen *et al*. (2009) observed a close correlation between the percent oil content and percent dry matter. The maturity index can then be calculated by either the oil content or dry matter. Hofman *et al*. (2002a) and Gamble *et al*. (2010) referred to percent dry matter determination as an alternative to oil content determination, when assessing the avocado maturity.

2.2.3.5 Dry matter content

An extended maturation stage of avocados allows for more oil accumulation and dry matter content, however, this introduces the risk of increased disease. Maturity standards are being used by avocado-producing countries to avoid the marketing of low quality, immature fruit. The standards adopted are the Californian minimum dry matter of 20.8% for ‘Hass’, or a slightly higher minimum dry matter content of approximately 25%, to decrease disorders during storage (Gamble *et al*., 2010). An oil content of 8% has been reported by Ozdemir and Topuz (2004) to be acceptable for the marketing of avocados. Villa-Rodriguez *et al*. (2011) found that the dry matter had increased from 31.65% to 36.52% over eight days at 15°C and thereafter decreased to 32.91% by Day 12. Hofman *et al*. (2000) found that the percent oil content and dry matter are not suitable indicators of avocado maturity in late-harvested ‘Hass’, due to inconsistent changes later in harvested fruit. No distinct relation could be found between the effect of varying temperature and relative humidity on the percent dry matter and oil content of avocados during storage, thus motivating research to be conducted in this field.

2.2.3.6 Total soluble sugars

Carbohydrates are an essential source of energy for growth, development and maintenance in avocados (Liu *et al*., 1999a; 1999b; Tesfay, 2009; Blakey, 2011). Five major soluble sugars have been identified within the avocado *viz*. the seven carbon (C7) reducing sugar (D-mannoheptulose), perseitol, sucrose, fructose and glucose (Liu *et al*., 1999b; Tesfay, 2009). These constitute approximately 98% of the total soluble sugars. Liu *et al*. (1999b) demonstrated that the ripening of avocados at 20°C resulted in a considerable decline in the total soluble sugars of the peel and flesh, particularly in the C7 sugars, which is concomitant with an increase in the oil content. A decrease in the total soluble sugars was observed at 1°C and 5°C storage, but this occurred at a slow rate. Similarly, Liu *et al*. (2002) found a decrease in the C7 sugars during the progression of the ripening process. However, studies by Blakey (2011) reported an increase in the glucose and fructose levels, while sucrose declined slightly during ripening. Carbohydrates are stored during the growth of the avocado. Once harvested, these carbohydrates are utilized for postharvest physiological processes, such as respiration via enzymatic mechanisms that metabolize the C7 sugars (Liu *et al*., 1999b). This suggests that the C7 sugars play an important role in avocado respiration during the ripening process.

2.3 Pre-Harvest and Harvesting Factors Affecting Avocado Quality

This section presents vital pre-harvest and harvesting techniques that affect postharvest avocado responses in terms of the physiological principles and the overall avocado quality.

2.3.1 Respiration and ripening

Respiration is described as a natural process occurring within all living organisms, whereby organic materials such as carbohydrates, proteins and fats are broken down. During respiration, oxygen is expended and carbon dioxide liberated, accompanied by the production of energy in the form of heat (Workneh and Osthoff, 2010). Ethylene is a plant hormone that is naturally produced by avocados. Both respiration and ethylene formation are, predominantly, responsible for the ripening of avocados. Starrett and Laties (1991), Jeong *et* *al*. (2002), Yahia (2002), Jeong *et al*. (2003), Workneh and Osthoff (2010) and Wu *et al*. (2011) describe avocados as being climacteric, characterised by a surge in ethylene production at the start of ripening, which is illustrated in Figure 2.2.

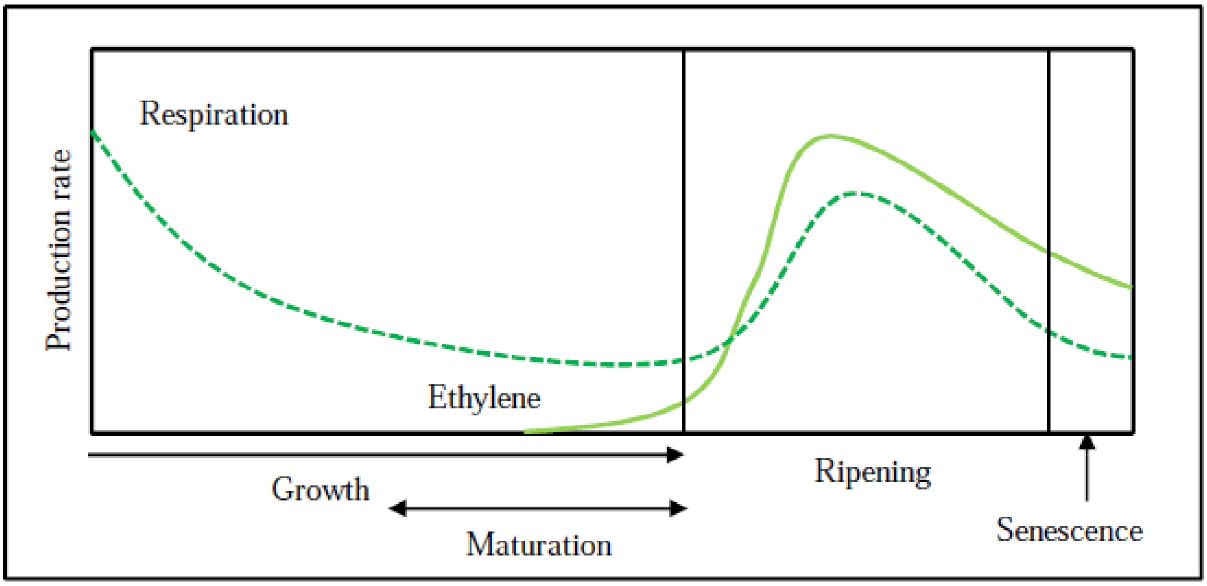

Figure 2.2 Physiological developmental stages of avocados (after Blakey, 2011)

The respiration of avocados follows three characteristic climacteric phases *viz*. preclimacteric minimum of least respiration, climacteric maximum of highest respiration and a postclimacteric phase synonymous with a decline in respiration. It is during the preclimacteric and climacteric phases, when many of the changes associated with ripening occur (Perez *et al*., 2004). The shelf life of fresh commodities is inversely related to the rate of respiration and ethylene production, as stated by Perez *et al*. (2004). An increase in the respiration rate hastens senescence, contributing to flavour loss and reduced dry weight (Workneh and Osthoff, 2010). Therefore, different handling methods, especially those associated with lowering respiration rates, should be favoured.

2.3.2 Pre-harvest factors

This section serves to highlight the significant interaction and impact of pre-harvest factors on postharvest requirements, development and the inherent quality characteristics of avocados.

2.3.2.1 Ambient heat exposure

Ferguson *et al*. (1999) observed the predominant pre-harvest factor influencing the postharvest quality of avocados during growth to be ambient temperature. This was confirmed by Woolf *et al*. (1999), who demonstrated that the side of the avocado exposed directly to sunlight while still on the tree was able to withstand higher temperatures during postharvest treatments, compared to the shaded side. Avocados exposed to high ambient temperatures in the field also demonstrated a tolerance to low postharvest temperatures and external chilling injury (Woolf and Ferguson, 2000). Results obtained by Woolf *et al*. (2000), further confirmed that avocados that had been exposed to direct sunlight were capable of tolerating postharvest hot water treatments of 50°C and 55ºC were found to be firmer. Sun-exposed fruit showed a higher endurance to chilling injury when stored at 0ºC for periods of between three to six weeks. The ethylene peak of sun-exposed fruit was delayed by two to five days during ripening at 20ºC. These findings indicate the benefit offered by sun exposure of the avocado fruit.

Heat treatments based on a similar principle of exposing avocados to high temperatures are being used to treat avocados. However, these treatments are for predetermined periods of time, using either heated air or water, in order to stimulate the production of heat-shock proteins (Florissen *et al*., 1996; Woolf *et al*., 1999; Woolf and Ferguson, 2000; Woolf *et al*., 2000; Fallik, 2004; Wu *et al*., 2011). This is further discussed in Section 2.4.3.

2.3.2.2 Water stress

Water-stressed ‘Hass’ avocado trees were found to bear more elongated fruit (Yahia, 2002). The reason for this is unknown and further studies are required to investigate this phenomenon, which is not included in the scope of this study. Water stress reduces the internal quality of avocados, due to the increased activity in polyphenol oxidase, which leads to the browning of the flesh. Lower concentrations of calcium, were found in water-stressed fruit, resulting in a high incidence of physiological disorders.

Adato and Gazit (1974) found that pre-harvest water stress resulted in premature fruit abscission and an increase in ethylene production, leading to accelerated ripening by 40% and 25%, depending on the degree of water stress. Both Kaluwa (2010) and Blakey (2011) confirm that water stress decreased the normal avocado ripening time, hence reducing the shelf life, which is accompanied by an increased risk of physiological disorders. The effect of water stress on avocados can further be linked to temperature. When a plant is water-stressed, the temperature of the fruit rises, because cooling results from water movement through the fruit stalks (Woolf and Ferguson, 2000). Once the fruit is harvested, this cooling effect is halted and the rise in temperature is often further exacerbated by exposure to the sun.

2.3.2.3 Harvesting techniques

Avocados, unlike other fruit, do not mature and ripen on the tree, but only once harvested (Lee *et al*., 1983; Hopkirk *et al*., 1994; Baryeh, 2000; Ozdemir and Topuz, 2004; Perez *et al*., 2004; Gamble *et al*., 2010; Osuna-Garcia *et al*., 2010). The time at which avocados are harvested contributes to the maturation and expected shelf life. Harvesting too early in the season contributes to low pulp dry matter. This is associated with irregular ripening, a watery texture, flavourless, shrivelled and blackened fruit (Gamble *et al*., 2010; Osuna-Garcia *et al*., 2010) and a low oil concentration (Blakey, 2011). Perez *et al*. (2004) state that harvesting prior to physiological maturity results in irregular softening, a poor taste and higher susceptibility to decay. Generally, if the avocados are not harvested at the appropriate time, the quality is compromised and the shelf life shortened (Wu *et al*., 2011).

The time of harvesting, among other factors, depends on the avocado cultivar. Whiley *et al*. (1996a) showed that early harvesting at 21% and 24% dry matter led to a higher cumulative and average yield in the early-maturing variety ‘Fuerte’. However, delaying harvesting till a value of 30% dry matter was attained, reduced yields by 26% and led to alternate bearing. Similar studies by Whiley *et al.* (1996b) indicated that the early harvesting of the late-maturing variety ‘Hass’ at 25-30% dry matter resulted in high productivity, whereas delayed harvesting till 35% dry matter, reduced yields, leading to alternate bearing. Chen *et al*. (2009) observed that late season ‘Sharwil’ were smaller in size, had higher oil and dry matter contents and demonstrated a shorter shelf life than early and mid-season fruit.

Hofman *et al*. (2002a) recommend that the picking of wet avocados should be avoided, as this increases the incidence of cold injury, pulp spot and lenticel damage. Fruit harvested in the morning or late afternoon tends to have less field heat. Colour, size or oil content generally serves as indications of the most appropriate time for harvesting (Ozdemir and

Topuz, 2004). Harvesting is mainly accomplished by manual techniques, such as clipping and snapping. Eaks (1973) investigated the influence of these two methods on the postharvest development of avocados and found no significant difference in terms of weight loss and the rate of ripening. Yahia (2002) states that clipping aided in reducing bruising and puncturing of adjacent fruit while in containers and reduced the onset of stem end rot. However, Hofshi and Witney (2002) disagree with the use of clipping. They referred to studies, which indicated that snapped avocados ripened at a faster rate than those that were clipped. Further studies can be carried out to establish the effect of snapping and clipping on the quality of avocados.

2.4 Pre-Packaging Treatments

Treatments prior to packaging have the added benefit of prolonging the shelf life and enhancing the quality of avocados, combined with suitable packaging and storage conditions (Tefera *et al*., 2007; Workneh *et al*., 2011a). This section presents some common pre-packaging techniques and technologies applied to avocados.

2.4.1 Surface coating and waxing

Postharvest water loss, resulting from transpiration has a detrimental effect on avocados, for example, weight loss, which leads to accelerated ripening and a higher degree of physiological disorders (Johnston and Banks, 1998). Waxes are impermeable to water, which addresses the challenge of water loss. Waxes allows for water retention, increased turgidity and maintaining the fruit weight for longer periods. Hagenmaier and Shaw (1992), Banks *et al*. (1997), Johnston and Banks (1998) and Maftoonazad and Ranaswamy (2008) describe waxes as also providing a surface barrier by hindering the movement of gases. This creates an internal modified atmosphere, resulting in lowered rates of respiration and delayed ripening. A polyethylene (PE)-based wax at 11% concentration visibly improved the exterior sheen and reduced mass loss (Johnston and Banks, 1998).

Waxes are able to contribute to the physiological characteristics of avocados and enhance the exterior aesthetic appeal by imparting a sheen and gloss to the fruit (Hagenmaier and Shaw, 1992; Johnston and Banks 1998; Yahia, 2002; Maftoonazad and Ramaswamy 2008). The use of pectin-based waxes by Maftoonazad and Ramaswamy (2008) demonstrated improved results, compared to their earlier use of methyl cellulose coatings to reduce respiration rates. In South Africa, the use of waxes is recommended prior to packaging, provided that no more than 140 mg of the compound per kg of avocados adheres (Agricultural Product Standards Act, 1990). 'StaFresh', a natural wax emulsion, produced equivalent and enhanced results, compared to a PE wax on South African avocados stored at 5.5°C for four weeks and ripened at 18°C (Kremer-Kohne and Duvenhage, 1997).

2.4.2 Application of 1-Methylcyclopropene

1-methylcyclopropene (1-MCP) is a synthetic cyclopropene used as an ethylene action inhibitor in many perishable fruit (Feng *et al*., 2000; Jeong *et al*., 2002; Jeong *et al*., 2003; Hershkovtiz *et al*., 2005; Zhang *et al*., 2011). Earlier work by Jeong *et al*. (2002) indicated that 'Simmonds' avocados, treated with 0.45 $\mu l.l^{-1}$ of gaseous 1-MCP for 24 hours at 20°C and 85% relative humidity, delayed ripening by four days. Later studies by Jeong *et al*. (2003) incorporated the use of wax and 1-MCP at a storage temperature of 13°C, which demonstrated less weight loss and a greener colour. Weight loss of fruit treated with 1-MCP and wax was found to be 3.8% after 19 days of storage (Jeong *et al*., 2003), whereas after eight days, the weight loss of fruit treated with only 1-MCP was found to be 3.9% (Jeong *et al*., 2002). 1-MCP has been used with success in South Africa (Lemmer *et al*., 2002; Vorster, 2005; Nelson, 2006). Lemmer *et al.* (2002) recommended that 500 ppb of 1-MCP be applied to avocados for 12 hours, at either 5°C or 10°C. However, it was further stated that additional studies may be beneficial to refine this application dosage and exposure time. 1-MCP, in combination with hypoxia conditions, alleviated the undesirable flavour as a result of the oxidation of the lipids within the avocado (Pathirana *et al*., 2011; Zhang *et al*., 2011).

2.4.3 Heat treatments

In Kenya, Ouma (2001) observed that heating avocados to 38°C for periods of 24, 48 and 72 hours improved the appearance and reduced the effects of chilling injury, as opposed to untreated fruit. The maximum ethylene evolution was delayed; however, the rate of respiration was unchanged. Furthermore, weight loss was reduced as the number of days of heating increased, leading to an improved shelf life.

In order to inhibit the attack of insect pests in avocados, cold disinfestation is often used (Florissen *et al*., 1996; Hofman *et al*., 2002b; Hofman *et al.*, 2003; Wu *et al*., 2011). This treatment requires exposing the fruit to 1°C for 16 days, but this induces chilling injury. To alleviate the onset of chilling injury, heating the avocados at various time and temperature regimes are applied. A summary of the regimes that have been researched are presented in Table 2.2.

Table 2.2 Heat treatment regimes of avocados

Temperature (°C)	**Heating Fluid**	**Exposure Time**	**Effects**	**Reference**
37-38	Air	17-18 hours	Reduced chilling injury	Sanxter *et al*., 1994
38	Air	6-12 hours	Reduced chilling injury and reduction in ripening time and flesh injury after ripening	Florissen *et al*., 1996
38	Water	2 hours	Reduced chilling injury	Woolf and Lay-Yee, 1997
40 and 41	Water	30 minutes	Reduced body rots and decreased vascular browning	Hofman *et al*., 2002b
38	Water	30 minutes	Good appearance and internal quality	Wu *et al*., 2011
38	Air	6 hours	Reduced chilling injury	Wu *et al*., 2011

Water is the favoured heating medium for thermal processes, due to the greater efficiency of heat transfer as opposed to air (Fallik, 2004). Heat treatment using air requires a longer heating time than with water. The variation in temperatures and associated treatment times differs among studies, depending on the cultivar, heating medium and pre-harvest environmental conditions such as sun exposure, among other factors (Woolf *et al*., 1999).

2.4.4 Low temperature conditioning

Low temperature conditioning consists of subjecting the avocado to temperatures slightly above those that cause chilling injury, in order to induce tolerance to low temperatures (Woolf *et al*., 2003). Low temperature conditioning of between 4-8°C for a period of four days, provided substantial protection against chilling injury, as demonstrated by Hofman *et al*. (2003). Similar findings were achieved by Woolf *et al*. (2003) at 6°C or 8°C for three to five days. Hard skin, tissue breakdown and the incidence of rot were reduced and even eliminated (Woolf *et al*., 2003) and skin damage and internal quality were improved (Hofman *et al*., 2003). Both these studies deduced that hot water treatments did not prove to be as successful in alleviating external chilling injury and improving the overall quality of the avocado, when compared to low temperature conditioning.

2.5 Packaging Methods

The basic functions of food packaging are storage, preservation and protection for prolonged periods of time (Garlic *et al*., 2011). This section provides a review of the past and current trends related to avocado packaging. The two most recognized avocado packaging techniques are modified atmosphere packaging (MAP) and controlled atmosphere storage (CAS), which are proven to extend the avocado shelf life and retain quality (Yahia and Gonzalez-Aguilar, 1998; Berrios, 2002).

2.5.1 Packaging films

Plastic materials that are primarily used for the MAP of whole fruit and vegetables are polybutylene, low density polyethylene, high density polyethylene, polypropylene, polyvinylchloride, polystyrene, ethylene vinyl acetate, ionomer, rubber hydrochloride (pliofilm) and polyvinylidine chloride (Workneh and Osthoff, 2010). A summary of the permeability characteristics of plastic films that are used in MAP is presented in Table 2.3.

Table 2.3 Packaging film permeabilities (after Workneh and Osthoff, 2010)

Film Type	**Transmission Rate**		
	Oxygen*	**Carbon Dioxide***	**Water Vapour****
Low Density Polyethylene (LDPE)	3900 - 13000	7700 - 77000	6 - 23.2
Linear Low Density Polyethylene (LL DPE)	7000 - 9300	-	16 - 31
Medium Density Polyethylene (MDPE)	2600 - 8293	7700 - 38750	8 - 15
High Density Polyethylene (HDPE)	52 - 4000	3900 - 10000	4 - 10
Polypropylene (PP)	1300 - 6400	7700 - 21000	4 - 10.8
Polyvinylchloride (PVC)	620 - 2248	4263 - 8138	> 8
Polyvinylchloride (PVC), plasticized	77 - 7750	770 - 55000	> 8
Polystyrene (PS)	2000 - 7700	10000 - 26000	108.5 - 155
Ethylene Vinyl Acetate Copolymer (12% VA)	8000 - 13000	35000 - 53000	60
Ionomer	3500 - 7500	9700 - 17800	22 - 30
Rubber Hydrochloride (Pliofilm)	130 - 1300	520 - 5200	> 8
Polyvinylidine Chloride (PVDC)	8 - 26	59	1.5 - 5

* Measured in units of $cm^3.m^{-2}.day^{-1}$ at 1 atmosphere.

**Measured in units of $g.m^{-2}.day^{-1}$ at 37.8°C and 90% relative humidity.

The storage of avocados in PE bags reduced chilling injury (Pesis *et al*., 1994; Meir *et al*., 1997). Thompson (2010) revealed that individually-sealed 'Fuerte' in PE bags of 0.025 mm thickness for 23 days at 14-17°C ripened normally once removed from the bags. The atmosphere within the bags after storage was found to be 8% carbon dioxide and 5% oxygen. Similarly, individually-sealed 'Hass' stored at 10ºC resulted in an increased storage life (Oudit and Scott, 1973). West Indian avocados stored in PE bags at 13ºC exhibited delayed softening and increased shelf life (Thompson *et al*., 1971). This study further demonstrated that perforated bags and unwrapped avocados have a similar effect on the storage life. LDPE packages displayed suitable MAP conditions of low oxygen and high carbon dioxide in retaining avocado, papaya and mango freshness, compared to oriented PP and oriented PS films (Xiao and Kiyoto, 2001).

Biodegradable films and coatings are becoming more valuable from an environmental perspective, as they are easily recyclable (Aguilar-Mendez *et al*., 2008). The composition of biodegradable films is essential in determining the postharvest behaviour of avocados and in the performance of the packaging itself. Gelatine-starch films and coatings are used on avocados with positive outcomes of firmer fruit pulps, skin colour retention and lower weight loss. Higher starch concentrations and pH of the biodegradable film cause greater carbon dioxide permeability, while lower levels of starch lead to higher film puncture strength (Aguilar-Mendez *et al*. 2008). Gelatine and starch based-films offer the benefit of being inexpensive and manufacturing is possible on a large scale.

2.5.2 Modified atmosphere packaging

A misconception exists that MAP and CAS are the same. However, MAP incorporates a lower degree of control over the concentration of gases, as it depends on the interaction between the commodity and the packaging (De Reuck *et al*., 2010; Workneh and Osthoff, 2010). The aim of MAP is to create an optimal micro-environment within the package, specific to the avocado requirements to delay ripening and maintain the quality. According to Meir *et al.* (1997), Mangaraj *et al*. (2009), Sandhya (2010) and Workneh and Osthoff (2010), an equilibrium must be established between the avocado and the packaging, based on the following factors:

(a) maturity stage and respiration rate of the commodity,

(b) storage temperature,

(c) film surface area to fruit volume or weight ratio, and

(d) the type of film (thickness and permeability to oxygen, carbon dioxide and water vapour).

An equilibrium is assumed to be established once the quantity of gas exchanged through the avocado is equivalent to that through the film (Mangaraj *et al*., 2009; De Reuck, 2010; Workneh and Osthoff, 2010). MAP is based on the principle of modifying the atmosphere within the package, to lower oxygen concentrations and raise carbon dioxide concentrations (Meir *et al*., 1997; Yahia and Gonzalez-Aguilar, 1998; Berrios, 2002; Hertog *et al*., 2003; Valle-Guadarrama *et al.*, 2004; Mangaraj *et al*., 2009; Workneh and Osthoff, 2010). This modified atmosphere suppresses respiration and ethylene formation, thereby promoting an extended avocado shelf life.

Gas concentrations for MAP were found to be 2-6% oxygen and 3-10% carbon dioxide at 5°C and 7°C (Meir *et al*., 1997). This combination inhibits avocado softening and decreases the effect of chilling injury. Meir *et al*. (1997) investigated the effect of MAP on the storage of 'Hass'. Optimum results were found when storing 3.2 kg of the avocados in 30µm PE bags (40 x 70cm) at 5ºC. The concentration of oxygen and carbon dioxide attained values of approximately 4% and 5%, respectively, at 5ºC and 7ºC. At 5ºC, lower ethylene evolution was detected with firmer fruit. These concentrations are in accordance with those prescribed by Sandhya (2010) of 2-5% oxygen, 3-10% carbon dioxide and 85-95% nitrogen. Berrios (2002) recommends similar CAS and MAP conditions of 2-5% oxygen and 3-10% carbon dioxide at 5-13ºC for the transportation and storage of avocados. A temperature variation of 7-14°C resulted in varying oxygen and carbon dioxide concentrations of between 2-6% and 3-7%, respectively (Meir *et al*., 1997). The avocados retained a good quality for up to seven weeks. Softening became evident within four weeks of storage, as oxygen levels exceeded 9%.

A web-based software tool, 'PACK-in-MAP', was developed and reported by Mahajan *et al*. (2006), which assists in designing the optimal modified atmosphere conditions of fresh commodities. This is achieved by a user input of the commodity type. The software is then able to define the optimum temperature, range of oxygen and carbon dioxide concentrations and the permeability of various packaging materials (Mahajan *et al*., 2006).

The modification of the storage environment can be accomplished either through the respiration of the commodity identified as natural or passive MAP, or by intentionally introducing a gas mixture into the packaging identified as artificial or active MAP (Yahia and Gonzalez-Aguilar, 1998; Mangaraj *et al*., 2009; De Reuck *et al*., 2010; Workneh and Osthoff, 2010). De Reuck (2010) stated that active MAP, as opposed to a passive mode, does not alter the gas composition at equilibrium, but rather, the time taken for equilibrium to be established is shortened. Few studies are presented on the MAP of avocados and the subsequent effect on the quality parameters, as well as the change in the gas concentration within the packaging over the storage period.

2.5.3 Controlled atmosphere storage

In CAS, the headspace gas is more precisely monitored and controlled on a continuous basis to suit the requirements of avocados (Berrios, 2002; Sandhya, 2010; Workneh and Osthoff, 2010), compared to that in MAP as discussed in Section 2.5.2. Oxygen and carbon dioxide concentrations of 2-6% and 3-10%, respectively, were recommended by Meir *et al*. (1997) at 5°C and 7°C, to reduce chilling injury and to inhibit the softening of avocados. The most effective results were attained with avocados stored at temperatures of between 5°C and 7°C and accompanied by oxygen and carbon dioxide concentrations of 2-3% and 8-10%, respectively (Meir *et al*., 1997). Reduction in mesocarp discolouration in 'Fuerte' was achieved with 2% oxygen and 10% carbon dioxide at 5.5°C for 28 days (Pesis *et al*., 2002). CAS is a capital intensive operation, as stated by Workneh and Osthoff (2010), and is suited for the bulk storage of commodities and for prolonged storage periods (Sadhya, 2010). The limited success of avocados under a controlled atmosphere for five to nine weeks has been presented by Meir *et al*. (1997).

2.6 Storage Conditions

The two common techniques for the storage of fruit are conventional refrigeration and controlled atmosphere (Kosiyachinda and Young, 1976). The conditions prevailing within these storage facilities are essential in attaining an extended shelf life and an enhanced quality of avocados. This section focuses on essential storage parameters and the subsequent effect on the avocado quality.

2.6.1 Temperature

Temperature is the most crucial factor to consider in the storage of fruit, due to its involvement in biological processes (Workneh *et al*., 2011b). Low temperature storage hinders the respiration rate and ethylene production, resulting in retarded metabolic rates and an extended shelf life (Hofman *et al*., 2002a; Perez *et al*., 2004; Workneh and Osthoff, 2010; Getinet *et al*., 2011; Workneh *et al*., 2011a). Theoretically, for every 10°C increase in temperature, a resultant doubling in the rate of respiration occurs (Workneh and Osthoff, 2010).

Zauberman and Jobin-Decor (1995) found that storage at 5°C and 8°C resulted in early ripening and mesocarp discolouration. However, Perez *et al*. (2004) report the optimum storage temperature for unripe avocados to be 5-13°C and 2-4°C for mature avocados, which result in two to four weeks of shelf life, depending on the cultivar. If mature avocados were stored at 5-8°C, the shelf life would be reduced to one to two weeks. According to Hopkirk *et al*. (1994), cool stored avocados at 6°C and thereafter ripened at 15°C, was most effective in enhancing the fruit quality. This compares with findings by Meir *et al*. (1995), which state that temperatures of between 5°C and 7°C yield successful results in prolonging the shelf life of 'Hass' by five to nine weeks. A combination of 7°C with 2% oxygen and > 4% carbon dioxide extended the storage time to nine weeks (Zauberman and Jobin-Decor, 1995). Storage at 2°C proved to be more successful, as the fruit remained unripe for up to four weeks and ripened normally when transferred to 22°C.

Van Rooyen and Bower (2006) discovered that the storage of 'Pinkerton' at below the recommended temperature of 5.5°C, reduced the severity of mesocarp discolouration, which was thought to be due to chilling injury, while storage at temperatures above 8°C intensified the disorder. Cold storage increased the occurrence of mesocarp discolouration and became more pronounced, with increasing maturity (Cutting *et al*., 1992). Temperatures that are too high are also undesirable, with fruit failing to ripen adequately and the proliferation of postharvest disorders at 30°C and 25°C, compared to a ripening temperature of 20°C (Eaks, 1978; Hopkirk *et al*., 1994). Flitsanov *et al*. (2000) demonstrated the effect of temperature on the firmness of 'Ettinger'. During the first four weeks of storage, at 2°C, 4°C, 6°C and 8°C, the firmness decreased to 89.2 N, 79.2 N, 12.5 N and 10.9 N, respectively, indicating that the higher temperature accelerated the ripening process. These results are in agreement with those found by Mizrach *et al*. (2000).

2.6.2 Relative humidity

Most fresh commodities require high relative humidity conditions during storage (Hofman *et al*., 2002a; Getinet *et al*., 2011). By increasing the relative humidity, the vapour pressure deficit is reduced, resulting in less water loss (Blakey, 2011). The negative effect of low relative humidity on texture and appearance can be attributed to water loss (Paull, 1999). Adato and Gazit (1974) demonstrated that avocados at 10-20% relative humidity lost water three times faster than those stored at 90-95% relative humidity and 21°C to 22°C. The ripening process was also hastened by approximately three days. Also, Hofman and Jobin-Decor (1999) discovered that holding avocados at 60% relative humidity, or less for four days resulted in an increase in the dry mass by 1.5% and reduced the days to ripen, compared to a 98% relative humidity. Storage conditions of mature avocados at 5°C and a relative humidity of 85-90% could result in a shelf life of two to three weeks (Perez *et al*., 2004). Table 2.4 lists optimum storage conditions for different avocado cultivars.

Table 2.4 Optimum temperature and relative humidity of avocado cultivars (Yahia, 2002)

Cultivar	Temperature (°C)	Relative Humidity (%)	Postharvest Life (weeks)
'Hass'	3 - 7	85 - 90	2 - 4
'Fuerte'	3 - 7	85 - 90	2 - 4
'Fuchs'	13	85 - 90	2
'Pollock'	13	85 - 90	2
'Lula'	4	90 - 95	4 - 8
'Booth I'	4	90 - 95	4 - 8

2.6.3 Gas concentration

Gases significantly contribute to the storage of fresh commodities, particularly oxygen, carbon dioxide, ethylene and nitrogen (Berrios, 2002). This section will discuss the effect of these gases on the quality of avocados.

2.6.4 Oxygen and carbon dioxide

Meir *et al.* (1995) describe oxygen and carbon dioxide as having a synergistic role in inhibiting the ripening process of avocados through the increase in carbon dioxide and the decrease in oxygen concentrations. Previous studies demonstrated that the most successful atmospheres were 2% oxygen and 10% carbon dioxide (Pesis *et al*., 2002). The study undertaken by Meir *et al*. (1995) showed that carbon dioxide and oxygen concentrations of 8% and 3%, respectively, yielded a storage time of nine weeks, with marketable fruit and no chilling injury. Similarly, carbon dioxide concentrations of 5% or 10% delayed the respiratory rise and decreased the respiration rate, contributing to a prolonged shelf life (Kosiyachinda and Young, 1976). 'Fuerte' exposed to 25% carbon dioxide for three days prior to storage at 5°C for 28 days, resulted in decreased disorders and lower levels of total phenols (Pesis *et al.*, 1994). Avocados subjected to excessively high carbon dioxide and too low oxygen concentrations induced exocarp and mesocarp injury (Yahia and Carrillo-Lopez, 1993; cited by Ke *et al*., 1995; Lange and Kader, 1997). Oxygen concentrations of less than 1% are likely to result in anaerobic respiration (Forero, 2007). Exposure to oxygen levels below 3% for prolonged periods is not recommended by Valle-Guadarrema *et al*. (2004). Lange and Kader (1995) showed that avocados stored in 40% carbon dioxide and 12.6% oxygen demonstrated increased respiration rates, when compared to 20% carbon dioxide and 16.8% oxygen. Meir *et al*. (1995) related peel injury with low concentrations of oxygen and slower softening rates of avocados to be associated with higher carbon dioxide levels.

2.6.5 Ethylene

Ethylene has the potential to induce over-ripening, accelerate quality loss and increase susceptibility to pathogens during the storage of fresh commodities (Martinez-Romero *et al*., 2007). The effect of ethylene on avocados can be identified as flesh softening, colour change and the development of distinct aromas (Gerard and Gouble, 2005; Martinez-Romero *et al*., 2007). Zauberman and Fuchs (1973) found that treatment of avocados with ethylene at a storage temperature of 6°C contributed to accelerated respiration rates and softening. Fruit treated continuously with exogenous ethylene produced the least amount of ethylene, compared to untreated fruit and those treated for 24 hours. It is suspected that the ethylene evolved is merely due to the diffusion of the exogenous ethylene that had initially been absorbed, rather than, the production of ethylene by the fruit (Zauberman and Fuchs, 1973).

Findings by Hatton and Reeder (1972) concur with those of Zauberman and Fuchs (1973), which proves that the removal of ethylene from storage atmospheres reduced the rate of softening. Eaks (1978) showed that avocados held at 35°C displayed the climacteric pattern and ripened with minute amounts of ethylene being evolved. Ethylene formation in avocados have, thus appeared to be independent of high temperatures, while at 40°C this process seems to be inhibited. Pesis *et al*. (2002) suggest that absorbent sachets are able to remove ethylene from the packaging after five weeks of storage at 5°C, to reduce mesocarp discolouration and decay in 'Hass'.

2.6.6 Nitrogen

Nitrogen is a tasteless, colourless, odourless gas and relatively unreactive (Sandya, 2010). As demonstrated by Ke *et al.* (1995) and Lange and Kader (1997), nitrogen is commonly used as a filler gas in the gas mixture to prevent the collapsing of packages, due to its low solubility in food. The storage of avocados in anoxia conditions of 100% nitrogen resulted in irreparable damage (Moriguchi and Romani, 1995). Gouble *et al.* (1995) demonstrated that continuous treatment with 80% of the nitrogen composite, nitrous oxide and 20% oxygen inhibited the ethylene production in avocado fruit. A summary of the pertinent postharvest conditions of avocados that were reviewed, are presented in Table 2.5, including the essential storage parameters, as discussed within the scope of this document.

Table 2.5 Summary of avocado storage conditions recommended by different authors

Cultivar/ Type	Storage Temperature (°C)	Ripening Temperature (°C)	Storage/ Ripening Time	Relative Humidity (%)	O_2* (%)	CO_2** (%)	Additional Information	Reference
'Hass'	6		10 days				Best quality fruit, less postharvest rots	Hopkirk *et al.*, 1994
		15						
'Hass'	5		<1 week				Increase in ethanol and acetaldehyde, reduction in pH values from 6.9 to 6.3	Ke *et al.*, 1995
		20	1-3 days		0.25	80		
'Hass'	5	20		90-95	3	8	Remained green after 9 weeks, retarded chilling injury	Meir *et al.*, 1995
'Hass'	5-7						Prolong the shelf life of 5-9 weeks	Meir *et al.*, 1995
'Hass'	2		4 weeks				Remained firm and green for 4 weeks, ripening was delayed	Zauberman and Jobin-Decor, 1995
		22						
'Hass'	5 and 8		4 weeks				Mesocarp discolouration and vascular browning, fruit ripening commenced during storage	Zauberman and Jobin-Decor, 1995
		22						
'Hass'	7				2	>4	Shelf life of 9 weeks	Zauberman and Jobin-Decor, 1995
'Hass'					12.6	40	Increased respiration rates	Lang and Kader, 1997
	5 and 7				2-6	3-10	CA and MA - softening and chilling injury inhibition	Meir *et al.*, 1997
	7-14				2-6	3-7	MA - Fruit retained good quality for 7 weeks	Meir *et al.*, 1997
	5-7				2-3	8-10	Recommended storage conditions	Meir *et al.*, 1997

Cultivar/ Type	Storage Temperature (°C)	Ripening Temperature (°C)	Storage/ Ripening Time	Relative Humidity (%)	O_2* (%)	CO_2** (%)	Additional Information	Reference
‘Hass’ avocado tree on clonal 'Duke 7' rootstock		20	12 days	85-90			Decline in the TSS content	Liu *et al*., 2002
‘Hass’	5-13				2-5	3-10	MA - For transport and storage	Berrios, 2002
‘Fuerte’	5.5		28 days		2	10	Reduced mesocarp discolouration	Pesis *et al*., 2002
Unripe	5-13						2-4 weeks shelf life	Perez *et al*., 2004
Mature	2-4						2-4 weeks shelf life	Perez *et al*., 2004
Mature	5			85-90			2-3 weeks shelf life	Perez *et al*., 2004
Mature	5-8						1-2 weeks shelf life	Perez *et al*., 2004
‘Pinkerton’	<5.5						Reduced mesocarp discolouration	Van Rooyen and Bower, 2006
‘Hass’	6		17 days		0.5	20	Increase in ethanol and acetaldehyde	Burdon *et al*., 2007
					2-5	3-10	MA - Recommended storage conditions	Sandhya, 2010
‘Hass’		20	12 days	75			Reduction in firmness from 75.43 N to 2.63 N	Arzate-Vazquez *et al*., 2011
‘Hass’		15	12 days				Firmness reduced from 130.51 N to 7.37 N and increase in dry matter from 31.65% to 36.52% and thereafter decrease to 32.91%	Villa-Rodriguez *et al*., 2011

Note: where entries are blank no information was available in literature.

* Oxygen and ** Carbon Dioxide concentrations.

2.7 Postharvest Supply Chain Management in South Africa

The South African avocado industry is predominantly export-based (Bower and Cutting, 1987), which necessitates the need to ensure that the avocado quality is capable of meeting international standards. This section briefly describes the postharvest supply chain of avocados in South Africa, the associated guidelines and recommendations provided by literature on storage regimes.

2.7.1 Quality standards

Eksteen (1995; 1999) and the Agricultural Product Standards Act (1990) provide guidelines and recommended procedures in South Africa for avocado export. The European Union Commission has provided a number of regulations governing the quality of produce deemed acceptable, such as EC 1935/2004 and 94/62/EC, which concern packaging of foodstuff and EC 178/2002, relating to food safety aspects (DAFF, 2010). The Codex Alimentarius is another set of codes used as a global reference of food standards and, specific to avocados, is the Codex Standard for Avocado (2005).

2.7.2 Avocado cold chain

The transportation of avocados from the growing regions in South Africa to the port in Cape Town and eventually, to European supermarkets, requires extensive logistical management (Bower and Cutting, 1987). The proper maintenance of the cold chain is essential in avoiding warm, soft fruit with physiological disorders (Nelson, 2006). The Perishable Products Export Control Board works in alliance with the South African Avocado Growers' Association to provide recommendations and guidelines for the handling of avocados during export (Eksteen, 1995; 1999).

Unpublished studies by Blakey and Bower (2009) and Kok *et al*. (2010) demonstrate to what extent a break in the avocado cold chain is detrimental to the quality of the fruit. These investigations indicate that the storage of avocados at 1°C for 28 days, to simulate shipping regimes, reduce the rate of softening and mass loss. However, Kok *et al.* (2010) state that additional studies are required to confirm these findings. Milne (1998) describes the vital role that the combination of time and temperature plays during the cold chain management of avocados, by reporting that a break later in the cold chain leads to greater fruit softening. Bezuidenhout (1992) conducted an analysis to address the softening of avocados experienced during export to Europe. It was found that an increase in temperature by 1°C during a transit time of 28 days resulted in increased softening, from a firmometer reading of 32 to 46.

Step-down temperature is an adopted technique, which gradually reduces the storage temperature of avocados (Milne, 1998). This was shown to reduce chilling injury and pulp spot symptoms. Early season 'Fuerte' stored at 7.5°C for week one, 5.5°C for weeks two and three, followed by 3.5°C for week four, resulted in reduced chilling injury, compared to 5.5°C for the total four week period. Milne (1998), however, reports that this step-down regime was not necessary for 'Fuerte' grown in KwaZulu-Natal, as a continuous storage temperature of 5.5°C was sufficient for both internal and external quality. It is not advised to subject avocados to temperatures below 5°C or greater than 10°C after harvest (Sekhune, 2012). Table 2.6 provides a summary of the recommended moisture content and air temperatures specific to 'Fuerte', 'Hass' and 'Ryan' avocados during export (Milne, 1998).

Table 2.6 Moisture content and temperature guidelines for avocado export

Moisture Content (%)	**Cold Room (°C)**	**Road Transport (°C)**	**Port Storage (°C)**	**Vessel - 1st week (°C)**	**Vessel - last week (°C)**
78.5 - 80.0	7.5	7.5	7.5	7.5	5.5
77.5 - 78.4	7.5	7.5	7.5	6.5	5.5
76.5 - 77.4	7.0	7.0	7.0	6.1	5.5
75.5 - 76.4	6.5	6.5	6.5	6.0	5.5
74.5 - 75.4	6.5	6.5	6.5	5.5	5.5
73.5 - 74.4	6.0	6.0	6.0	5.5	5.5
72.5 - 73.4	6.0	6.0	6.0	5.5	5.5
71.5 - 72.4	5.5	5.5	5.5	5.5	5.5
69.5 - 71.4	5.5	5.5	5.5	5.5	4.5
69.4 and less	5.5	5.5	5.5	5.5	3.5

An example of an avocado cold chain is illustrated in Figure 2.3, where T_X represents the temperature at each stage X in °C (eg. T_H is the temperature at harvest).

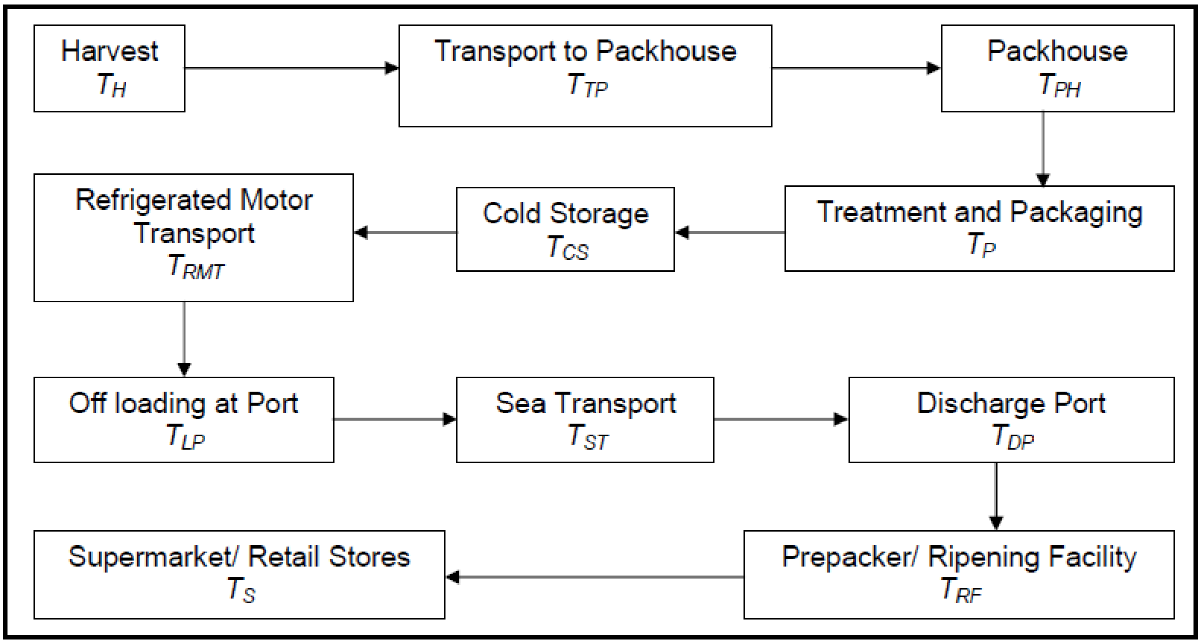

Figure 2.3 Avocado cold chain (after Eksteen, 1999)

Each stage depicted in Figure 2.3 requires a specific temperature, in order to maintain avocado quality from the point of harvest till its final market destination. These conditions vary, depending on the time of harvest and the cultivar (Sekhune, 2012). It is, therefore, essential to apply the appropriate temperature regime, depending on the prevailing circumstances.

2.8 References

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3. THE EFFECT OF POSTHARVEST HANDLING AND FIXED TEMPERATURE STORAGE CONDITIONS ON THE AVOCADO QUALITY – EXPERIMENT I

3.1 Abstract

To maintain avocado fruit quality, proper integrated postharvest technologies are required. The aim of this experiment was to quantify the extent of avocado (‘Hass’) quality changes subjected to different pre-packaging, packaging and fixed temperature and relative humidity storage conditions. This experiment focused on subjecting avocados to fixed temperature (5°C or 10°C) and relative humidity (85% or 90%) storage conditions for 12 days, in combination with pre-packaging and packaging treatments. A randomised complete block design with pre-packaging (hot water and Avoshine® wax coating), packaging (low density polyethylene (LDPE) and corn starch biodegradable films) and storage conditions (controlled and ambient) with three replications was used. The quality parameters that were evaluated included physiological weight loss (PWL), respiration rate, marketability, skin colour, firmness (exterior and pulp), puree colour, puree viscosity, moisture content (MC), dry matter (DM), pH, total soluble solids (TSS) and total titratable acidity (TTA). Storage conditions and the storage period significantly ($P \leq 0.001$) affected the PWL, respiration rate, marketability, skin colour, firmness (exterior and pulp), puree colour, MC, DM, pH, TSS and TTA. Low temperature storage (5°C + 85% and 5°C + 90%) offered the greatest benefit in maintaining higher fruit marketability, when compared to the other storage conditions. Control samples that were subjected to ambient conditions without any pre-packaging or packaging treatments exhibited increased rates of ripening, which was evident in increased PWL, reduced firmness (exterior and pulp), darkened skin colour, rapid decline in pH, increased TTA and TSS. The Avoshine® coating, combined with LDPE packaging, was most beneficial in maintaining better fruit quality and delaying the ripening process. In addition the application of cold storage was essential in maintaining avocado quality, preventing excessive deterioration and extending the avocado shelf life. The hot water treatment promoted a darkening of the skin, decreased pulp firmness and lower fruit marketability.

3.2 Introduction

"Shelf life is defined as the period in which a product should maintain a predetermined level of quality under specified storage conditions" (Perez *et al*., 2004). Postharvest technologies are primarily aimed at minimising fruit losses by controlling metabolic reactions, such as respiration through the manipulation of the external conditions (Mashau *et al*., 2012). Some of these technologies include controlled atmosphere storage, modified atmosphere packaging or the use of waxes applied to the exterior of the avocado. Respiration occurring within fruit is a necessary and natural process, but once harvested, excessive respiration rates can be detrimental to the avocado quality by depleting valuable stored carbohydrates. This induces high physiological weight loss, wilting, shrivelling, reduced firmness, a darkening of the skin and off flavours, which may result in a reduced shelf life. The shelf life is, therefore, inversely proportional to the respiration rate (Perez *et al*., 2004).

Pre-packaging methods, such as heat treatments have been used as a non-chemical disinfection technique to control insect pests and micro-organisms that promote fungal decay. Numerous studies have demonstrated the positive effects of heat treatments on the quality of avocados (Sanxter *et al*., 1994; Florissen *et al*., 1996; Lurie, 1998; Fallik, 2004; Wu *et al*., 2011). The most apparent influence of heat treatments on avocados was a reduction in the chilling associated with cold storage. Other benefits included reduced body rots, the preservation of the green skin colour and firmer fruit. However, the type of heating method, including the heating fluid, temperature and exposure duration, contributes significantly to the final fruit quality. After harvest, loss in mass as a result of moisture loss via transpiration and/or carbon loss through respiration is a major contributor to hastening the ripening process (Johnston and Banks, 1998). The application of coatings or waxes to the surface of avocado fruit has the ability to modify the internal environment by inhibiting the escape of moisture from the fruit to the surrounding environment and lowering the intake rate of oxygen and the expulsion rate of carbon dioxide. Maftoonazad and Ramaswamy (2005; 2008) found that coated avocados displayed superior quality with respect to enhanced colour, firmer fruit and reduced respiration, when compared to non-coated fruit.

Packaging films with relatively low gas permeability have the ability to create a modified atmosphere for fruit by increasing the carbon dioxide and lowering the oxygen concentrations as the fruit respires (Meir *et al*., 1997; Illeperuma and Nikapitiya, 2002). The application of suitable packaging, with the added benefit of optimum storage conditions, has been proven to be successful in maintaining avocado quality and improving the shelf life. 'Hass' avocados, packaged in polyethylene films and stored at 5°C demonstrated an extension in the shelf life (Illeperuma and Nikapitiya, 2002). The avocado cultivars 'Fuerte' and 'Nabal' stored in sealed polyethylene bags, demonstrated a shelf life of three and six and a half weeks, respectively (Meir *et al*., 1997). The use of biodegradable materials as a form of packaging have also been proven to be beneficial in delaying the ripening process and reducing fruit quality loss (Aguilar-Mendez *et al*., 2008). The use of biodegradable packaging has the added advantage of minimizing the detrimental impact of pollution and the encumbrance of landfills by, being easily disposed of in the form of compost.

Temperature is the predominant environmental factor that influences the various physiological and biochemical changes during the ripening process of horticultural commodities (Paull, 1999; Perez *et al*., 2004). The shelf life of avocados is most commonly extended by low temperature storage because of the reduced respiration and enzyme activity . The relative humidity has also been shown to affect the quality of avocados. A loss of moisture tends to increase with a decrease in the surrounding relative humidity. This is due to a water vapour pressure deficit that exists between the fruit and the surrounding environment, prompting the loss in moisture (Maftoonazad and Ramaswamy, 2008; Li *et al*., 2010).

Based on the provided evidence, the use of suitable pre-packaging, packaging and storage conditions have been found to influence the ripening process of avocados and, hence, the fruit quality. In this experiment, the effect of integrated postharvest technology of pre-packaging, packaging and storage conditions (temperature and relative humidity) on the physical, chemical and subjective sensory quality parameters were investigated for avocado fruit. A combination of these postharvest treatments could bring about a synergistic effect on the shelf life extension of fruits. However, limited work has been done on the integrative effectiveness of these particular postharvest handling techniques on avocados.

The aim of this experiment was to investigate the combined effect of postharvest treatments on the quality of avocados and to provide practical recommendations for avocado postharvest handling in South Africa based on the results obtained. The specific objective formulated for this experiment was to investigate the combined effect of pre-packaging treatments, packaging films and multiple fixed temperature and relative humidity storage conditions on the physical, chemical and subjective sensory quality of avocados in South Africa.

3.3 Growing Site Description

Avocados (*Persea americana* Mill.), belonging to the 'Hass' cultivar, were obtained from the Everdon Estate located in the Karkloof Valley in Howick, KwaZulu-Natal (29°27'S, 30°16'E). The Everdon Estate is primarily an organic farm owned by Westfalia, which is South Africa's largest producer of avocados. The orchard is located in the Phillips' Bioclimatic Group 3, characteristic of cool mesic conditions typical of 'mist belt' areas (Moore-Gordon *et al*., 1995; Moore-Gordon and Wolstenholme, 1996). The orchard experiences mean minimum temperatures of approximately 15°C in January and approximately 6.7°C in July, with corresponding mean maximum temperatures of approximately 26.1°C and 19.4°C (Moore-Gordon and Wolstenholme, 1996). The area receives an average annual rainfall of 1052 mm. Micro-jet irrigation systems supply water to the scheme that has been installed with tensiometers (Moore-Gordon and Wolstenholme, 1996). The predominant soil is a well-drained Hutton prepared by deep-ripping only once. Two mulch dressings are placed around trees annually, which have resulted in a marked improvement to the fruit borne. The farm produces an average of 10 $tons.ha^{-1}$ per year of avocados, of which approximately 70% are exported. Due to the cooler subtropical climate in Howick, the avocados grown in these orchards mature at a later stage, compared to those grown in the Limpopo province. This enables the Everdon Estate to lengthen its export season, particularly in the case of 'Hass', which has a harvest season starting from early July and extending into October or early November each year.

3.4 Plant Material

Green mature 'Hass' avocados were manually harvested commercially by trained harvesters early in the morning to reduce field heat and minimise mechanical injury. Once harvested, avocados within a mass range of 203-243 g were then selected and packed into single layer standard Count 18 corrugated cardboard boxes (18 avocados per box) with ventilation. A total of 420 avocados were acquired for this experiment. From the commercially harvested avocados, samples were selected based on their uniformity of weight, shape, colour, size and whether they were bruise- and blemish-free to be used in the experiment (Mohammed *et al*., 1999; Maftoonazad and Ramaswamy, 2008; Getinet *et al*., 2011). The selected samples were then immediately transported to the University of KwaZulu-Natal Food Science and Agricultural Engineering laboratory, which is located 37 km from the packhouse, where sample preparation, treatment and storage trials were carried out.

3.5 Experimental Design

A factorial design consisting of three pre-packaging treatments (Avoshine®, hot water immersion and no pre-packaging treatments), three packaging treatments (low density polyethylene (LDPE), corn starch biodegradable films and no packaging), five temperature and relative humidity (RH) storage regimes (5°C + 85%, 5°C + 90%, 10°C + 85%, 10°C + 90% and ambient) and three replications were arranged in a Randomised Complete Block Design (Mohammed *et al*., 1999; Getinet *et al*., 2011). This method groups experimental units into blocks as uniform as possible, ensuring that the differences between treatments are 'true' differences, thus accounting for any variations in the samples (Compton, 1994). The samples were randomly treated and evaluated for their quality immediately after harvest.

3.6 Sample Preparation and Treatments

The avocado samples were visually inspected at the laboratory to ensure that they were not subjected to any damage during transportation and, if they were, the damaged avocados were excluded from the samples (Getinet *et al*., 2011). All work surfaces, tools and utensils were cleaned and disinfected using a cloth and disinfectant. A total of 84 avocados were treated and tested per storage regime with three samples tested on Day 0 and 27 samples on Days 4, 8 and 12. The samples were gently hand-washed under running tap water and were then surface-dried. The following sections contain a detailed description of the pre-packaging and packaging treatment procedures that were followed for each storage condition.

3.7 Pre-packaging Treatments

The use of a wax (Kremer-Kohne and Duvenhage, 1997) and hot water pre-packaging treatments (Wu *et al*., 2011) were selected, as these are extensively used for avocados. Avoshine® is a liquid polyethylene wax emulsion that is used to coat avocados, which aids in replacing the natural coat that can be removed, either through washing or damage (Hall, 2011). Avoshine® is composed of 0-30% polyethylene, ≈10% vegetable based emulsifiers, ≈1% antifoam and ≈60% water (Hall, 2011). A wax coating was evenly applied to the surface of 27 cleaned and dried avocado samples, using a soft bristle 38 mm Basic® Paint Brush. Approximately 0.4 ml of Avoshine® was used per 250 g of avocado fruit (Blakey, 2012). Once the wax completely covered the fruit in a single coat, the avocados were placed on a single layer of paper towels on a metal tray to absorb any excess wax that may be present and left to surface-dry for 40 seconds (Hall, 2011).

For the hot water pre-treatment a hot water bath was first cleaned before adding approximately seven litres of tap water. The water was initially heated to 80°C for 30 minutes to destroy most heat-sensitive micro-organisms and, thereafter, reduced to 38°C. A digital thermometer (CEG8106) was used to monitor the water temperature. Twenty-seven other avocado samples were immersed in the hot water for five minutes, then removed and dried. Twenty-seven different avocados were used as a control, which were neither coated with the wax nor dipped in the water bath. Figure 3.1 represents the pre-treatments used. All samples were then clearly labelled using an Artline® 400 XF white permanent marker.

Figure 3.1 Pre-treated avocado samples

3.8 Packaging Treatments

LDPE and biodegradable packaging films were selected as they were shown to be beneficial for avocados (Xiao and Kiyoto, 2001; Aguilar-Mendez *et al*., 2008). LDPE bags with 20 µm thickness and 250 × 150 mm were used. LDPE films are soft, flexible and strong, with a high ratio of CO_2 to O_2 permeability (Mangaraj *et al*., 2009). LDPE bags have a higher water vapour transmission rate of between 375-500 g.µm.m^{-2}.day^{-1}, compared to high density polyethylene bags, which have a rate of 100-300 g.µm.m^{-2}.day^{-1} (Mangaraj *et al*., 2009). Two rows with four micro perforations at 30 mm intervals were made along the bottom of each bag, using a needle with a diameter of 1.13×10^{-3} m (Figure 3.2). This was to allow for the movement of gases and moisture between the mico-environment inside the bag and the surroundings. Nine avocados that had been coated with Avoshine®, nine avocados subjected to hot water treatment and nine avocados that had not been pre-treated were each placed in a single LDPE bag and sealed, using a SLE Sealex heat sealer. In essence each bag had one avocado. The bags were uniformly sealed at 164.3 mm from the base, to ensure that the volume within the both the LDPE and biodegradable bags were approximately equal.

The other flexible film used in this experiment was a transparent biodegradable corn starch cellulose bag of 30 µm thickness, 240 × 100 mm and 45 mm gussets. These bags have a high barrier to air and micro-organisms, which is ideal for the packaging of food (Aguila-Mendez *et al*., 2008). Biodegradable bags are a natural renewable resource due to its ability to be composted, compared to conventional plastics (Kantola and Helen, 2000; Aguila-Mendez *et al*., 2008). Two rows containing four micro perforations at 20 mm intervals were made along the bottom of each bag, using a needle with a diameter of 1.13×10^{-3} m (Figure 3.2). The interval spacing of the micro-perforations were smaller compared to the LDPE bags, due to the difference in width of the bags and to allow for evenly-spaced micro-perforations in both types of bags. Nine avocados that had been coated with Avoshine®, nine avocados subjected to hot water treatment and nine avocados that had not been pre-treated were each placed in a single biodegradable bag and sealed. The bags were uniformly sealed at 170 mm from the base, to ensure that the volume within both the LDPE and biodegradable bags were approximately equal.

Nine avocados that had been coated with Avoshine®, nine avocados subjected to hot water treatment and nine avocados that had not been pre-treated were not packaged to serve as a control. A summary of the different treatment combinations are presented in Table 6.1 in Appendix A.

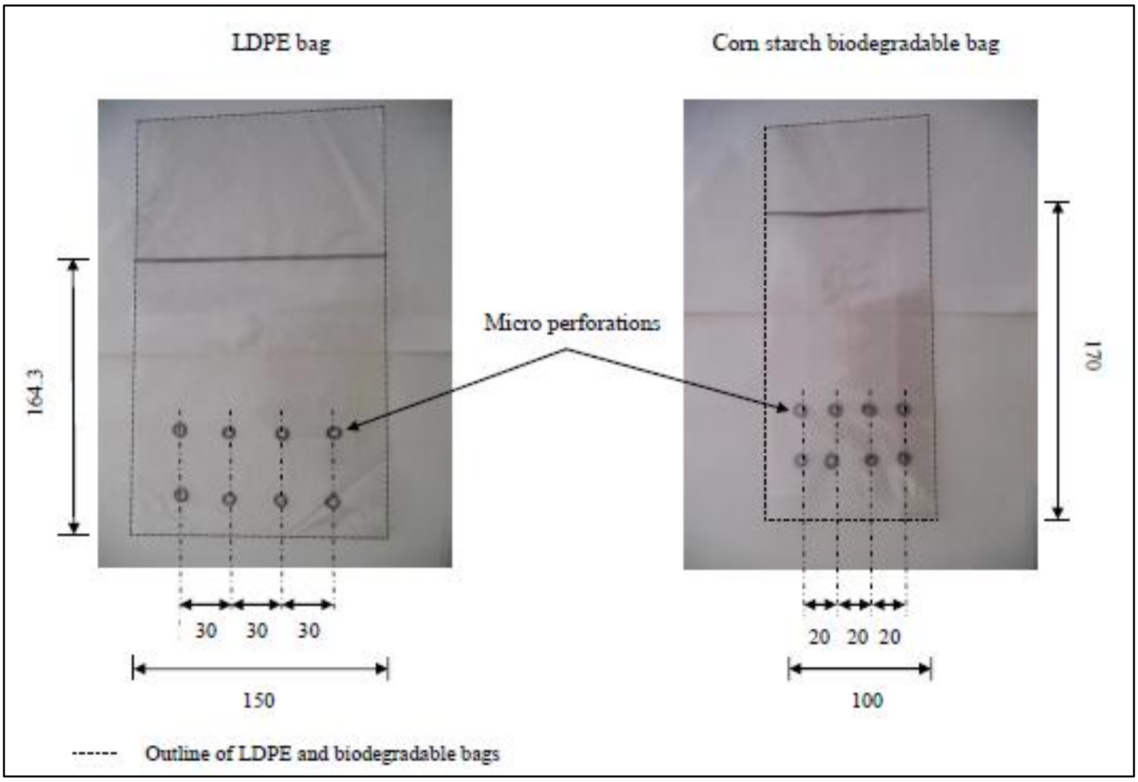

Figure 3.2 LDPE and corn starch biodegradable flexible films with micro-perforations

3.9 Temperature and Relative Humidity

The treated avocado samples were stored at temperatures of 5°C and 10°C. These temperatures were selected based on the recommendations of (Sekhune, 2012). The two relative humidity conditions selected were 85% and 90%, as recommended for 'Hass' by (Yahia, 2002). These conditions were controlled in a CTS Climate Test Chamber (Model C-40/100) with a temperature range of -40°C to +180°C and a humidity range of 10% to 98%. Theoretical temperature and relative humidity fluctuations of the chamber for climatic testing are ± 0.3K and 1.5%, respectively. The total capacity of the storage chamber is 100 litres (500 × 500 × 400 mm). The temperature and relative humidity storage conditions of 5°C + 85%, 5°C + 90%, 10°C + 85% and 10°C + 90% were programmed to operate for 12 days each. Each storage condition was programmed to start consecutively as the Climate Test Chamber is able to only simulate one regime of temperature and relative humidity at a time. The avocado samples were placed in the chamber on trays. The dimensions and a schematic of the CTS Climate Test Chamber are provided in Figure 3.3.

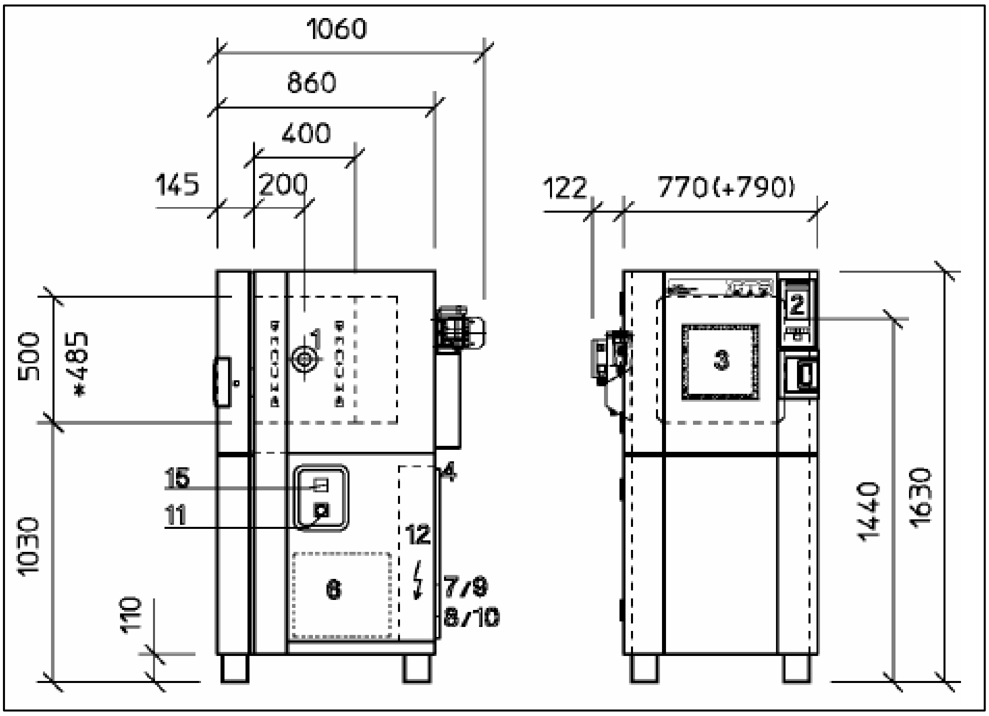

Figure 3.3 Schematic of the CTS Climate Test Chamber used to maintain temperatures and relative humidity during storage trials (Clima Temperatur Systeme Gmbh, 2010). All dimensions are in millimetres

Eighty four different avocado samples were placed in six corrugated cardboard boxes in a single layer and placed in an undisturbed area of the Food Science and Agricultural Engineering laboratory, exposed to ambient temperature (± 24.09°C) and relative humidity (± 53.40%) conditions (Figures 7.1 and 7.2 in Appendix B). This represented the control conditions for a duration of 12 days. Two HOBO data loggers, one placed on the avocado samples and the other placed in close proximity, were used to record the environmental conditions. Once the storage period had concluded, the BoxCar® Pro 4.3 software was used to retrieve the environmental data from the data loggers for analysis.

3.10 Data Collection and Analysis

Data was collected on Days 0, 4, 8 and 12 during storage, in order to determine the change in the avocado quality (Arzate-Vazquez *et al.*, 2011). The differences between treatments were determined by an analysis of variance (ANOVA) by means of the MSTAT-C statistical software, Version 2.10 (MSTAT, Michigan State University). The means were separated using the Duncan's Multiple Range Test, with a significance level of 0.05. The following parameters were used to evaluate the change in the quality of the avocados: physiological weight loss, respiration rate, marketability, skin colour, firmness (exterior and pulp), puree colour, puree viscosity, moisture content, dry matter, pH, total soluble solids and total titratable acidity. More detail on each quality parameter is contained in the sections to follow.

3.10.1 Physiological weight loss

Stored avocados from each treatment were individually weighed using a Mettler PJ 300 scale at the start of the experiment and at the specified intervals. The differential weight loss was calculated for each sample in each interval and converted to a percentage. The percentage cumulative weight loss could then be determined by summing the respective physiological weight losses (Getinet *et al*., 2008; Awole *et al*., 2011).

3.10.2 Respiration rate

Treated avocados were sealed in 1000 ml jars for 27 minutes. The carbon dioxide released by the avocados was measured, using an EGM-4 Environmental Gas Analyzer with an error of less than 1% of span concentration over the calibrated range. The carbon dioxide concentration within an empty sealed jar of the same size was also measured. The volume of each avocado sample was obtained by the water displacement method (Maftoonanzad and Ramaswamy, 2008). Figure 3.4 is a schematic diagram of the experimental apparatus and setup for determining the levels of carbon dioxide released by the avocados.

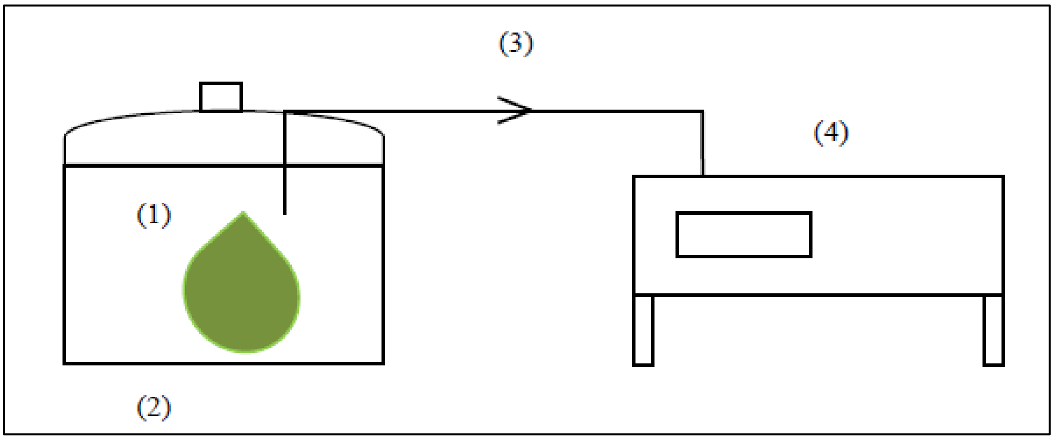

Figure 3.4 Carbon dioxide determination: (1) avocado sample; (2) 1000 ml jar to contain the sample; (3) duct conveying gas into the EGM-4 Environmental Gas Analyzer; (4) EGM-4 Environmental Gas Analyzer

The carbon dioxide ($ml.kg^{-1}.h^{-1}$) released was calculated using the following equation:

$$CO_2 = \frac{Net\ CO_2}{1000} \times Headspace \times \frac{1000}{m} \times \frac{60}{t} \tag{3.1}$$

where:

CO_2 = carbon dioxide released from avocado fruit

Net CO_2 = fruit CO_2 – ambient CO_2 [ml],

Headspace = container volume – fruit volume [l],

m = fruit mass [g], and

t = time of 1 minute.

3.10.3 Percent marketability

Marketable quality was evaluated according to the scoring method used by Awole *et al.* (2011) based on a rating, with 1 being ‘unusable’, 3 being ‘unsalable’, 5 being ‘fair’, 7 being ‘good’ and 9 being ‘excellent’. The number of fruit receiving a rating of 5 and above was used to calculate the percent of marketable fruit. Descriptive quality attributes were determined subjectively, based on observing the level of visible mould, decay, shriveling and shine (Tefera *et al.*, 2007). Figure 3.5 provides examples of typical marketability ratings.

Avocado sample					
Rating	1	3	5	7	9
Description	Unusable	Unsalable	Fair	Good	Excellent

Figure 3.5 Marketability rating system used for avocado fruit (after Awole *et al*., 2011)

3.10.4 Skin colour

A HunterLab ColorFlex EZ Spectrophotometer (Model 45/0 LAV) was used in assessing L*, a* and b* colour parameters of the avocado peel (Coronel *et al*., 2005; Maftoonazad and Ramaswamy, 2008). The spectrophotometer was calibrated using a black calibration tile and a white calibration tile, in order to standardize the equipment before readings could be done. Unpeeled whole samples were placed on the sample port and covered, using the opaque sample cup cover to avoid external light from interfering with the xenon flash, which illuminates the sample necessary for colour determination. The lens wipe or lens brush was used between each sample to remove any dirt or dust accumulation on the lens.

3.10.5 Exterior firmness (puncture force)

The Instron Universal Testing Machine (Model 3345) was used in conjunction with the Instron Bluehill 2 Version 2.25 software to determine the firmness of the avocados by means of puncturing the surface. Unpeeled avocados were placed horizontally on a curved platform which conformed to the avocado's shape, to ensure stability during the compression test. A probe of 1.5 mm diameter was used to make two punctures per avocado sample on opposite sides of the equatorial region. The cross head speed was set at 5 $mm.s^{-1}$ to travel to a depth of 5 mm. The maximum force required to puncture the fruit was taken as the exterior fruit firmness (Aguilar-Mendez *et al*., 2008).

3.10.6 Pulp firmness (Kramer Shear)

The same equipment used for the exterior firmness was used for the pulp firmness, however a Kramer Shear cell fitting was used instead of a puncture probe. The fruit were peeled and disks of 20 × 30 mm were cut from the pulp of each sample, using a stencil. The disks were then cut to a thickness of 10 mm. The disks were weighed and placed in the sample chamber while the metal plates were positioned to travel through the disk at a speed of 10 $mm.min^{-1}$. A low speed was used so as to reduce the frictional force between the blades and the sample chamber (Harker *et al*., 1997). The maximum force applied was divided by the weight of the disk to accommodate for the difference in the area of tissue cut by the plates. Harket *et al*. (1997) explains the Kramer Shear cell to be most closely associated with a combination of compression and extrusion and/or shear and extrusion. Therefore, the values obtained are considered to be empirical values, rather than fundamental measurements of shear and compression.

3.10.7 Puree preparation

The avocado samples were diced into smaller pieces. A Braun 300 W MR 400 hand blender was used to blend the diced avocados for two to three minutes until a fine paste was formed. The blender and utensils were washed and hand-dried between samples to avoid contamination (Jacobo-Velazquez and Hernandez-Brenes, 2011). This puree was sampled for colour, moisture content, dry matter, total titratable acidity, total soluble solids, pH and viscosity.

3.10.8 Puree colour

The same spectrophotometer that was used for the skin colour determination was used for the puree colour. A plastic ring with a thickness of 13 mm was placed inside a 64 mm glass sample cup and filled with the avocado puree until it reached the top of the plastic ring. The puree was immediately covered with a ceramic disk, with the white side facing the sample.
The cup was then placed on the sample port and covered using the sample cup cover.

3.10.9 Moisture content and dry matter

Moisture content (wet basis) and dry matter were determined using 3 g of the avocado puree placed in small foil containers. The samples were dried in an oven at 70°C for 48 h or until a constant weight. Constant weight was obtained by weighing the samples every hour after the 48 hour duration until there was no longer a change in the sample weights. Samples were re-weighed and the moisture content and dry matter were expressed as a percentage of the original weight of the puree (Chen *et al*., 2009).

3.10.10 Total titratable acidity, total soluble solids and pH

Approximately 25 g of the avocado puree was added to a beaker containing 25 g of distilled water. The samples were homogenized and frozen at -4°C. The samples were then allowed to thaw, after which the homogenate was filtered through muslin to collect the juice (Maftoonazad and Ramaswamy, 2008). An aliquote of 3 ml of juice was pipetted to a 50 ml beaker, into which two drops of phenolphthalein indicator solution was added. The juice aliquote was titrated with 0.1 N Sodium Hydroxide (NaOH) till a pink colour was formed and persisted for five seconds while the solution was being stirred, using a magnetic stirrer. Titratable acidity was calculated as the number of milliliters of 0.1 N sodium hydroxide multiplied by an appropriate conversion factor (Equation 3.2). A conversion factor of 0.28 was selected, based on linoleic acid, a predominant acid in avocados, as used by Maftoonazad and Ramaswamy (2008).

$$TTA = \frac{0.1N\ NaOH \times NaOH \times 0.28 \times 100}{S} \tag{3.2}$$

where:

$TTA =$ total titratable acidity,

$0.1N\ NaOH =$ 0.1 moles of NaOH [N],

$NaOH =$ amount of NaOH added [ml],

$0.28 =$ conversion factor, and

$S =$ juice sample [ml]

The pH was measured using a standard pH meter that had already been calibrated using pH 4 and pH 7 buffer solutions. The pH probe was dipped into the avocado juice until the reading stabilized (Getinet *et al*., 2008; Maftoonazad and Ramaswamy, 2008; Jacobo-Velazquez and Hernandez-Brenes, 2011).

The total soluble solids were determined using a ATAGO digital portable palette style refractometer (± 0.1% Brix) by placing one to two drops of the juice on the prism (Getinet *et* *al*., 2008; Maftoonazad and Ramaswamy, 2008). The prism was cleaned with 99.9% ethanol and then with distilled water, using a soft cloth between samples.

3.10.11 Puree viscosity

Puree viscosity was measured using the Anton PaarRheolab QC Rheometer basic unit (Model 13000) with Rheoplus V3.40 software. The remaining puree was passed through a piece of muslin. Approximately 16 g of puree was used to fill the measuring cup until the preset marker. The viscosity was measured as a function of shear rate, which was ramped from 0.01 s^{-1} to 100 s^{-1} for 250 seconds (Tabilo-Munizaga *et al*., 2005). The cup was inserted into the coupling ring with the threads facing up. The cup was then attached to the flange by fastening the coupling ring on the flange. All measurements were carried out at a room temperature of approximately 24°C.

3.10.12 Subjective quality attributes

Once the avocados were removed from storage, they were inspected and examined visually as well as by hand feel for any physical changes such as mould development/decay, colour and firmness. Any other variances with regard to the physical appearance were also observed and recorded.

3.11 Results and Discussion

This section presents the results and discusses the changes in the physical, chemical and subjective sensory quality parameters of avocado fruit as a result of the treatment combinations.

3.11.1 Physiological weight loss

Table 3.1 displays the cumulative physiological weight loss (PWL) of avocados subjected to the various pre-packaging, packaging and storage treatments. The storage conditions and the storage period had a significant ($P \leq 0.001$) influence on the PWL. The PWL increased with storage time, but was most pronounced in control samples. Storage at the lower temperature of 5°C + 90% RH and 5°C + 85% RH were found to reduce the PWL. However, storage at

5°C + 90% RH proved to be more effective in reducing the PWL. The lower weight loss can be due to the lower temperature, which reduces respiration rates and consequently the ripening, leading to a delay in senescence, compared to higher temperatures. In addition, higher temperatures and/or lower RH result in an increased vapour pressure deficit between the fruit and the surroundings, leading to an increase in moisture loss from the fruit to the surroundings (Maftoonazad and Ramaswamy, 2008; Awole *et al*., 2011).

Pre-packaging and packaging treatments were found to be less significant (P≤0.05), compared to storage conditions and the storage period. Avoshine® wax-coated samples and those pre-treated with hot water, generally displayed a lower PWL, compared to the control samples without any pre-packaging treatments. The wax coating appeared to have created a partially permeable layer, limiting the loss of moisture from the fruit to the surroundings, hence the fruit retained more moisture for the 12-day storage period (Johnston and Banks, 1998; Maftoonazad and Ramaswamy, 2008). The wax coating is able to seal any natural cracks or breaks in the fruit's exterior surface (Fallik, 2004). Avocado samples subjected to ambient conditions succumbed to the greatest loss in physiological weight, compared to those samples stored under controlled temperatures and relative humidity conditions. This is in agreement with the findings of Aguilar-Mendez *et al*. (2008), Maftoonazad and Ramaswamy (2008) and Awole *et al*. (2011). Avocado samples that had not been pre-packaged, experienced severe PWL of 19.23% by Day 12. LDPE and biodegradable packaging films appeared to have induced the lowest weight loss at 5°C and 90%, which could be attributed to the high air barrier and moisture property of the films, thus creating a high humidity micro-environment within the bags and limiting the loss of moisture from the avocado fruit (Kantola and Helen, 2000; Aguila-Mendez *et al*., 2008).

The two-way interactions between (a) packaging and storage conditions, and (b) storage conditions and the storage period were highly significant (P≤0.001) with regard to the PWL. The two-way interaction between (a) pre-packaging treatment and the storage conditions, and (b) the pre-packaging treatment and the storage period had a significant (P≤0.05) effect on the PWL during the 12-day storage period. Generally, all samples treated with the Avoshine® wax and LDPE or biodegradable films exhibited lower PWL, compared to the other treatments. This loss in the physiological weight was exceptionally low at the lowest temperature of 5°C. The primary mechanism for PWL is loss through moisture, which occurs as a result of the vapour pressure deficit. This indicates that the application of the wax was effective in acting as a barrier in preventing the loss in moisture, which is in agreement with the findings of Aguilar-Mendez *et al.* (2008). The storage of the waxed samples at 5°C + 90% resulted in the least cumulative weight loss of 1.94%, which was most effective in inhibiting the PWL.

Table 3.1 The physiological weight loss (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			**Storage Period (Days)**			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	0.00^{l}	2.49^{hi}	4.19^{gh}	6.01^{fg}
		5°C, 90%	0.00^{l}	3.05^{ghi}	3.23^{ghi}	3.40^{ghi}
		10°C, 85%	0.00^{l}	6.79^{efg}	6.80^{efg}	9.73^{cd}
		10°C, 90%	0.00^{l}	6.40^{fg}	8.63^{de}	8.70^{de}
		AT, ARH	0.00^{l}	5.80^{fg}	6.90^{efg}	9.87^{cd}
	Bio	5°C, 85%	0.00^{l}	3.05^{ghi}	4.67^{fgh}	5.65^{fg}
		5°C, 90%	0.00^{l}	0.78^{ij}	3.37^{ghi}	3.74^{gh}
		10°C, 85%	0.00^{l}	2.39^{hi}	4.50^{fgh}	6.88^{efg}
		10°C, 90%	0.00^{l}	6.12^{fg}	6.13^{fg}	7.43^{ef}
		AT, ARH	0.00^{l}	5.35^{fgh}	7.10^{efg}	12.16^{bc}
	NP	5°C, 85%	0.00^{l}	2.11^{hi}	4.43^{gh}	4.46^{fgh}
		5°C, 90%	0.00^{l}	2.02^{hij}	3.70^{gh}	4.10^{gh}
		10°C, 85%	0.00^{l}	7.13^{efg}	8.41^{def}	8.42^{def}
		10°C, 90%	0.00^{l}	6.77^{efg}	7.60^{ef}	7.64^{ef}
		AT, ARH	0.00^{l}	6.05^{fg}	12.91^{abc}	16.35^{ab}
Avoshine®	LDPE	5°C, 85%	0.00^{l}	2.50^{hi}	3.91^{gh}	4.23^{gh}
		5°C, 90%	0.00^{l}	1.87^{hij}	1.90^{hij}	1.94^{hij}
		10°C, 85%	0.00^{l}	4.23^{gh}	6.62^{fg}	9.66^{cd}
		10°C, 90%	0.00^{l}	2.18^{hi}	6.48^{fg}	6.53^{fg}
		AT, ARH	0.00^{l}	4.57^{fgh}	7.23^{efg}	8.01^{ef}
	Bio	5°C, 85%	0.00^{l}	0.20^{k}	0.93^{hij}	2.48^{hi}
		5°C, 90%	0.00^{l}	0.47^{j}	0.93^{hij}	2.48^{hi}
		10°C, 85%	0.00^{l}	2.68^{hi}	5.28^{fgh}	7.53^{ef}
		10°C, 90%	0.00^{l}	3.89^{gh}	4.89^{fgh}	7.27^{efg}
		AT, ARH	0.00^{l}	5.76^{fg}	8.64^{de}	10.87^{cd}
		5°C, 85%	0.00^{l}	1.20^{hij}	1.38^{hij}	2.77^{hi}
		5°C, 90%	0.00^{l}	1.70^{hij}	4.32^{gh}	4.66f^{gh}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
	NP	10°C, 85%	0.00^{l}	3.32^{ghi}	3.35^{ghi}	8.59^{def}
		10°C, 90%	0.00^{l}	6.27^{fg}	7.21^{efg}	8.52^{def}
		AT, ARH	0.00^{l}	6.63^{efg}	11.06^{c}	12.20^{abc}
NPP	LDPE	5°C, 85%	0.00^{l}	3.94^{gh}	5.93^{fg}	8.62^{de}
		5°C, 90%	0.00^{l}	2.40^{hi}	4.01^{gh}	8.02^{ef}
		10°C, 85%	0.00^{l}	2.54^{hi}	6.28^{fg}	8.43^{def}
		10°C, 90%	0.00^{l}	6.70^{efg}	7.10^{efg}	7.12^{efg}
		AT, ARH	0.00^{l}	7.07^{efg}	7.29^{efg}	10.00^{cd}
	Bio	5°C, 85%	0.00^{l}	2.07^{hij}	3.40^{ghi}	4.61^{fgh}
		5°C, 90%	0.00^{l}	0.35^{jk}	4.42^{gh}	4.83^{fgh}
		10°C, 85%	0.00^{l}	0.93^{hij}	5.21^{fgh}	8.70^{de}
		10°C, 90%	0.00^{l}	5.75^{fg}	6.02^{fg}	8.50^{def}
		AT, ARH	0.00^{l}	7.031^{efg}	8.34^{def}	12.77^{abc}
	NP	5°C, 85%	0.00^{l}	4.43^{gh}	4.91^{fgh}	7.50^{ef}
		5°C, 90%	0.00^{l}	4.73^{fgh}	4.76^{fgh}	6.03^{fg}
		10°C, 85%	0.00^{l}	4.55^{fgh}	5.23^{fgh}	8.45^{def}
		10°C, 90%	0.00^{l}	5.75^{fg}	7.68^{ef}	8.52^{def}
		AT, ARH	0.00^{l}	8.5^{def}	12.56^{abc}	19.23^{a}
Significance						
Pre-packaging (A)	*					
Packaging (B)	*					
Storage Condition (C)	**					
Storage Period (D)	**					
AB	NS					
AC	*					
BC	**					
AD	*					
BD	NS					
CD	**					
ABC	NS					
ABD	NS					
ACD	NS					
BCD	*					
ABCD	NS					

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interaction between packaging, storage conditions and storage period significantly (P≤0.05) influenced the PWL. Packaged samples (LDPE and biodegradable films) induced a lower PWL, compared to non-packaged samples under all storage conditions. The combination of LDPE packaging films with Avoshine® wax coating at low temperature storage (5°C + 90% RH) proved to be beneficial in reducing the total PWL, compared to other pre-packaging and packaging treatments at controlled or ambient storage conditions. Moisture loss is one of the primary factors contributing to the deterioration and poor quality of fruit, not only in salable weight loss, but also loss of firmness and nutritional value (Kader, 2002). The quality of many fruit is reliant upon the postharvest water loss, which is affected by the storage temperature and humidity (Awole *et al*., 2011). Weight loss is mainly due to moisture loss through the transpiration process and, to a lesser degree, the carbon loss via gas exchange (Maftoonazad and Ramaswamy, 2008). Increased respiration rates can also result in a decrease in the weight due to the degradation of sugars during the process (Maftoonazad and Ramaswamy, 2008). The results obtained for the respiration rate of avocados in this experiment (Section 3.11.2) stored at ambient conditions were excessively higher than at low temperatures, which compares to the results obtained for PWL, as the greatest PWL was also encountered at ambient conditions. Based on the results, it is apparent that controlled low temperature storage results in better quality fruit in terms of the PWL. The application of Avoshine® wax to the surface of the avocado, then packaged in a LDPE film and stored at a low temperature and high relative humidity (5°C + 90%) reduced the cumulative PWL.

3.11.2 Respiration rate

The storage conditions and the storage period had a significant (P≤0.001) effect on the respiration rate of the avocados (Table 3.2). All avocado samples demonstrated respiration rates characteristic to those of climacteric fruit, with a preclimacteric minimum of the least respiration followed by a climacteric maximum of the highest respiration and a postclimacteric phase of a gradual declination in the respiration rate (Starrett and Laties, 1991; Jeong *et al*., 2002; Yahia, 2002; Jeong *et al*., 2003; Blakey and Bower, 2009; Workneh and Osthoff, 2010; Wu *et al*., 2011). This is explained in the literature review contained in Chapter 2. The climacteric peaks were observed to be higher at the higher storage temperatures. Storage at 5°C + 85% RH and 5°C + 90% RH substantially reduced the occurrence of peak respiration rates, which can be attributed to the lower temperatures capable of inactivating enzymes associated with avocado ripening. Similar trends were obtained for the PWL (Section 3.11.1), where the higher temperatures promoted a substantial loss in weight, either as moisture loss or due to the loss of carbon dioxide gas. Avocados subjected to ambient conditions displayed much larger peaks in the respiration rate. According to Villa-Rodriguez *et al.* (2011), once the climacteric peak is reached, an increase in the avocado metabolism ensues, resulting in the exhaustion of valuable carbohydrates. This hastens the ripening process and reduces the quality and nutritive value of the fruit. Therefore, a delay and/or reduction in respiration are required, in order to extend the shelf life and preserve the quality. The peak respiration rate for samples treated with (a) Avoshine® and LDPE, (b) Avoshine® and bio, (c) Avoshine® and NP, (d) NPP and LDPE, (e) NPP and bio and (f) NPP and NP, and subjected to storage at ambient conditions, occurred on Day 4. However, samples with the same treatment combinations, as mentioned above, that were exposed to the controlled refrigerated storage conditions (5°C + 85%, 5°C + 90%, 10°C + 85% and 10°C + 90%) resulted in a 4-day delay in the peak respiration, which occurred only on Day 8.

Pre-packaging was found to have a significant ($P \leq 0.001$) influence on the respiration rate, while the packaging treatment was found to be less significant ($P \leq 0.05$). Avocados pre-treated with Avoshine® wax demonstrated a reduction in the occurrence of the climacteric peak, compared to hot water treated and control samples. Studies have indicated that the wax coating may have formed a partially permeable barrier on the exterior surface of the avocado, which has the potential of delaying the ripening process by modifying the internal environment of the fruit, similar to that of modified atmosphere storage (Maftoonazad and Ramaswamy, 2008). The peak in the respiration rate for all hot water treated avocado stored at ambient conditions was excessively higher than other treatments and was observed on Day 12. The highest peak in the respiration rate of 2411 $ml.kg^{-1}.h^{-1}$ was found in hot water treated samples without any packaging and stored in ambient conditions. During the hot water treatment, the heat may have resulted in damage to the avocado tissue, giving rise to increased respiration rates and the onset of early ripening. Both LDPE and biodegradable packaging were effective in reducing the respiration rates, compared to the samples devoid of packaging.

Table 3.2 The respiration rate ($ml.kg^{-1}.h^{-1}$) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	193^{l}	579^{ghi}	690^{fg}	537^{hi}
		5°C, 90%	171^{l}	646^{gh}	1084^{def}	516^{hi}
		10°C, 85%	188^{l}	833^{efg}	1179^{de}	736^{fg}
		10°C, 90%	206^{l}	804^{efg}	1138^{de}	463^{ij}
		AT, ARH	178^{l}	1115^{de}	1587^{cd}	2146^{ab}
	Bio	5°C, 85%	193^{l}	587^{ghi}	654^{gh}	455^{ij}
		5°C, 90%	171^{l}	595^{ghi}	1090^{def}	558^{h}
		10°C, 85%	188^{l}	709^{fg}	1125^{de}	556^{h}
		10°C, 90%	206^{l}	595^{ghi}	1039^{def}	441^{ij}
		AT, ARH	178^{l}	921^{ef}	1682^{cd}	2309^{a}
	NP	5°C, 85%	193^{l}	663^{gh}	648^{gh}	407^{ijk}
		5°C, 90%	171^{l}	668^{gh}	1061^{def}	517^{hi}
		10°C, 85%	188^{l}	780^{efg}	1077^{def}	540^{h}
		10°C, 90%	206^{l}	792^{efg}	966^{ef}	498^{hij}
		AT, ARH	178^{l}	1030^{def}	1042^{def}	2411^{a}
Avoshine®	LDPE	5°C, 85%	193^{l}	322^{jk}	415^{ijk}	253^{kl}
		5°C, 90%	171^{l}	331^{jk}	522^{hi}	436^{ij}
		10°C, 85%	188^{l}	519^{hi}	645^{gh}	435^{ij}
		10°C, 90%	206^{l}	338^{jk}	655^{gh}	310^{jk}
		AT, ARH	178^{l}	1499^{cd}	1085^{def}	950^{ef}
	Bio	5°C, 85%	193^{l}	326^{jk}	426^{ij}	298^{k}
		5°C, 90%	171^{l}	308^{jk}	586^{ghi}	273^{k}
		10°C, 85%	188^{l}	525^{hi}	559^{h}	404^{ijk}
		10°C, 90%	206^{l}	394^{ijk}	588^{ghi}	298^{k}
		AT, ARH	178^{l}	1560^{cd}	1680^{cd}	763^{fg}
	NP	5°C, 85%	193^{l}	363^{ijk}	467^{ij}	276^{k}
		5°C, 90%	171^{l}	321^{jk}	521^{hi}	403^{ijk}
		10°C, 85%	188^{l}	513^{hi}	663^{gh}	423^{ij}
		10°C, 90%	206^{l}	369^{ijk}	878^{efg}	327^{jk}
		AT, ARH	178^{l}	1753^{c}	806^{efg}	1406^{cd}
NPP	LDPE	5°C, 85%	193^{l}	503^{hij}	793^{efg}	399^{ijk}
		5°C, 90%	171^{l}	574^{ghi}	1160^{de}	755^{efg}
		10°C, 85%	188^{l}	752^{fg}	1233^{de}	759^{efg}
		10°C, 90%	206^{l}	699^{fg}	1212^{de}	598^{ghi}
		AT, ARH	178^{l}	1645^{bc}	1410^{cd}	905^{ef}
	Bio	5°C, 85%	193^{l}	495^{hij}	657^{gh}	409^{ijk}
		5°C, 90%	171^{l}	517^{hij}	1026^{def}	565^{h}
		10°C, 85%	188^{l}	744^{efg}	1234^{de}	602^{ghi}
		10°C, 90%	206^{l}	675^{gh}	1107^{de}	493^{hij}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		AT, ARH	178^{l}	1564^{cd}	1472^{cd}	1205^{de}
	NP	5°C, 85%	193^{l}	396^{ijk}	832^{efg}	505^{hij}
		5°C, 90%	171^{l}	561^{h}	1158^{de}	540^{h}
		10°C, 85%	188^{l}	795^{efg}	1322^{d}	652^{gh}
		10°C, 90%	206^{l}	624^{gh}	1188^{de}	530^{hi}
		AT, ARH	178^{l}	2307^{a}	1915^{b}	681^{fg}
Significance						
Pre-packaging (A)		**				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		NS				
BC		NS				
AD		**				
BD		NS				
CD		**				
ABC		NS				
ABD		NS				
ACD		**				
BCD		NS				
ABCD		*				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interaction between pre-packaging treatments, storage conditions and storage period were highly significant (P≤0.001) with regard to the avocado respiration rates. Avoshine® coated samples stored at 5°C + 85% RH displayed the least respiration rates during the 12 days of storage. Samples that had been hot water treated only, and control samples stored under ambient conditions displayed accelerated respiration rates. These findings are in agreement with studies by Maftoonazad and Ramaswamy (2008) and Villa-Rodriguez *et al*. (2011). The low temperature storage was effective in delaying and reducing the respiration peaks. However, the supplemental use of the Avoshine® wax further reduced the respiration rates by acting as a barrier film for gas diffusion, thereby creating a modified atmosphere inside the fruit.

The four-way interaction of pre-packaging, packaging, storage conditions and storage period had a significant ($P \leq 0.05$) effect on the avocado respiration rate. Storage at a low temperature reduced the occurrence of peak respiration rates, which are desired, in order to delay the ripening process and increase the overall shelf life. Avoshine® wax demonstrated the greatest benefit for avocados, combined with LDPE and biodegradable films in refrigerated storage conditions throughout the 12 days of storage. The combination of Avoshine® coated samples and LDPE packaging resulted in the lowest peak respiration value of 415 $ml.kg^{-1}.h^{-1}$ on Day 8 at 5°C + 85% RH.

From the results obtained, it can be deduced that samples treated with Avoshine® demonstrated lower climacteric peak respiration rates, with a delay of four days in samples subjected to refrigerated conditions. The integrated use of Avoshine®, LDPE and low temperature storage was found to be the most beneficial in avocado fruit by giving rise to the lowest peak respiration value. This, in turn produces fruit of superior quality with an extended shelf life.

3.11.3 Percent marketability

Table 3.3 shows the percentage of marketable avocados subjected to different treatment combinations over the 12-day period. The statistical analysis indicated that the main response of the storage conditions and the storage period were highly significant ($P \leq 0.001$) with regard to the percent marketability of avocado samples. At ambient conditions, the temperature was much higher with lower relative humidity. In comparison, the lower temperature and higher relative humidity within the controlled conditions assisted in maintaining the marketability of the avocados samples by reducing the water vapour pressure deficit, thus preventing excessive moisture loss (Workneh *et al*., 2009; Li *et al*., 2010). This, in turn creates turgid cells and promotes fruit firmness.

Pre-packaging and packaging treatments had no significant ($P > 0.05$) influence on the avocado marketability. However, Avoshine® coated samples resulted in fruit that had greater marketability and were aesthetically more appealing due to the glossy exterior imparted by the wax. Avoshine® coated samples without packaging and stored at ambient conditions, resulted in a marketability of 33.3%, compared to 0% for hot water treated avocado and control samples after 12 days of storage. Hot water treated avocados did not show a significant difference from control samples. Heat treatments are primarily used for insect pest eradication or the prevention of fungal development, but can also lead to internal and/or external tissue damage (Lurie, 1998). External damage can result in peel browning and even the onset of decay, while internal damage can cause poor colour development and abnormal softening in fruit, which could account for the low marketability of hot water treated avocados. LDPE packaging films maintained 100% marketable fruit from Day 1 to Day 12 at all controlled refrigerated storage conditions (5°C + 85%, 5°C + 90%, 10°C + 85% and 10°C + 90%). However, avocado samples treated with (a) HWT and Bio, (b) HWT and NP, (c) NPP and Bio and (d) NPP and NP, resulted in 0% marketability by Day 12 at ambient storage conditions.

Exposure of the avocados to controlled low temperature storage conditions resulted in 100% marketable fruit for the entire storage period. The avocados showed little variation from the green skin colour, firmness and were without any decay or localised darkening. These specific observed attributes of the avocado represent fruit of high quality (Maftoonazad and Ramaswamy, 2008). Storage at ambient conditions had a negative influence on marketability by reducing the amount of samples that were deemed marketable. Table 3.3 clearly illustrates the rapid decline in the marketability of avocados subjected to ambient conditions by Day 12 for all pre-packaging and packaging treatments. The rapid decline can be attributed to the higher ambient temperatures, which promote the biochemical processes, hence leading to accelerated ripening, compared to storage at 5°C or 10°C.

The three-way interaction of pre-packaging treatments, packaging films and storage conditions was found to significantly ($P \leq 0.05$) influence the avocado marketability. Avoshine® coated samples, in combination with LDPE packaging produced 100% marketable fruit at both refrigerated and ambient conditions. The use of Avoshine® wax created a partially permeable barrier, which limits the amount of moisture escaping via transpiration or the movement of gases such as carbon dioxide and oxygen. In addition, the LDPE film created a high RH micro-environment within the bag, while the lower temperature assisted in reducing enzymatic activity responsible for promoting the ripening process. The main features observed in avocado samples stored at ambient condition were a decrease in firmness and extreme darkening of the skin.

Table 3.3 The marketability (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	100[a]	100[a]
	Bio	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	66.67[b]	0[d]
	NP	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	66.67[b]	0[d]
Avoshine®	LDPE	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	100[a]	100[a]
	Bio	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	100[a]	33.33[c]
	NP	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	100[a]	33.33[c]
		5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
NPP	LDPE	10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	66.67[b]	33.33[c]
	Bio	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	33.33[c]	0[d]
	NP	5°C, 85%	100[a]	100[a]	100[a]	100[a]
		5°C, 90%	100[a]	100[a]	100[a]	100[a]
		10°C, 85%	100[a]	100[a]	100[a]	100[a]
		10°C, 90%	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	33.33[c]	0[d]

Significance	
Pre-packaging (A)	NS
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	NS
BD	NS
CD	**
ABC	*
ABD	NS
ACD	NS
BCD	NS
ABCD	NS

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

Avocado marketability was based on a subjective analysis technique and did not show significant variation among the different pre-packaging, packaging and low temperature storage treatments. However, it is apparent that a significant difference in the percentage of marketable fruit exists between avocado samples at refrigerated storage conditions and those at ambient storage conditions. The integrated application of Avoshine® and LDPE packaged resulted in 100% marketability even at ambient conditions and is, therefore, a beneficial practice to preserve the aesthetic quality of avocados.

3.11.4 Skin colour

The lightness (L*) values of the avocado skin, as a result of the interaction of different pre-packaging, packaging and storage condition treatments, are shown in Table 3.4. The statistical analysis indicated a significant ($P \leq 0.001$) effect of the storage condition and the storage period on the lightness of the avocado skin colour. A reduction in the skin lightness was observed for all treatments, which is synonymous with change from green toward a purple/black for the 'Hass' cultivar during ripening. However, the reduction in the L* value was more pronounced in fruit that had been subjected to ambient conditions, compared to those stored at low temperature conditions for all treatments. Storage at 5°C + 90% RH and 5°C + 85% RH maintained a lighter skin colour for the entire storage duration, compared to 10°C + 90% RH and 10°C + 85% RH. Both the pre-packaging and packaging treatments significantly ($P \leq 0.05$) influenced the lightness colour index of the avocado skin. Avocados coated with Avoshine® showed a slower decline in the lightness, compared to the avocado samples that were hot water treated and control samples at the lower temperatures. Samples devoid of packaging and pre-packaging treatments experienced a higher degree of skin darkening. Studies by Woolf (1997) and Blakey and Bower (2007) have demonstrated the beneficial use of hot water treatments at 38°C for 60 minutes and 15 minutes, respectively, with regard to improved avocado skin colour. However, Hofman *et al*. (2002) found that avocados without hot water treatments illustrated less skin darkening, compared to those treated at 38°C. There is variation in the literature pertaining to the effect of hot water treatments on the quality of avocados. However, the majority of the literature recommends the use of hot water treatments with differing exposure times (Paull and Chen, 2000; Woolf *et al*., 2004; Wu *et al*., 2011). In this experiment, the exposure time of five minutes to hot water at 38°C was not found to be beneficial for the quality parameters measured. This could be as a result of tissue damage. Cox *et al*. (2004) attributed a change in the avocado skin colour to an initial reduction in the chlorophyll content and an increase in the anthocyanin cyanidin 3-*O*-glucoside synthesis. The concentration of anthocyanin cyanidin 3-*O*-glucoside has been found to be affected by temperature, where higher temperatures increase the concentration (Cox *et al.*, 2004).

Table 3.4 The lightness (L*) of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			**Storage Period (Days)**			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	41.52^{a}	37.46^{cde}	36.61^{ef}	35.90^{efg}
		5°C, 90%	41.38^{a}	38.98^{bc}	36.85^{def}	36.67^{ef}
		10°C, 85%	41.33^{a}	36.00^{efg}	36.15^{efg}	34.67^{gh}
		10°C, 90%	41.63^{a}	35.57^{fg}	34.64^{gh}	33.93^{hij}
		AT, ARH	41.47^{a}	33.41^{jk}	29.52^{mn}	30.48^{m}
	Bio	5°C, 85%	41.52^{a}	37.51^{cde}	36.90^{def}	35.43^{fg}
		5°C, 90%	41.38^{a}	39.03^{bc}	37.88^{cde}	36.25^{ef}
		10°C, 85%	41.33^{a}	37.00^{cde}	36.14^{efg}	34.92^{fgh}
		10°C, 90%	41.63^{a}	37.26^{cde}	36.08^{efg}	34.31^{hi}
		AT, ARH	41.47^{a}	35.33^{fg}	33.94^{hij}	26.40^{op}
	NP	5°C, 85%	41.52^{a}	36.48^{ef}	35.44^{fg}	34.16^{hi}
		5°C, 90%	41.38^{a}	38.77^{c}	36.65^{ef}	35.03^{fgh}
		10°C, 85%	41.33^{a}	35.41^{fg}	34.94^{fgh}	33.42^{jk}
		10°C, 90%	41.63^{a}	35.76^{fg}	34.29^{hi}	32.79^{jkl}
		AT, ARH	41.47^{a}	37.15^{cde}	32.03^{kl}	24.80^{pq}
Avoshine®	LDPE	5°C, 85%	41.52^{a}	39.10^{bc}	38.80^{c}	36.51^{ef}
		5°C, 90%	41.38^{a}	40.20^{ab}	40.50^{ab}	38.17^{cd}
		10°C, 85%	41.33^{a}	35.19^{fg}	34.56^{gh}	34.73^{gh}
		10°C, 90%	41.63^{a}	36.67^{ef}	36.26^{ef}	33.88^{ij}
		AT, ARH	41.47^{a}	37.56^{cde}	34.10^{hi}	29.79^{mn}
	Bio	5°C, 85%	41.52^{a}	38.90^{c}	38.71^{c}	35.96^{efg}
		5°C, 90%	41.38^{a}	39.49^{abc}	38.98^{bc}	37.58^{cde}
		10°C, 85%	41.33^{a}	36.50^{ef}	35.32^{fg}	35.63^{fg}
		10°C, 90%	41.63^{a}	36.05^{efg}	35.59^{fg}	33.76^{j}
		AT, ARH	41.47^{a}	35.34^{fg}	32.05^{kl}	25.20^{opq}
	NP	5°C, 85%	41.52^{a}	37.22^{cde}	35.77^{fg}	34.58^{gh}
		5°C, 90%	41.38^{a}	39.49^{abc}	35.95^{efg}	35.73^{fg}
		10°C, 85%	41.33^{a}	36.67^{ef}	36.53^{ef}	33.49^{jk}
		10°C, 90%	41.63^{a}	37.52^{cde}	32.90^{jkl}	32.99^{jkl}
		AT, ARH	41.47^{a}	32.74^{jkl}	32.87^{jkl}	23.86^{r}
NPP	LDPE	5°C, 85%	41.52^{a}	38.48^{cd}	37.53^{cde}	35.26^{fg}
		5°C, 90%	41.38^{a}	38.97^{bc}	36.40^{ef}	36.55^{ef}
		10°C, 85%	41.33^{a}	35.78^{fg}	34.50^{gh}	34.51^{gh}
		10°C, 90%	41.63^{a}	34.82^{fgh}	33.82^{ij}	33.53^{j}
		AT, ARH	41.47^{a}	33.82^{ij}	31.31^{m}	29.17^{mno}
	Bio	5°C, 85%	41.52^{a}	38.12^{cd}	35.30^{fg}	35.17^{fg}
		5°C, 90%	41.38^{a}	38.36^{cd}	38.62^{cd}	36.00^{efg}
		10°C, 85%	41.33^{a}	34.65^{gh}	34.49^{gh}	34.22^{hi}
		10°C, 90%	41.63^{a}	35.89^{efg}	35.12^{fg}	32.34^{kl}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		AT, ARH	41.47[a]	34.03[hi]	26.70[nop]	24.39[qr]
	NP	5°C, 85%	41.52[a]	35.99[efg]	34.48[gh]	34.02[hi]
		5°C, 90%	41.38[a]	36.27[ef]	36.09[efg]	34.73[gh]
		10°C, 85%	41.33[a]	36.32[ef]	35.19[fg]	33.01[jkl]
		10°C, 90%	41.63[a]	34.27[hi]	33.41[jk]	31.57[lm]
		AT, ARH	41.47[a]	33.26[jk]	28.89[no]	23.29[rs]
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		*				
BC		NS				
AD		NS				
BD		NS				
CD		**				
ABC		*				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interaction involving pre-packaging treatment, packaging films and storage conditions had a significant (P≤0.05) influence on the L* colour parameter. Avocados coated with Avoshine® wax, packaged in LDPE and stored at 5°C and 90% RH, resulted in a reduction of 7.8% in lightness form Day 0 to Day 12. A reduction in the lightness of 43.8% was observed in control samples without any pre-packaging, packaging and stored at ambient conditions from Day 0 to Day 12, which is approximately more than five times the reduction for samples with the treatment combination of Avoshine®, LDPE and 5°C + 90% RH.

Storage conditions and storage period had a significant (P≤0.001) influence of the a* values as indicated in Table 3.5. Negative a* values are indicative of green fruit while an increase in a* values implies fruit ripening as the colour shifts toward red. Avocados subjected to controlled refrigerated storage conditions displayed a greener colour as indicated by the lower a* values. Avocado samples subjected to ambient storage conditions exhibited a rapid increase in the a* values by Day 12, which could be due to the darkening of the skin and loss of the green colour. The change of the skin colour from green to purple/black in 'Hass' avocados is one of the primary indicators of ripening (Cox *et al*., 2004; Osuna-Garcia *et al*., 2010). As in the case of the lightness (L*) colour index, the storage conditions of 5°C + 90% RH and 5°C and 85% RH were most effective in maintaining the greenness of the skin colour, indicative of slow ripening and an improved shelf life.

Pre-packaging and packaging treatments were found to be significant ($P \leq 0.05$), although the degree of influence was slightly lower than the effect of the storage conditions and the storage period. All avocados coated with the Avoshine® wax displayed a glossy and greener exterior enhancing the visual appeal throughout the storage period, compared to unwaxed fruit. These findings are in agreement with the studies conducted by Aguilar-Mendez *et al.* (2008) and Maftoonazad and Ramaswamy (2008). All packaged samples displayed a greener colour, compared to control samples, which could be attributed to the increased carbon dioxide concentration within the packaging preventing chlorophyll degradation and, therefore, a subsequent retention of a greener colour (Aguilar-Mendez *et al*., 2008; Maftoonazad and Ramaswamy, 2008). However, LDPE films were the most effective packaging film in maintaining a greener avocado skin colour.

The a* colour index of the avocado skin was significantly ($P \leq 0.05$) affected by the two-way interaction between the pre-packaging and packaging treatments. The combined treatment of Avoshine® wax and LDPE films was most effective in maintaining the green skin colour of the avocado fruit. Similarly, the two-way interaction between pre-packaging and the storage conditions was found to be significant ($P \leq 0.05$) on the a* colour index. Avocado samples coated with the Avoshine® wax and stored at 5°C + 90% RH and 5°C + 85% RH, exhibited lower a* values, indicating more green fruit. The combined effect of waxing and LDPE packaging can also be observed in the avocado samples subjected to ambient conditions, resulting in an a* value of -5.74, which is still negative, compared to other samples subjected to ambient conditions. The two-way interactions between (a) packaging and the storage conditions, (b) pre-packaging and the storage period, and (c) packaging and the storage period were not significant ($P > 0.05$).

Table 3.5 The a* colour parameter of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	-8.34^{hi}	-8.44^{hi}	-8.21^{h}	-7.38^{gh}
		5°C, 90%	-8.49^{hi}	-8.41^{hi}	-8.14^{h}	-7.50^{gh}
		10°C, 85%	-8.41^{hi}	-8.28^{h}	-7.57^{gh}	-7.48^{gh}
		10°C, 90%	-8.43^{hi}	-7.13^{gh}	-6.95^{gh}	-6.69^{fgh}
		AT, ARH	-8.36^{hi}	-6.88^{fgh}	-4.78^{ef}	-0.46^{cd}
	Bio	5°C, 85%	-8.34^{hi}	-8.20^{h}	-7.86^{h}	-7.11^{gh}
		5°C, 90%	-8.49^{hi}	-7.89^{h}	-8.23^{h}	-7.10^{gh}
		10°C, 85%	-8.41^{hi}	-7.69^{gh}	-7.61^{gh}	-6.73^{fgh}
		10°C, 90%	-8.43^{hi}	-7.58^{gh}	-6.43^{fgh}	-6.31^{fgh}
		AT, ARH	-8.36^{hi}	-6.14^{fg}	-3.19^{e}	2.37^{ab}
	NP	5°C, 85%	-8.34^{hi}	-8.23^{h}	-7.64^{gh}	-7.30^{gh}
		5°C, 90%	-8.49^{hi}	-8.29^{h}	-7.99^{h}	-7.08^{gh}
		10°C, 85%	-8.41^{hi}	-7.10^{gh}	-7.15^{gh}	-6.84^{fgh}
		10°C, 90%	-8.43^{hi}	-7.25^{gh}	-6.68^{fgh}	-6.04^{fg}
		AT, ARH	-8.36^{hi}	-6.00^{fg}	-0.13^{c}	3.07^{a}
Avoshine®	LDPE	5°C, 85%	-8.34^{hi}	-8.04^{h}	-8.24^{h}	-8.00^{h}
		5°C, 90%	-8.49^{hi}	-8.43^{hi}	-8.34^{hi}	-7.96^{h}
		10°C, 85%	-8.41^{hi}	-7.23^{gh}	-6.88^{fgh}	-6.58^{fgh}
		10°C, 90%	-8.43^{hi}	-8.40^{hi}	-7.38^{gh}	-7.63^{gh}
		AT, ARH	-8.36^{hi}	-8.02^{h}	-7.23^{gh}	-5.74^{fg}
	Bio	5°C, 85%	-8.34^{hi}	-8.42^{hi}	-7.98^{h}	-7.55^{gh}
		5°C, 90%	-8.49^{hi}	-8.48^{hi}	-8.34^{hi}	-7.87^{h}
		10°C, 85%	-8.41^{hi}	-7.43^{gh}	-7.05^{gh}	-6.80^{fgh}
		10°C, 90%	-8.43^{hi}	-6.92^{fgh}	-6.85^{fgh}	-6.32^{fgh}
		AT, ARH	-8.36^{hi}	-7.21^{gh}	-1.30^{de}	2.07^{bc}
	NP	5°C, 85%	-8.34^{hi}	-8.03^{h}	-7.45^{gh}	-7.64^{gh}
		5°C, 90%	-8.49^{hi}	-8.03^{h}	-7.93^{h}	-7.47^{gh}
		10°C, 85%	-8.41^{hi}	-7.39^{gh}	-7.19^{gh}	-6.48^{fgh}
		10°C, 90%	-8.43^{hi}	-7.00^{gh}	-7.04^{gh}	-6.75^{fgh}
		AT, ARH	-8.36^{hi}	-7.14^{gh}	-0.26^{c}	2.42^{ab}
NPP	LDPE	5°C, 85%	-8.34^{hi}	-8.14^{h}	-7.65^{gh}	-7.32^{gh}
		5°C, 90%	-8.49^{hi}	-8.00^{h}	-7.77^{gh}	-7.47^{gh}
		10°C, 85%	-8.41^{hi}	-7.91^{h}	-7.42^{gh}	-6.85^{fgh}
		10°C, 90%	-8.43^{hi}	-7.68^{gh}	-7.31^{gh}	-6.57^{fgh}
		AT, ARH	-8.36^{hi}	-5.31^{f}	-0.81^{de}	1.90^{bc}
	Bio	5°C, 85%	-8.34^{hi}	-8.45^{hi}	-7.76^{gh}	-6.79^{fgh}
		5°C, 90%	-8.49^{hi}	-7.97^{h}	-7.33^{gh}	-7.33^{gh}
		10°C, 85%	-8.41^{hi}	-7.12^{gh}	-7.46^{gh}	-6.87^{fgh}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		10°C, 90%	-8.43^hi^	-7.06^gh^	-6.81^fgh^	-5.99^fg^
		AT, ARH	-8.36^hi^	-6.26^fgh^	-0.10^c^	2.48^ab^
		5°C, 85%	-8.34^hi^	-8.32^h^	-7.19^gh^	-6.73^fgh^
		5°C, 90%	-8.49^hi^	-7.77^gh^	-7.21^gh^	-6.64^fgh^
	NP	10°C, 85%	-8.41^hi^	-7.19^gh^	-6.85^fgh^	-6.32^fgh^
		10°C, 90%	-8.43^hi^	-6.97^fgh^	-6.56^fgh^	-5.23^f^
		AT, ARH	-8.36^hi^	-8.00^h^	-2.08^de^	3.54^a^
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		*				
AC		*				
BC		NS				
AD		NS				
BD		NS				
CD		**				
ABC		**				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interaction between pre-packaging, packaging and the storage conditions had a highly significant (P≤0.001) influence on the a* colour index of the avocado skin. The integration of Avoshine® wax with LDPE films and storage at 5°C + 90% RH and 5°C + 85% RH resulted in an increase of a* by a mere 6.2% and 4.1%, respectively. In comparison, control samples devoid of pre-packaging, packaging and stored at ambient conditions resulted in a rapid increase in a* by 142.3%. This, therefore, indicates that the combined application of Avoshine® wax, LDPE and cold storage were found to be highly beneficial in maintaining the colour characteristics of avocado fruit. The interactions of (a) pre-packaging, packaging and the storage period, (b) pre-packaging, storage conditions and storage period, (c) packaging, storage conditions and storage period, and (d) pre-packaging, packaging, storage conditions and the storage period were not significant (P>0.05).

The third colour parameter that was analyzed was b*, which represents the yellowness or blueness with +b* and –b*, respectively. As indicated in Table 3.6, both the storage conditions and the storage period were found to be highly significant (P≤0.001) with regard to the b* values. A general decrease in the b* values was observed in all treatments indicative of the reduction in the yellowness and conversion into a darker chroma of the avocado skin colour. The storage of samples at the lower temperature of 5°C resulted in a slower decline in the b* value for both 85% and 90% RH conditions, compared to 10°C and ambient conditions. These trends are similar to those obtained for the L* and a* colour indices in this experiment. Storage at ambient conditions led to a rapid decline of the b* values of avocados that were subjected to all treatments, with the control samples exhibiting the greatest reduction of 96.7%. Pre-packaging and packaging treatments were found to be significant (P≤0.05) with regard to the b* values. As in the analysis of the a* colour index, Avoshine® waxed avocados showed a glossy and greener exterior, compared to hot water treated and control samples. The Avoshine® wax formed a partially permeable layer on the avocado skin, which limited the entry and exit of gases and moisture. This may have, therefore, limited the ripening catalyzed by an excessive moisture loss and an absorption of oxygen required in the respiration process. LDPE films maintained greener fruit for a longer period of time. Similar observations were obtained by Meir *et al*. (1997), in which the storage of avocado fruit in polyethylene bags at 5°C exhibited a decrease in black pigmentation development of the peel.

Table 3.6 The b* colour parameter of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			**Storage Period (Days)**			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	25.24^{a}	22.04^{c}	21.69^{c}	20.31^{cd}
		5°C, 90%	24.27^{a}	22.70^{bc}	22.60^{bc}	21.25^{c}
		10°C, 85%	24.98^{a}	18.33^{cd}	18.35^{cd}	17.64^{d}
		10°C, 90%	24.06^{a}	18.25^{cd}	17.57^{d}	17.21^{de}
		AT, ARH	25.01^{a}	17.31^{de}	14.93^{f}	7.99^{h}
	Bio	5°C, 85%	25.24^{a}	22.39^{bc}	20.04^{cd}	19.16^{cd}
		5°C, 90%	24.27^{a}	24.27^{a}	24.28^{a}	18.89^{cd}
		10°C, 85%	24.98^{a}	19.05^{cd}	19.46^{cd}	16.70^{ef}
		10°C, 90%	24.06^{a}	21.02^{cd}	18.95^{cd}	16.77^{ef}
		AT, ARH	25.01^{a}	16.37^{ef}	10.53^{gh}	3.17^{i}
	NP	5°C, 85%	25.24^{a}	23.54^{b}	16.49^{ef}	17.65^{d}
		5°C, 90%	24.27^{a}	23.12^{b}	18.32^{cd}	16.77^{ef}
		10°C, 85%	24.98^{a}	19.62^{cd}	19.04^{cd}	17.15^{de}
		10°C, 90%	24.06^{a}	19.85^{cd}	16.68^{ef}	16.12^{ef}
		AT, ARH	25.01^{a}	21.30^{c}	13.30^{g}	1.63^{k}
Avoshine®	LDPE	5°C, 85%	25.24^{a}	24.76^{a}	23.84^{ab}	21.56^{c}
		5°C, 90%	24.27^{a}	23.37^{b}	23.35^{b}	22.30^{bc}
		10°C, 85%	24.98^{a}	23.84^{ab}	20.41^{cd}	19.83^{cd}
		10°C, 90%	24.06^{a}	25.17^{a}	21.26^{c}	20.16^{cd}
		AT, ARH	25.01^{a}	23.39^{b}	20.93^{cd}	17.82^{d}
	Bio	5°C, 85%	25.24^{a}	24.61^{a}	22.69^{bc}	21.47^{c}
		5°C, 90%	24.27^{a}	24.36^{a}	21.13^{cd}	21.05^{cd}
		10°C, 85%	24.98^{a}	21.01^{cd}	19.99^{cd}	18.99^{cd}
		10°C, 90%	24.06^{a}	20.00^{cd}	17.37^{d}	18.15^{cd}
		AT, ARH	25.01^{a}	19.61^{cd}	14.56^{fg}	4.02^{hi}
	NP	5°C, 85%	25.24^{a}	23.39^{b}	22.44^{bc}	20.36^{cd}
		5°C, 90%	24.27^{a}	24.62^{a}	19.98^{cd}	17.98^{d}
		10°C, 85%	24.98^{a}	20.03^{cd}	18.59^{cd}	16.85^{ef}
		10°C, 90%	24.06^{a}	18.81^{cd}	17.14^{de}	16.60^{ef}
		AT, ARH	25.01^{a}	19.25^{cd}	13.42^{g}	2.32^{j}
NPP	LDPE	5°C, 85%	25.24^{a}	25.01^{a}	20.03^{cd}	19.54^{cd}
		5°C, 90%	24.27^{a}	23.87^{ab}	20.16^{cd}	21.01^{cd}
		10°C, 85%	24.98^{a}	19.29^{cd}	21.10^{cd}	17.73^{d}
		10°C, 90%	24.06^{a}	20.00^{cd}	18.27^{cd}	17.01^{de}
		AT, ARH	25.01^{a}	18.12^{cd}	16.48^{ef}	6.47^{gh}
	Bio	5°C, 85%	25.24^{a}	22.93^{b}	20.84^{cd}	18.09^{cd}
		5°C, 90%	24.27^{a}	24.05^{a}	21.91^{c}	18.66^{cd}
		10°C, 85%	24.98^{a}	17.42^{d}	17.98^{d}	17.24^{de}
		10°C, 90%	24.06^{a}	19.92^{cd}	18.39^{cd}	14.42^{fg}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		AT, ARH	25.01[a]	15.83[f]	9.13[gh]	2.00[j]
		5°C, 85%	25.24[a]	21.42[c]	18.26[cd]	15.82[f]
		5°C, 90%	24.27[a]	19.20[cd]	19.74[cd]	14.93[f]
	NP	10°C, 85%	24.98[a]	18.20[cd]	17.88[d]	16.20[ef]
		10°C, 90%	24.06[a]	18.99[cd]	15.80[f]	14.35[fg]
		AT, ARH	25.01[a]	16.80[ef]	8.81[h]	0.82[l]
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		*				
BC		NS				
AD		NS				
BD		NS				
CD		**				
ABC		*				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interaction between pre-packaging and the storage conditions was found to be significant (P≤0.05) on the b* of the avocado skin. Samples coated with the Avoshine® wax and stored at 5°C + 90% RH and 5°C + 85% RH showed reduced rates of a decrease in b*. Avocado samples treated with Avoshine® and LDPE and subjected to ambient storage conditions, resulted in a b* value of 17.82 on Day 12, compared to control samples having a b* value of 0.82 on Day 12. The large difference in the b* values indicates the benefit offered by pre-packaging and packaging treatments, as opposed to no treatment. Bhaskaran *et al*. (2002) also found that LDPE packaging could retain the green colour of avocado fruit. The two-way interactions between (a) pre-packaging and packaging, (b) packaging and storage conditions, (c) pre-packaging and storage period, and (d) packaging and storage period were not found to be significant (P>0.05) on the avocado b* values.

The three-way interaction between the pre-packaging treatments, the packaging films and the storage conditions had a significant ($P \leq 0.05$) influence on the b* colour index. The integration of Avoshine® wax with LDPE films and storage at 5°C + 90% RH resulted in a reduction in b* by 8.1%, compared to a reduction of 96.7% in control samples. This, therefore, indicates the beneficial application of Avoshine® wax, LDPE and cold storage in maintaining the colour characteristics of avocado fruit. The interactions of (a) pre-packaging, packaging and storage period, (b) pre-packaging, storage conditions and storage period, (c) packaging, storage conditions and storage period, and (d) pre-packaging, packaging, storage conditions and storage period were not significant ($P > 0.05$) on the avocado b* values.

The skin colour changed from an initial green colour on Day 0 to a dark purple by Day 12, losing liightness at a faster rate when exposed to ambient conditions, compared to the controlled low temperature and high RH conditions and as illustrated in Figure 3.6. This colour change manifests as a result of a reduction in the chlorophyll content and an increase in the anthocyanin cyanidin 3-*O*-glucoside (Cox *et al.*, 2004; Aguilar-Mendez *et al*., 2008; Toivonen and Brummell, 2008; Osuna-Garcia *et al*., 2010) synonymous with a decrease in L*, increase in a* and reduction in b* values.

Figure 3.6 Change in the skin colour of control avocado samples subjected to ambient storage conditions during the 12 days of storage

3.11.5 Exterior firmness (puncture force)

The exterior firmness of the sampled avocados are presented in Table 3.7. The firmness of whole avocados decreased with the progression of storage time from Day 0 to Day 12. The storage conditions and the storage period had a significant ($P \leq 0.001$) effect on the firmness.

Avocado samples subjected to storage at ambient conditions displayed a rapid decline in the firmness by Day 12, which is synonymous with accelerated ripening. Storage at 5°C + 85% RH and 5°C + 90% RH resulted in the least reduction in the avocado firmness. Similar trends were obtained for PWL and the respiration rate, where at the lower storage temperature of 5°C minimal changes in these quality parameters occurred, compared to at ambient conditions and at 10°C. This similarity can be attributed to the higher temperatures responsible for increased enzyme activity, which hastens the ripening process evident in increased respiration rates (Section 3.11.2). The vapour pressure deficit between the fruit and surrounding environment is also higher at higher temperatures and/or lower RH, promoting excessive moisture loss from the avocado. This moisture loss, in turn reduces the turgidity of the fruit, leading to loss in firmness. These results compare with the findings of Maftoonazad and Ramaswamy (2008) and Li *et al*. (2010). The RH did not have a considerable effect on the firmness, despite it having an effect on the PWL in this experiment.

Pre-packaging and packaging treatments were found to have a significant ($P \leq 0.05$) effect on the avocado firmness. The Avoshine® wax pre-packaging treatment proved to be more beneficial, compared to the hot water treated and control samples in maintaining firmer avocado fruit throughout the storage period, which, in effect could contribute to an extended shelf life. Many studies have shown that the hot water treatment of avocados is beneficial in terms of reduced softening, subject to the temperature and exposure times that have been applied (Lurie, 1998; Paull and Chen, 2000; Fallik, 2004). However, there still exists the possibility of tissue damage (Lurie, 1998), which may be the cause for the hot water treated avocados to experience increased softening. In addition to increased softening, fruit can experience abnormal softening, where individual fruit will not soften at the same rate. Loss in moisture has a direct effect on the firmness and textural changes by encouraging the loss in turgidity (Li *et al*., 2010). LDPE and biodegradable packaging films reduced avocado softening, compared to samples that had no packaging films. The reduction in firmness was found to be concomitant with a change in colour (Paull, 1999).

Table 3.7 The firmness (N) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		5°C, 85%	17.65^{a}	15.43^{efg}	15.14^{fgh}	15.05^{fgh}
		5°C, 90%	17.58^{a}	17.18^{bc}	16.00^{de}	15.75^{ef}
	LDPE	10°C, 85%	17.60^{a}	16.80^{cd}	16.00^{de}	15.95^{de}
		10°C, 90%	17.55^{a}	16.31^{de}	16.00^{de}	15.75^{ef}
		AT, ARH	17.71^{a}	15.36^{fg}	11.62^{jkl}	9.96^{klm}
		5°C, 85%	17.65^{a}	15.83^{def}	15.05^{fgh}	15.38^{fg}
		5°C, 90%	17.58^{a}	16.72^{cd}	16.50^{cde}	15.52^{efg}
HWT	Bio	10°C, 85%	17.60^{a}	16.21^{de}	15.57^{efg}	15.36^{fg}
		10°C, 90%	17.55^{a}	16.15^{de}	15.26^{fgh}	15.51^{efg}
		AT, ARH	17.71^{a}	14.66^{hi}	8.38^{mn}	5.57^{op}
		5°C, 85%	17.65^{a}	15.62^{efg}	15.25^{fgh}	14.95^{gh}
		5°C, 90%	17.58^{a}	17.14^{bc}	16.27^{de}	14.51^{hi}
	NP	10°C, 85%	17.60^{a}	15.73^{ef}	15.43^{efg}	15.34^{fg}
		10°C, 90%	17.55^{a}	16.58^{cde}	16.51^{cde}	15.20^{fgh}
		AT, ARH	17.71^{a}	14.12^{hi}	7.62^{mno}	4.51^{pqr}
		5°C, 85%	17.65^{a}	16.30^{de}	16.20^{de}	16.19^{de}
		5°C, 90%	17.58^{a}	17.27^{ab}	16.88^{cd}	16.53^{cde}
	LDPE	10°C, 85%	17.60^{a}	16.55^{cde}	16.38^{de}	16.37^{de}
		10°C, 90%	17.55^{a}	16.38^{de}	16.30^{de}	16.01^{de}
		AT, ARH	17.71^{a}	15.39^{fg}	14.84^{gh}	13.93^{hij}
		5°C, 85%	17.65^{a}	16.30^{de}	16.29^{de}	15.51^{efg}
		5°C, 90%	17.58^{a}	16.78^{cd}	16.52^{cde}	16.37^{de}
Avoshine®	Bio	10°C, 85%	17.60^{a}	17.16^{bc}	16.38^{de}	16.00^{de}
		10°C, 90%	17.55^{a}	16.59^{cde}	16.58^{cde}	16.22^{de}
		AT, ARH	17.71^{a}	16.07^{de}	12.52^{jk}	7.45^{nop}
		5°C, 85%	17.65^{a}	15.54^{efg}	15.44^{efg}	15.43^{efg}
		5°C, 90%	17.58^{a}	17.82^{ab}	15.53^{efg}	15.08^{fgh}
	NP	10°C, 85%	17.60^{a}	16.56^{cde}	15.72^{ef}	15.47^{efg}
		10°C, 90%	17.55^{a}	15.99^{de}	15.72^{ef}	15.24^{fgh}
		AT, ARH	17.71^{a}	15.80^{def}	10.62^{kl}	4.93^{p}
		5°C, 85%	17.65^{a}	16.02^{de}	15.60^{efg}	15.47^{efg}
		5°C, 90%	17.58^{a}	17.95^{a}	15.51^{efg}	15.35^{fg}
	LDPE	10°C, 85%	17.60^{a}	16.53^{cde}	16.10^{de}	15.53^{efg}
		10°C, 90%	17.55^{a}	15.77^{ef}	15.53^{efg}	15.52^{efg}
		AT, ARH	17.71^{a}	15.44^{efg}	13.22^{ij}	8.69^{lmn}
		5°C, 85%	17.65^{a}	16.09^{de}	15.48^{efg}	14.56^{hi}
		5°C, 90%	17.58^{a}	15.74^{ef}	15.46^{efg}	15.05^{fgh}
NPP	Bio	10°C, 85%	17.60^{a}	16.78^{cd}	16.73^{cd}	15.19^{fgh}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		10°C, 90%	17.55[a]	16.72[cd]	15.47[efg]	15.50[efg]
		AT, ARH	17.71[a]	15.24[fgh]	14.35[hi]	4.80[pq]
	NP	5°C, 85%	17.65[a]	16.17[de]	15.50[efg]	14.05[hi]
		5°C, 90%	17.58[a]	15.58[efg]	15.13[fgh]	14.30[hi]
		10°C, 85%	17.60[a]	16.48[cde]	16.24[de]	14.17[hi]
		10°C, 90%	17.55[a]	16.26[de]	16.24[de]	15.08[fgh]
		AT, ARH	17.71[a]	15.66[efg]	9.19[lm]	3.84[q]

Significance	
Pre-packaging (A)	*
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	**
AD	NS
BD	NS
CD	**
ABC	*
ABD	NS
ACD	NS
BCD	*
ABCD	NS

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interaction between packaging and storage conditions was highly significant (P≤0.001) in terms of the avocado firmness. Avocado samples packaged in LDPE films and subjected to cold storage conditions, specifically that of 5°C + 90% RH displayed the least reduction in firmness. Control samples devoid of any pre-packaging treatments and packaging films succumbed to the greatest loss in firmness. The loss in firmness of control samples was further exacerbated when they were exposed to ambient conditions, resulting in a reduction in firmness of 78% from Day 0 to Day 12.

The three-way interaction between pre-packaging, packaging and storage conditions had a significant (P≤0.05) effect on the avocado firmness. The combination of Avoshine® wax, LDPE packaging and refrigerated storage resulted in firmer fruit. A reduction of a mere 6.0% was observed for samples that had been wax-coated, packaged in LDPE and stored at 5°C + 90% RH. Samples with the same pre-packaging and packaging treatments and stored at ambient conditions, displayed the least reduction in firmness of only 21.3%, compared to other treatment combinations of avocados stored at ambient conditions. The results from this experiment indicate that the use of pre-packaging and/or packaging treatments have reduced the rate of softening of avocado fruit, when combined with low temperature storage. The use of Avoshine® wax as a pre-packaging treatment and LDPE films as a packaging treatment offered the least reduction in avocado fruit firmness. The implementation of low temperature storage of 5°C further contributed to increased firmness.

3.11.6 Pulp firmness (Kramer shear)

Table 3.8 displays the avocado pulp firmness as a result of the various postharvest treatment techniques. The storage conditions and the storage period were found to have a significant ($P \leq 0.001$) effect on the avocado pulp firmness by shear. The exposure of avocado samples to low temperature storage reduced the rate of softening throughout the storage period, when compared to ambient conditions. The reduction in the rate of pulp softening was slower at 5°C + 90% RH than at 10°C + 85% RH and 10°C + 90% RH for controlled temperature storage. The loss in firmness ranged from 6.5%-16.5%, 15.7%-21.6%, 10.5%-26.9% and 45.0%-99.6% for 5°C + 90% RH, 10°C + 85% RH, 10°C + 90% RH and ambient conditions, respectively. The minimum loss in firmness was observed for avocado samples subjected to combined Avoshine® wax pre-treatment and LDPE packaging films, while the maximum reduction in the pulp firmness was associated with control samples devoid of pre-packaging and packaging. These findings are in accordance with those of Aguilar-Mendez *et al.* (2008) and Maftoonazad and Ramaswamy (2008). The comparatively higher temperatures and lower RH at ambient conditions contributed to a loss of moisture from the avocados as a result of the vapour pressure deficit created between the fruit and the surrounding environment. The greater the deficit, the greater the amount of moisture which escapes from the fruit (Li *et al*., 2010). This loss in moisture results in decreased pulp firmness due to the loss in turgidity of the cell tissue. The higher temperatures also stimulate the activity of enzymes promoting the ripening process, resulting in fruit softening.

Table 3.8 The pulp firmness ($N.g^{-1}$) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	200.0^{abc}	204.9^{abc}	184.2^{bcd}	244.6^{a}
		5°C, 90%	210.4^{abc}	221.1^{abc}	210.4^{abc}	194.5^{bc}
		10°C, 85%	210.4^{abc}	212.8^{abc}	203.8^{abc}	171.4^{cde}
		10°C, 90%	204.0^{abc}	216.5^{abc}	194.9^{bc}	189.1^{bcd}
		AT, ARH	200.3^{abc}	182.3^{bcd}	116.4^{gh}	74.13^{ijk}
	Bio	5°C, 85%	200.0^{abc}	219.2^{abc}	174.2^{cde}	215.7^{abc}
		5°C, 90%	210.4^{abc}	223.2^{abc}	212.7^{abc}	196.1^{bc}
		10°C, 85%	210.4^{abc}	215.5^{abc}	179.4^{cde}	167.0^{de}
		10°C, 90%	204.0^{abc}	219.3^{abc}	213.9^{abc}	177.1^{cde}
		AT, ARH	200.3^{abc}	199.8^{bc}	135.6^{efg}	4.62^{m}
	NP	5°C, 85%	200.0^{abc}	183.2^{bcd}	167.4^{de}	227.5^{ab}
		5°C, 90%	210.4^{abc}	218.3^{abc}	219.4^{abc}	187.8^{bcd}
		10°C, 85%	210.4^{abc}	219.3^{abc}	176.3^{cde}	167.2^{de}
		10°C, 90%	204.0^{abc}	193.4^{bc}	187.3^{bcd}	170.0^{cde}
		AT, ARH	200.3^{abc}	194.2^{bc}	107.5^{hi}	1.03^{no}
Avoshine®	LDPE	5°C, 85%	200.0^{abc}	207.8^{abc}	193.6^{bc}	228.3^{ab}
		5°C, 90%	210.4^{abc}	222.2^{abc}	220.4^{abc}	196.7^{bc}
		10°C, 85%	210.4^{abc}	203.4^{abc}	193.0^{bc}	177.3^{cde}
		10°C, 90%	204.0^{abc}	209.2^{abc}	202.4^{abc}	188.3^{bcd}
		AT, ARH	200.3^{abc}	218.9^{abc}	129.1^{fg}	110.2^{hi}
	Bio	5°C, 85%	200.0^{abc}	194.0^{bc}	209.4^{abc}	224.1^{abc}
		5°C, 90%	210.4^{abc}	224.0^{abc}	217.3^{abc}	187^{bcd}
		10°C, 85%	210.4^{abc}	212.1^{abc}	174.8^{cde}	168.6^{de}
		10°C, 90%	204.0^{abc}	219.6^{abc}	183.0^{bcd}	170.3^{cde}
		AT, ARH	200.3^{abc}	215.8^{abc}	59.07^{jk}	45.58^{kl}
	NP	5°C, 85%	200.0^{abc}	172.0^{cde}	187.7^{bcd}	230.7^{ab}
		5°C, 90%	210.4^{abc}	222.1^{abc}	191.0^{bc}	178.4^{cde}
		10°C, 85%	210.4^{abc}	199.6^{bc}	178.2^{cde}	169.1^{cde}
		10°C, 90%	204.0^{abc}	196.6^{bc}	176.4^{cde}	166.6^{de}
		AT, ARH	200.3^{abc}	185.7^{bcd}	55.46^{jk}	1.09^{mn}
NPP	LDPE	5°C, 85%	200.0^{abc}	183.4^{bcd}	187.6^{bcd}	227.8^{ab}
		5°C, 90%	210.4^{abc}	201.2^{abc}	199.0^{bc}	191.8^{bc}
		10°C, 85%	210.4^{abc}	203.1^{abc}	200.3^{abc}	170.2^{cde}
		10°C, 90%	204.0^{abc}	214.6^{abc}	181.2^{bcd}	170.8^{cde}
		AT, ARH	200.3^{abc}	200.3^{abc}	83.46^{ij}	3.89^{mn}
	Bio	5°C, 85%	200.0^{abc}	191.2^{bc}	172.8^{cde}	213.9^{abc}
		5°C, 90%	210.4^{abc}	205.2^{abc}	190.6^{bc}	188.3^{bcd}
		10°C, 85%	210.4^{abc}	211.5^{abc}	192.6^{bc}	179.6^{cde}
		10°C, 90%	204.0^{abc}	205.4^{abc}	182.8^{bcd}	163.9^{def}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		AT, ARH	200.3^{abc}	184.1^{bcd}	64.78^{jk}	1.08^{no}
		5°C, 85%	200.0^{abc}	175.3^{cde}	184.6^{bcd}	221.5^{abc}
		5°C, 90%	210.4^{abc}	198.2^{bc}	197.2^{bc}	175.7^{cde}
	NP	10°C, 85%	210.4^{abc}	193.6^{bc}	167.7^{de}	165.0^{de}
		10°C, 90%	204.0^{abc}	202.5^{abc}	188.3^{bcd}	153.8^{ef}
		AT, ARH	200.3^{abc}	174.8^{cde}	73.60^{ijk}	0.79^{no}
Significance						
Pre-packaging (A)		NS				
Packaging (B)		NS				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		NS				
BC		*				
AD		NS				
BD		NS				
CD		**				
ABC		NS				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

Pre-packaging and packaging treatments were not found to be significant (P>0.05) in terms of the pulp firmness. An average increase of approximately 11.4% in the pulp firmness was observed for avocados that were subjected to storage at 5°C + 85% RH between Day 8 and Day 12. This is uncommon as the fruit firmness generally decreases with time at varying rates, depending on the treatment (Mizrach and Flitsanov, 1999; Maftoonazad and Ramaswamy, 2005; Aguilar-Mendez, 2008; Arzate-Vazquez *et al*., 2011), as discussed in Section 3.11.5. However, it was reported by Li *et al*. (2010) that firmness has been noted to increase after harvest. This can be due to physical damage, storage at low temperatures or at temperatures up to 20°C, resulting in cell wall secondary lignification in the flesh (Li *et al*., 2010). This phenomenon results in firm flesh, dry pulp, peel adhesion and ultimate deterioration, which could be the reason for the increase in firmness observed (Li *et al*., 2010).

The two-way interaction between packaging films and storage conditions had a significant ($P \leq 0.05$) influence on the pulp firmness. The presence of low oxygen and high carbon dioxide gas concentrations are able to retard the action of enzymes, consequently retaining the fruit firmness (Maftoonazad and Ramaswamy, 2008). Fruit that had been packaged with either LDPE or biodegradable films remained firmer than fruit that had not been packaged due to the accumulation of carbon dioxide within the packaging. However, LDPE films were more beneficial in retaining the pulp firmness, which was most apparent at 5°C + 90% RH, with a reduction in firmness of only 6.5%.

3.11.7 Puree colour

The avocado pulp colour can be classified as being yellow-green to light yellow, with a smooth buttery consistency (Ramtahal *et al*., 2007). The pulp is of concern as this is the portion of the avocado fruit that is consumed either directly or in a processed form. The storage conditions and storage period significantly ($P \leq 0.001$) influenced the L* colour index of the pureed avocado pulp, as presented in Table 3.9. A decrease in the pulp lightness was observed for all treatment combinations. A greater decline in the L* value was observed for the higher ambient temperature. Storage at 5°C + 85% RH and 5°C + 90% RH reduced the rate at which L* decreased. In comparison the degree at which the decrease in L* occurred, was found to be greater in samples subjected to ambient storage conditions, which is representative of the higher temperatures and lower RH. Similar observations were obtained in the analysis of the avocado skin colour in this experiment (Section 3.11.4).

Pre-packaging and packaging treatments were both found to have a significant ($P \leq 0.05$) effect on the avocado puree lightness. Hot water treated and Avoshine® coated samples displayed the least decrease in the lightness, compared to control samples without any pre-treatment. As previously mentioned in Section 3.11.4, hot water treatment resulted in excessive skin darkening. However, the pulp had not been detrimentally affected, but rather the hot water pre-treatment proved to be beneficial in maintaining the lightness of the avocado puree colour. It can, therefore, be deduced that the presumed tissue damage that may have been as a result of the hot water treatment is isolated only to the exterior of the avocado, encompassing the skin and not the pulp. This could be due to the short exposure time of five minutes, which did not allow enough time for the pulp to reach the desired treatment temperature of 38°C. LDPE and biodegradable packaged avocado samples showed higher L* values, compared to unpackaged control samples

The two-way interactions between (a) the pre-packaging and packaging, (b) pre-packaging and storage conditions, (c) packaging and the storage conditions, and (d) packaging and storage period had a significant ($P \leq 0.05$) influence on the L* colour parameter of the avocado puree. The treatment combinations of (a) HWT and LDPE, (b) HWT and Bio, (c) Avoshine® and LDPE, and (d) Avoshine® and Bio displayed higher L* values on Day 12, compared to (a) HWT only, (b) Avoshine® only, (c) LDPE only, (d) Bio only and (e) control samples devoid of any pre-packaging and packaging treatments. This indicates the beneficial use of combined pre-packaging and packaging treatments, as opposed to only pre-packaging or only packaging. Both (a) HWT and Bio, and (b) Avoshine® and Bio treated avocados stored at 5°C + 85% RH, exhibited a decrease in the L* value of 3.0% from Day 0 to Day 12, compared to a decrease of 23.8% observed in control avocado samples.

The three-way interaction between pre-packaging, storage conditions and storage period was found to have a significant ($P \leq 0.001$) effect on the L* value of the avocado puree. Avocado samples treated with (a) HWT and LDPE, (b) HWT and Bio, (c) Avoshine® and LDPE and (d) Avoshine® and Bio stored at 5°C + 85% RH and 5°C + 90% RH displayed higher L* values, compared to other treatment and storage combinations, as indicated in Table 3.9. Control avocado samples devoid of pre-packaging and packaging and exposed to ambient storage conditions, resulted in the lowest L* value of 54.36 on Day 12, indicative of darkening.

Table 3.9 The lightness (L*) of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	72.23^{a}	71.62^{ab}	70.26^{bc}	69.18^{de}
		5°C, 90%	72.50^{a}	69.07^{de}	66.34^{hi}	68.15^{efg}
		10°C, 85%	71.98^{a}	68.57^{ef}	68.36^{ef}	66.82^{gh}
		10°C, 90%	72.05^{a}	68.90^{def}	68.76^{def}	67.68^{fg}
		AT, ARH	71.37^{a}	68.46^{ef}	66.72^{gh}	65.28^{jk}
	Bio	5°C, 85%	72.23^{a}	71.62^{ab}	70.88^{abc}	70.04^{cd}
		5°C, 90%	72.50^{a}	69.99^{cd}	69.20^{de}	68.29^{efg}
		10°C, 85%	71.98^{a}	68.10^{efg}	67.88^{fg}	67.73^{fg}
		10°C, 90%	72.05^{a}	68.75^{def}	68.58^{ef}	67.52^{fg}
		AT, ARH	71.37^{a}	66.18^{hi}	65.75^{ij}	64.99^{jk}
	NP	5°C, 85%	72.23^{a}	71.58^{ab}	71.19^{abc}	69.15^{de}
		5°C, 90%	72.50^{a}	68.39^{ef}	68.67^{ef}	67.63^{fg}
		10°C, 85%	71.98^{a}	67.18^{g}	67.37^{g}	66.84^{gh}
		10°C, 90%	72.05^{a}	69.31^{de}	68.94^{def}	66.83^{gh}
		AT, ARH	71.37^{a}	69.30^{de}	66.07^{hi}	59.57^{n}
Avoshine®	LDPE	5°C, 85%	72.23^{a}	71.57^{ab}	71.19^{abc}	69.07^{de}
		5°C, 90%	72.50^{a}	67.87^{fg}	66.64^{gh}	69.80^{cd}
		10°C, 85%	71.98^{a}	68.89^{def}	68.59^{ef}	68.27^{efg}
		10°C, 90%	72.05^{a}	68.65^{ef}	68.53^{ef}	67.88^{fg}
		AT, ARH	71.37^{a}	68.10^{efg}	67.71^{fg}	66.50^{gh}
	Bio	5°C, 85%	72.23^{a}	71.72^{ab}	70.32^{bc}	70.07^{cd}
		5°C, 90%	72.50^{a}	69.29^{de}	67.04^{g}	67.36^{g}
		10°C, 85%	71.98^{a}	68.00^{efg}	67.09^{g}	66.37^{hi}
		10°C, 90%	72.05^{a}	66.63^{gh}	66.26^{hi}	65.52^{ijk}
		AT, ARH	71.37^{a}	71.43^{ab}	69.29^{de}	64.26^{l}
	NP	5°C, 85%	72.23^{a}	69.58^{d}	68.47^{ef}	67.28^{g}
		5°C, 90%	72.50^{a}	67.28^{g}	66.78^{gh}	66.21^{hi}
		10°C, 85%	71.98^{a}	66.92^{gh}	66.91^{gh}	67.09^{g}
		10°C, 90%	72.05^{a}	66.57^{gh}	65.74^{ij}	65.96^{ij}
		AT, ARH	71.37^{a}	66.82^{gh}	65.71^{ij}	64.00^{lm}
NPP	LDPE	5°C, 85%	72.23^{a}	72.00^{a}	69.65^{d}	66.79^{gh}
		5°C, 90%	72.50^{a}	67.66^{fg}	67.92^{fg}	66.93^{gh}
		10°C, 85%	71.98^{a}	68.12^{efg}	66.92^{gh}	65.97^{ij}
		10°C, 90%	72.05^{a}	66.87^{gh}	66.17^{hi}	65.71^{ij}
		AT, ARH	71.37^{a}	66.73^{gh}	66.13^{hi}	63.27^{m}
	Bio	5°C, 85%	72.23^{a}	72.01^{a}	69.28^{de}	67.97^{fg}
		5°C, 90%	72.50^{a}	67.59^{fg}	67.17^{g}	66.61^{gh}
		10°C, 85%	71.98^{a}	67.63^{fg}	67.11^{g}	65.31^{jk}
		10°C, 90%	72.05^{a}	68.45^{ef}	66.03^{hi}	65.33^{jk}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		AT, ARH	71.37[a]	66.51[gh]	65.97[ij]	62.95[mn]
	NP	5°C, 85%	72.23[a]	69.82[cd]	69.97[cd]	66.74[gh]
		5°C, 90%	72.50[a]	67.32[g]	66.34[hi]	66.34[hi]
		10°C, 85%	71.98[a]	67.49[fg]	66.25[hi]	65.19[jk]
		10°C, 90%	72.05[a]	66.38[hi]	65.32[jk]	64.79[kl]
		AT, ARH	71.37[a]	68.15[efg]	65.44[ijk]	54.36[o]
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		*				
AC		*				
BC		*				
AD		NS				
BD		*				
CD		**				
ABC		*				
ABD		*				
ACD		**				
BCD		*				
ABCD		**				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The four-way interaction between pre-packaging, packaging, storage conditions and storage periods was found to be highly significant (P≤0.001) on the avocado puree lightness. In the two-way analysis, avocado samples treated with the combination of pre-packaging and packaging and subjected to refrigerated conditions showed less darkening, compared to control samples. This indicates the benefit offered by integrating suitable pre-packaging, packaging and storage conditions in improving the internal quality of the avocado fruit.

The storage conditions and storage period were found to be highly significant (P≤0.001) with regard to the a* values of the avocado puree. An increase in the value of a* was observed during the 12-day storage period, indicating that the puree colour tended to move away from green toward red according to the CIELAB colour coordinate system (Table 3.10). This, in turn implies that the pulp became darker with increasing storage time. The increase in the value of the a* colour index was more apparent at ambient conditions representative of higher temperatures and a lower relative humidity, which has been proven to increase the rate of ripening. Storage at 5°C + 85% RH and 5°C + 90% RH maintained the a* values to a higher degree for all treatments throughout the storage period, compared to other storage conditions.

Pre-packaging and packaging treatments were both found to have a significant ($P \leq 0.05$) influence on the a* value. Avocado samples that had been packaged in either the LDPE or biodegradable films also represented lower a* values, compared to samples without any packaging. Similar results were obtained for the L* colour index of the avocado puree.

The two-way interactions between (a) pre-packaging and packaging, and (b) pre-packaging and storage conditions had a significant ($P \leq 0.05$) effect on the a* value of the avocado puree. A higher significance ($P \leq 0.001$) was found for the interaction between packaging and storage conditions. Packaged samples generally demonstrated lower a* values, compared to unpackaged samples, which was most evident at the low temperature of 5°C. The combination of LDPE or biodegradable packaging with low temperature storage was found to be suitable in preventing a hastened darkening of the avocado puree.

The benefit of the pre-packaging treatments supplementary to low temperature storage can be seen in the highly significant ($P \leq 0.001$) four-way interaction of pre-packaging, packaging, storage conditions and storage period on with a* values. Control samples demonstrated the greatest increase in a* from -8.83 on Day 0 to 3.10 on Day 12. This, therefore, indicates the enhancement of the colour characteristics of the avocado puree by implementing suitable pre-packaging, packaging and storage conditions, compared to fruit devoid of any pre-packaging or packaging treatments.

Table 3.10 The a* colour parameter of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	-8.60^{p}	-7.84^{o}	-7.30^{mn}	-7.08^{l}
		5°C, 90%	-8.80^{p}	-8.47^{op}	-8.25^{op}	-7.03^{l}
		10°C, 85%	-8.64^{p}	-6.54^{k}	-6.30^{jk}	-5.75^{ij}
		10°C, 90%	-8.77^{p}	-7.09^{l}	-4.89^{h}	-4.41^{fgh}
		AT, ARH	-8.83^{p}	-4.65^{gh}	-4.35^{fgh}	-2.13^{cd}
	Bio	5°C, 85%	-8.60^{p}	-8.03^{op}	-7.48^{n}	-6.74^{kl}
		5°C, 90%	-8.80^{p}	-8.47^{op}	-7.33^{mn}	-7.12^{lm}
		10°C, 85%	-8.64^{p}	-7.94^{op}	-7.34^{mn}	-6.93^{l}
		10°C, 90%	-8.77^{p}	-5.82^{j}	-5.32^{hij}	-5.11^{hi}
		AT, ARH	-8.83^{p}	-4.13^{fg}	-3.52^{ef}	-2.07^{cd}
	NP	5°C, 85%	-8.60^{p}	-7.43^{n}	-6.68^{k}	-6.32^{jk}
		5°C, 90%	-8.80^{p}	-8.41^{op}	-7.89^{o}	-6.73^{kl}
		10°C, 85%	-8.64^{p}	-6.99^{l}	-5.98^{j}	-3.71^{f}
		10°C, 90%	-8.77^{p}	-6.20^{jk}	-4.90^{h}	-2.88^{de}
		AT, ARH	-8.83^{p}	-6.89^{l}	-5.63^{hij}	-0.45^{b}
Avoshine®	LDPE	5°C, 85%	-8.60^{p}	-7.48^{n}	-7.06^{l}	-5.85^{j}
		5°C, 90%	-8.80^{p}	-7.32^{mn}	-7.25^{lm}	-7.00^{l}
		10°C, 85%	-8.64^{p}	-7.22^{lm}	-6.23^{jk}	-4.89^{h}
		10°C, 90%	-8.77^{p}	-7.49^{n}	-6.40^{jk}	-5.84^{j}
		AT, ARH	-8.83^{p}	-5.01^{h}	-4.93^{h}	-4.86^{h}
	Bio	5°C, 85%	-8.60^{p}	-8.30^{op}	-6.60^{k}	-5.83^{j}
		5°C, 90%	-8.80^{p}	-7.63^{no}	-7.28^{lm}	-6.99^{l}
		10°C, 85%	-8.64^{p}	-5.20^{hi}	-4.08^{fg}	-4.97^{h}
		10°C, 90%	-8.77^{p}	-5.72^{ij}	-4.90^{h}	-4.21^{fg}
		AT, ARH	-8.83^{p}	-4.51^{gh}	-4.43^{fgh}	-2.00^{cd}
	NP	5°C, 85%	-8.60^{p}	7.45^{n}	-6.47^{jk}	-4.81^{h}
		5°C, 90%	-8.80^{p}	-8.23^{op}	-7.45^{n}	-6.61^{k}
		10°C, 85%	-8.64^{p}	-5.31^{hij}	-5.57^{hij}	-3.39^{ef}
		10°C, 90%	-8.77^{p}	-7.44^{n}	-5.77^{ij}	-3.12^{def}
		AT, ARH	-8.83^{p}	-5.05^{h}	-3.36^{ef}	-1.64^{bcd}
NPP	LDPE	5°C, 85%	-8.60^{p}	-8.14^{op}	-7.03^{l}	-3.79^{f}
		5°C, 90%	-8.80^{p}	-7.68^{no}	-7.04^{l}	-4.71^{h}
		10°C, 85%	-8.64^{p}	-4.57^{gh}	-4.17^{fg}	-3.09^{def}
		10°C, 90%	-8.77^{p}	-5.76^{ij}	-4.30^{fgh}	-3.80^{f}
		AT, ARH	-8.83^{p}	-7.41^{n}	-4.84^{h}	-2.41^{cde}
	Bio	5°C, 85%	-8.60^{p}	-7.70^{no}	-7.18^{lm}	-5.64^{hij}
		5°C, 90%	-8.80^{p}	-6.29^{jk}	-6.21^{jk}	-3.58^{ef}
		10°C, 85%	-8.64^{p}	-4.30^{fgh}	-3.93^{f}	-3.80^{f}
		10°C, 90%	-8.77^{p}	-3.58^{ef}	-2.75^{de}	-2.75^{de}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		AT, ARH	-8.83[p]	-4.54[gh]	-2.88[de]	-0.92[bc]
	NP	5°C, 85%	-8.60[p]	-8.16[op]	-7.41[n]	-4.58[gh]
		5°C, 90%	-8.80[p]	-7.22[lm]	-7.28[mn]	-5.64[hij]
		10°C, 85%	-8.64[p]	-5.77[ij]	-4.85[h]	-3.05[def]
		10°C, 90%	-8.77[p]	-6.34[jk]	-5.62[hij]	-2.98[de]
		AT, ARH	-8.83[p]	-4.20[fg]	-2.93[de]	3.10[a]
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		*				
AC		*				
BC		**				
AD		NS				
BD		NS				
CD		**				
ABC		*				
ABD		*				
ACD		**				
BCD		*				
ABCD		**				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

The b* colour index of the avocado puree as a result of the application of different pre-packaging, packaging and storage conditions for a 12-day storage period are presented in Table 3.11. Storage conditions and the storage period were found to be highly significant (P≤0.001) on the b* values of the avocado puree. A steady decline in the b* value was observed for all treatments. This decrease in the b* value is synonymous with the colour change from yellow to blue, indicative of darkening of the puree, which was most apparent at ambient storage conditions, while storage at 5°C reduced the rate at which a reduction in b* occurred. Similar results were found by Zauberman and Jobin-Decor (1995), where the darkening of the mesocarp was most apparent at higher temperatures, while storage of avocados at 5°C extended the avocado shelf life and reduced mesocarp discolouration. Pre-packaging and packaging treatments were found to be significant (P≤0.05). As previously discussed for the L* and a* colour indices of the avocado puree, samples that had been pre-treated with Avoshine® wax or with hot water, demonstrated reduced rates of darkening, as opposed to control samples. Once again samples that had been packaged in either LDPE or biodegradable films showed less darkening than control samples without packaging.

All interactions, with the exception of the two-way interaction between pre-packaging and packaging treatments, which was found to have a significance of $P \leq 0.05$, were found to be highly significant ($P \leq 0.001$). Avoshine® coated samples, packaged in LDPE films and stored at 5°C + 90% RH demonstrated the least reduction in b* of 43.98 on Day 0 to 41.93 on Day 12. In comparison, control samples demonstrated the greatest reduction in b* of 44.00 on Day 0 to 33.12 on Day 12.

The results obtained for the colour of the avocado puree indicate that low temperature storage at 5°C + 85% RH or at 5°C + 90% RH proved to be beneficial in reducing the rate at which the avocado colour puree changed. Furthermore, the use of pre-packaging treatments such as Avoshine® or hot water treatments, in combination with LDPE or biodegradable films improved the colour of avocado samples, compared to control samples. The combined use of pre-packaging treatments (HWT or Avoshine®) with packaging films (LDPE or biodegradable) proved to be beneficial in preventing the darkening of the puree, which is associated with the ripening process specifically at refrigerated conditions (5°C).

Table 3.11 The b* colour parameter of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	44.21^{a}	40.53^{fgh}	40.49^{gh}	40.58^{fgh}
		5°C, 90%	43.98^{a}	42.92^{b}	41.74^{de}	41.59^{de}
		10°C, 85%	44.64^{a}	41.11^{ef}	39.94^{hi}	39.26^{jk}
		10°C, 90%	44.78^{a}	42.66^{bc}	42.42^{bcd}	40.08^{h}
		AT, ARH	44.00^{a}	40.58^{fgh}	38.65^{lm}	36.94^{p}
	Bio	5°C, 85%	44.21^{a}	42.05^{cd}	41.38^{def}	40.68^{fgh}
		5°C, 90%	43.98^{a}	42.12^{cd}	41.31^{def}	41.50^{de}
		10°C, 85%	44.64^{a}	40.85^{fg}	40.81^{fg}	39.58^{ij}
		10°C, 90%	44.78^{a}	40.88^{fg}	40.46^{gh}	39.94^{hi}
		AT, ARH	44.00^{a}	41.31^{def}	41.13^{ef}	37.17^{op}
	NP	5°C, 85%	44.21^{a}	41.13^{ef}	40.58^{fgh}	40.13^{h}
		5°C, 90%	43.98^{a}	42.00^{cd}	41.87^{d}	41.40^{def}
		10°C, 85%	44.64^{a}	39.87^{hi}	39.40^{ijk}	38.16^{n}
		10°C, 90%	44.78^{a}	41.83^{d}	40.97^{ef}	39.00^{jk}
		AT, ARH	44.00^{a}	42.66^{bc}	42.42^{bcd}	34.28st
Avoshine®	LDPE	5°C, 85%	44.21^{a}	41.33^{def}	40.37^{gh}	40.15^{h}
		5°C, 90%	43.98^{a}	42.04^{cd}	42.38^{bcd}	41.93^{d}
		10°C, 85%	44.64^{a}	40.81^{fg}	39.92^{hi}	39.61^{hij}
		10°C, 90%	44.78^{a}	40.88^{fg}	39.97^{hi}	39.73^{hij}
		AT, ARH	44.00^{a}	41.62^{de}	40.29^{gh}	39.54^{ij}
	Bio	5 °C, 85 %	44.21^{a}	41.45^{de}	41.04^{ef}	40.90^{fg}
		5 °C, 90 %	43.98^{a}	41.84^{d}	41.61^{de}	41.44^{de}
		10°C, 85%	44.64^{a}	42.17^{cd}	41.12^{ef}	40.73^{fgh}
		10°C, 90%	44.78^{a}	41.58^{de}	40.83^{fg}	40.53^{fgh}
		AT, ARH	44.00^{a}	40.08^{h}	38.81^{k}	35.80^{qr}
	NP	5°C, 85%	44.21^{a}	40.56^{fgh}	39.12^{jk}	39.94^{hi}
		5°C, 90%	43.98^{a}	42.02^{cd}	41.96^{d}	41.72^{de}
		10°C, 85%	44.64^{a}	38.88^{k}	38.72^{kl}	37.58^{no}
		10°C, 90%	44.78^{a}	40.01^{h}	39.12^{jk}	38.65^{l}
		AT, ARH	44.00^{a}	38.70^{kl}	35.92^{q}	34.00^{s}
NPP	LDPE	5 °C, 85 %	44.78^{a}	41.40^{def}	39.92^{hi}	43.98^{a}
		5 °C, 90 %	43.98^{a}	41.00^{ef}	40.63^{fgh}	44.78^{a}
		10°C, 85%	44.64^{a}	41.47^{de}	39.26^{jk}	44.64^{a}
		10°C, 90%	44.21^{a}	40.90^{fg}	40.75^{fgh}	39.52^{ij}
		AT, ARH	44.00^{a}	39.47^{ijk}	38.13^{n}	44.00^{a}
	Bio	5°C, 85%	44.78^{a}	41.49^{de}	41.50^{de}	41.49^{de}
		5°C, 90%	43.98^{a}	41.35^{def}	41.00^{ef}	40.46^{gh}
		10°C, 85%	44.64^{a}	42.38^{bcd}	41.15^{ef}	40.81^{fg}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		10°C, 90%	44.21[a]	40.41[gh]	39.72[hij]	39.43[ijk]
		AT, ARH	44.00[a]	40.79[fg]	40.03[h]	33.57[t]
	NP	5°C, 85%	43.98[a]	40.26[gh]	39.61[hij]	39.69[hij]
		5°C, 90%	44.78[a]	41.66[de]	40.80[fg]	40.11[h]
		10°C, 85%	44.64[a]	39.63[hij]	39.37[ijk]	38.40[mn]
		10°C, 90%	44.21[a]	41.29[def]	41.35[def]	38.13[n]
		AT, ARH	44.00[a]	40.28[gh]	35.24[rs]	33.12[tu]
Significance						
Pre-packaging (A)	*					
Packaging (B)	*					
Storage Condition (C)	**					
Storage Period (D)	**					
AB	*					
AC	**					
BC	**					
AD	**					
BD	**					
CD	**					
ABC	**					
ABD	**					
ACD	**					
BCD	**					
ABCD	**					

NS, *, ** Non-significant or significant at P ≤ 0.05 or P ≤ 0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P ≤ 0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.11.8 Puree viscosity

The avocado puree viscosity was significantly (P≤0.05) affected by the storage conditions and the storage period (Table 3.12). The increase in the puree viscosity was more apparent in avocados stored at ambient conditions, compared to avocados stored at controlled low temperature. Packaging treatments had a significant (P≤0.05) influence on the pulp viscosity, while pre-packaging treatments were not found to be significant (P>0.05). Packaged samples in both the LDPE and biodegradable films showed the least increase in the viscosity, compared to control samples devoid of packaging. It has been found that changes in the viscosity and elasticity of avocado tissue are as a result of fruit ripening (Sakurai and Nevins, 1997). A decrease in the elasticity of the cell walls was observed during the ripening of avocados mediated by endo-type hydrolytic enzymes in the breakdown of xyloglucan molecules (Sakurai and Nevins, 1997). This decrease in the elasticity could account for the increase in the viscosity of the pureed avocado pulp as result of ripening. A decrease in the elasticity and resultant breakdown of the cell wall due to the ripening process also leads to a decrease in the firmness, which is accelerated at higher storage temperatures. This trend can be observed for avocado samples subjected to ambient conditions, resulting in a drastic decrease in the pulp firmness (Section 3.11.6).

The two-way interaction between packaging films and storage conditions was found to be significant ($P \leq 0.05$) with regard to the avocado pulp viscosity. LDPE films resulted in the least increase in viscosity. LDPE packaged samples that had been coated with Avoshine® maintained the viscosity throughout the storage period, even when subjected to ambient conditions. Control samples without any pre-packaging or packaging and stored at ambient conditions led to an increase in the viscosity by 98.5%. This can be attributed to the increased vapour pressure deficit between the fruit and the external surrounding promoting the loss of moisture from the fruit. This reduction in the moisture may also contribute to the increased viscosity of the pulp as a result of fruit ripening due to the partial deficiency of water.

The results obtained in this experiment demonstrate the beneficial use of low temperature storage conditions in maintaining the avocado pulp viscosity for all pre-packaging and packaging treatment combinations from Day 0 to Day 12. At ambient conditions, a significant increase in the viscosity was observed, specifically in control samples without any pre-packaging and packaging treatments. The Avoshine® and LDPE treatment combination maintained a consistent pulp viscosity throughout the storage period at refrigerated and ambient conditions. Therefore, this treatment combination offers the greatest benefit to avocado fruit. Rheological properties of food products are essential in quality control and the efficient design of process equipment such as pumps, piping and heat exchangers (Sanchez *et al*., 2009; Balestra *et al*., 2011). From an engineering perspective, an understanding of the rheological properties of fruit enables the energy consumption of the pump in process equipment to be determined and to solve problems relating to air incorporation into the fruit pulp mixture, resulting in undesirable reactions such as oxidation (Sanchez *et al*., 2009).

Table 3.12 The puree viscosity (Pa.s) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	0.05^{g}	0.08^{g}	0.07^{g}	0.09^{g}
		5°C, 90%	0.02^{g}	0.10^{g}	0.06^{g}	0.14^{g}
		10°C, 85%	0.02^{g}	0.05^{g}	0.06^{g}	0.09^{g}
		10°C, 90%	0.05^{g}	0.07^{g}	0.04^{g}	0.08^{g}
		AT, ARH	0.06^{g}	0.02^{g}	0.49^{f}	2.82^{b}
	Bio	5°C, 85%	0.09^{g}	0.08^{g}	0.05^{g}	0.12^{g}
		5°C, 90%	0.02^{g}	0.09^{g}	0.17^{g}	0.15^{g}
		10°C, 85%	0.02^{g}	0.07^{g}	0.07^{g}	0.06^{g}
		10°C, 90%	0.05^{g}	0.07^{g}	0.04^{g}	0.06^{g}
		AT, ARH	0.06^{g}	0.02^{g}	1.41^{d}	2.54^{bc}
	NP	5°C, 85%	0.09^{g}	0.11^{g}	0.16^{g}	0.03^{g}
		5°C, 90%	0.02^{g}	0.08^{g}	0.13^{g}	0.08^{g}
		10°C, 85%	0.02^{g}	0.07^{g}	0.12^{g}	0.06^{g}
		10°C, 90%	0.05^{g}	0.08^{g}	0.07^{g}	0.09^{g}
		AT, ARH	0.06^{g}	0.02^{g}	3.35^{ab}	3.86^{a}
Avoshine®	LDPE	5°C, 85%	0.09^{g}	0.05^{g}	0.05^{g}	0.05^{g}
		5°C, 90%	0.02^{g}	0.06^{g}	0.05^{g}	0.07^{g}
		10°C, 85%	0.02^{g}	0.11^{g}	0.06^{g}	0.07^{g}
		10°C, 90%	0.05^{g}	0.07^{g}	0.02^{g}	0.07^{g}
		AT, ARH	0.06^{g}	0.02^{g}	0.06^{g}	0.11^{g}
	Bio	5°C, 85%	0.09^{g}	0.08^{g}	0.06^{g}	0.06^{g}
		5°C, 90%	0.02^{g}	0.07^{g}	0.05^{g}	0.06^{g}
		10°C, 85%	0.02^{g}	0.06^{g}	0.06^{g}	0.05^{g}
		10°C, 90%	0.05^{g}	0.07^{g}	0.03^{g}	0.05^{g}
		AT, ARH	0.06^{g}	0.02^{g}	0.77^{ef}	2.00^{c}
	NP	5°C, 85%	0.09^{g}	0.36^{g}	0.07^{g}	0.25^{g}
		5°C, 90%	0.02^{g}	0.04^{g}	0.08^{g}	0.09^{g}
		10°C, 85%	0.02^{g}	0.06^{g}	0.07^{g}	0.04^{g}
		10°C, 90%	0.05^{g}	0.08^{g}	0.02^{g}	0.08^{g}
		AT, ARH	0.06^{g}	0.02^{g}	2.55^{bc}	3.29^{ab}
NPP	LDPE	5°C, 85%	0.09^{g}	0.13^{g}	0.10^{g}	0.06^{g}
		5°C, 90%	0.02^{g}	0.06^{g}	0.06^{g}	0.04^{g}
		10°C, 85%	0.02^{g}	0.08^{g}	0.07^{g}	0.12^{g}
		10°C, 90%	0.05^{g}	0.04^{g}	0.04^{g}	0.07^{g}
		AT, ARH	0.06^{g}	0.02^{g}	0.95^{def}	3.12^{ab}
	Bio	5°C, 85%	0.09^{g}	0.12^{g}	0.13^{g}	0.03^{g}
		5°C, 90%	0.02^{g}	0.06^{g}	0.12^{g}	0.02^{g}
		10°C, 85%	0.02^{g}	0.09^{g}	0.08^{g}	0.07^{g}
		10°C, 90%	0.05^{g}	0.04^{g}	0.04^{g}	0.12^{g}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		AT, ARH	0.06^{g}	0.02^{g}	1.74^{cd}	2.99^{b}
		5°C, 85%	0.09^{g}	0.10^{g}	0.04^{g}	0.03^{g}
		5°C, 90%	0.02^{g}	0.07^{g}	0.06^{g}	0.04^{g}
	NP	10°C, 85%	0.02^{g}	0.16^{g}	0.08^{g}	0.14^{g}
		10°C, 90%	0.05^{g}	0.05^{g}	0.04^{g}	0.10^{g}
		AT, ARH	0.06^{g}	0.03^{g}	1.77^{cd}	3.88^{a}
Significance						
Pre-packaging (A)		NS				
Packaging (B)		*				
Storage Condition (C)		*				
Storage Period (D)		*				
AB		NS				
AC		NS				
BC		*				
AD		NS				
BD		NS				
CD		**				
ABC		NS				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.11.9 Moisture content

The avocado moisture content (MC) for different treatments and storage conditions and storage durations, are presented in Table 3.13. The storage conditions and the storage period significantly (P≤0.001) affected the avocado MC. A comparison of the different storage conditions indicated that the reduction in the MC occurred at a slower rate at the lower temperatures (5°C) and the higher RH (90%). The difference in the avocado moisture reduction rate between the different temperature and RH conditions can be attributed to a higher water vapour pressure deficit created at higher temperatures and/or low RH, resulting in a greater loss of moisture to the external environment (Hofman and Jobin-Decor, 1997; Maftoonazad and Ramaswamy, 2008; Li *et al*., 2010; Valle-Guadarrama *et al*., 2004). A general reduction in the MC was observed in all samples. However, control avocado samples depicted the greatest reduction in the MC, due to excessively higher exposure temperatures and no pre-packaging and packaging treatments. The main effects of pre-packaging and packaging treatments were found to be significant ($P \leq 0.05$). A similar trend was also observed by Li *et al*. (2010) for peach fruit, in which fruit stored at a RH range of 77 - 83% displayed greater mass loss, compared to those stored at 95-99% RH.

The two-way interactions between (a) pre-packaging and packaging, (b) pre-packaging and storage conditions, and (c) packaging and storage conditions were found to have a significant ($P \leq 0.001$) effect on the moisture loss. Avocado samples coated with Avoshine® showed the least reduction in the MC, followed by hot water treated avocado samples and control samples. The integration of Avoshine® with LDPE or biodegradable packaging showed a lower reduction in the MC, compared to hot water treated samples packaged in LDPE or biodegradable films. This could be due to tissue damage that may have occurred as a result of exposing the avocado samples to the hot water pre-treatment. The use of waxing by Maftoonazad and Ramaswamy (2005; 2008) has been shown to reduce the rate of ripening by creating a partially permeable layer surrounding the avocado inhibiting the loss of moisture and reducing the respiration process.

The three-way interactions of (a) pre-packaging, packaging and storage conditions, and (b) packaging, storage conditions and storage period, had high statistical significance ($P \leq 0.001$). Samples that had not been pre-treated, nor packaged and subjected to ambient conditions, demonstrated the greatest loss in MC of 39.3%, while samples that had been pre-treated with Avoshine® wax, thereafter packaged in LDPE films and stored at 5°C and 90% RH, demonstrated the least reduction in the MC of 4.4%.

As the avocado matures and ripens, the water content within the mesocarp of the fruit declines with an increase in the oil content (Lee *et al*., 1983; Bower and Cutting, 1988; Chen *et al*., 2009). Therefore, a reduction in the MC can be associated with the ripening of avocados. LDPE films illustrated the least reduction in the MC, followed by biodegradable films and thereafter control samples devoid of packaging. The packaging is effective in creating a low vapour pressure deficit between the fruit and the micro-environment within the packaging due to the initial build-up of moisture from the fruit. This then further reduces the rate of moisture loss form the avocados. A reduction in the MC and associated increase in the

DM (and oil content) is closely linked to the ripening of avocado fruit (Clark *et al*., 2003; Ozdemir and Topuz, 2004; Villa-Rodriguez *et al*., 2011).

The results obtained for MC indicate that the storage of avocado samples at lower temperatures and higher RH limits the reduction in the MC, thereby delaying the ripening process. In addition, the integrated use of Avoshine® wax with LDPE packaging was found to be beneficial in delaying the ripening of avocado samples by reducing the MC and, as previously demonstrated, resulting in a reduction in the PWL, reduced and delayed peak respiration rates, higher percentage of marketable avocados, superior colour and reduced firmness.

Table 3.13 The moisture content (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			**Storage Period (Days)**			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	62.81^{a}	61.93^{a}	56.53^{bc}	55.67^{bcd}
		5°C, 90%	62.01^{a}	54.46^{bcd}	52.58^{cde}	56.63^{bc}
		10°C, 85%	62.54^{a}	53.13^{cde}	48.54^{defg}	47.95^{efg}
		10°C, 90%	61.50^{a}	53.38^{cde}	48.24^{efg}	47.47^{efg}
		AT, ARH	61.60^{a}	48.45^{defg}	44.50^{ghi}	42.79^{hi}
	Bio	5°C, 85%	62.81^{a}	60.88^{ab}	59.56^{ab}	57.50^{bc}
		5°C, 90%	62.01^{a}	56.78^{bc}	52.12c^{de}	51.64^{cdef}
		10°C, 85%	62.54^{a}	50.00^{def}	49.49^{defg}	49.01^{defg}
		10°C, 90%	61.50^{a}	50.51^{def}	50.00^{def}	48.99^{defg}
		AT, ARH	61.60^{a}	53.50^{bcd}	49.25^{defg}	41.67^{hij}
	NP	5°C, 85%	62.81^{a}	59.85^{ab}	56.92^{bc}	45.13^{gh}
		5°C, 90%	62.01^{a}	50.00^{def}	49.24^{defg}	48.86^{defg}
		10°C, 85%	62.54^{a}	49.74^{def}	49.22^{defg}	43.43^{hi}
		10°C, 90%	61.50^{a}	50.10^{def}	48.50^{defg}	43.94^{ghi}
		AT, ARH	61.60^{a}	44.95^{gh}	47.30^{efg}	40.61^{hij}
Avoshine®	LDPE	5°C, 85%	62.81^{a}	62.70^{a}	57.55^{bc}	57.10^{bc}
		5°C, 90%	62.01^{a}	58.57^{abc}	59.39^{abc}	59.30^{abc}
		10°C, 85%	62.54^{a}	53.11^{cde}	50.42^{def}	50.00^{def}
		10°C, 90%	61.50^{a}	53.74^{bcd}	51.50^{cdef}	47.76^{efg}
		AT, ARH	61.60^{a}	55.28^{bcd}	48.67^{defg}	47.74^{efg}
	Bio	5°C, 85%	62.81^{a}	56.80^{bc}	54.68^{bcd}	54.82^{bcd}
		5°C, 90%	62.01^{a}	53.20^{cde}	51.27^{cdef}	49.50^{defg}
		10°C, 85%	62.54^{a}	56.70^{bc}	46.91^{efg}	46.59^{fg}
		10°C, 90%	61.50^{a}	47.65^{efg}	47.06^{efg}	47.88^{efg}
		AT, ARH	61.60^{a}	45.18^{gh}	44.39^{ghi}	43.22^{hi}
	NP	5°C, 85%	62.81^{a}	54.78^{bcd}	54.77^{bcd}	54.04^{bcd}
		5°C, 90%	62.01^{a}	51.24^{cdef}	50.00^{def}	48.47^{defg}
		10°C, 85%	62.54^{a}	48.00^{efg}	48.54^{defg}	46.46^{fg}
		10°C, 90%	61.50^{a}	53.55^{bcd}	48.94^{defg}	46.19^{fg}
		AT, ARH	61.60^{a}	51.47^{cdef}	45.70^{gh}	44.72^{gh}
NPP	LDPE	5°C, 85%	62.81^{a}	60.64^{ab}	51.98^{cde}	50.25^{def}
		5°C, 90%	62.01^{a}	52.18^{cde}	51.92^{cde}	51.71^{cdef}
		10°C, 85%	62.54^{a}	44.14^{ghi}	40.64^{hij}	39.35^{hij}
		10°C, 90%	61.50^{a}	50.42^{def}	47.15^{efg}	38.62^{ij}
		AT, ARH	61.60^{a}	52.33^{cde}	50.00^{def}	41.84^{hij}
	Bio	5°C, 85%	62.81^{a}	58.16^{abc}	56.90^{bc}	49.07^{defg}
		5 °C, 90 %	62.01^{a}	49.75^{def}	49.85^{def}	49.63^{defg}
		10°C, 85%	62.54^{a}	49.15^{defg}	46.50^{fg}	44.44^{ghi}
		10°C, 90%	61.50^{a}	51.11^{cdef}	47.02^{efg}	42.01^{hij}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		AT, ARH	61.60^{a}	47.42^{efg}	46.72^{fg}	43.00^{hi}
		5°C, 85%	62.81^{a}	54.81^{bcd}	49.62^{defg}	47.19^{efg}
		5°C, 90%	62.01^{a}	48.40^{defg}	47.07^{efg}	46.80^{fg}
	NP	10°C, 85%	62.54^{a}	49.75^{def}	45.92^{gh}	42.10^{hij}
		10°C, 90%	61.50^{a}	52.85^{cde}	52.38^{cde}	45.45^{fg}
		AT, ARH	61.60^{a}	44.53^{ghi}	40.40^{hij}	37.39^{j}
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		**				
AC		**				
BC		**				
AD		NS				
BD		NS				
CD		**				
ABC		**				
ABD		*				
ACD		*				
BCD		**				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.11.10 Dry matter

The storage conditions and the storage period were found to be highly significant (P≤0.001) with regard to the DM content (Table 3.14). A general increase in the DM of the avocado samples were detected with the progression of storage time. It can be observed that the decrease in the MC (Table 3.13) of the avocado samples on Days 0, 4, 8 and 12 corresponds with an increase in the DM, so that the total MC and DM equates to approximately 100%. As a result a reduction in the MC will be proportional to an increase in the DM on a percentage basis. A comparison of the different storage conditions indicated that storage at 5°C resulted in the least increase in the DM, compared to at 10°C and at ambient conditions. Storage at ambient conditions promoted the ripening process, due to the excessively higher temperatures, therefore, increasing the percent DM. Storage at the different RH affected the DM. However, this was more pronounced at 5°C than at 10°C with an increase in the DM being greater at the lower RH of 85%. This can be due to the lower RH creating a higher water vapour pressure deficit, thus promoting the loss of moisture from the fruit and a subsequent increase in the DM. Storage at ambient conditions accelerated the rate of DM accumulation. Studies by Hofman and Jobin-Decor (1997) have indicated that the storage of avocados at 22°C and a RH of between 80% and 98% did not have a significant effect the DM after four days of storage, compared to storage at a RH of 60% and lower, which could represent the ambient conditions in the present experiment. As with the MC in Section 3.11.9, the higher temperature and lower RH created an environment promoting the loss in moisture from the avocado fruit, thereby increasing the DM content (Hofman and Jobin-Decor, 1997) and promoting fruit ripening. The main effects of the pre-packaging treatments and packaging films were found to have a significance level of $P \leq 0.05$ with regards to the DM.

The two-way interactions between (a) pre-packaging treatments and packaging films, (b) pre-packaging treatments and storage conditions, and (c) packaging and storage conditions were found to have a significant ($P \leq 0.001$) effect on the percent DM. Avocado samples coated with Avoshine® showed the least increase in the DM, followed by hot water treated samples and samples without any pre-treatment. The combined use of the Avoshine® wax with LDPE or biodegradable films showed a slower rate of DM accumulation, compared to hot water treated samples in LDPE or biodegradable packaging. This could be due to tissue damage that may have occurred as a result of exposing the avocado samples to the heating medium during pre-treatment. LDPE films illustrated the least increase in the DM, followed by biodegradable films and, thereafter control samples devoid of packaging. The use of wax coatings has been shown to reduce the ripening of avocados in many studies (Maftoonazad and Ramaswamy, 2005; 2008), which is in agreement with the findings of the current experiment.

The three-way interactions between (a) pre-packaging, packaging and storage conditions, and (b) packaging, storage conditions and storage period, had a highly significant ($P \leq 0.001$) influence on the DM content. Control samples devoid of pre-packaging and packaging treatments and subjected to ambient conditions were found to have a DM accumulation of 38.7%. Avocado samples that had been pre-treated with Avoshine® wax and, thereafter packaged in LDPE films and stored at 5°C + 90% RH, demonstrated the least reduction in the DM of 6.7%.

Table 3.14 The dry matter (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
HWT	LDPE	5°C, 85%	37.19^{j}	38.07^{j}	43.47^{hi}	44.33^{ghi}
		5°C, 90%	37.99^{j}	45.54^{ghi}	47.42^{fgh}	43.37^{hi}
		10°C, 85%	37.46^{j}	46.87^{fgh}	51.46^{defg}	52.05^{def}
		10°C, 90%	38.50^{j}	46.62^{fgh}	51.76^{def}	52.53^{def}
		AT, ARH	38.40^{j}	51.55^{defg}	55.50^{bcd}	57.21^{bc}
	Bio	5°C, 85%	37.19^{j}	39.12^{ij}	40.44^{ij}	42.50^{hi}
		5°C, 90%	37.99^{j}	43.22^{hi}	47.88^{fgh}	48.36^{efgh}
		10°C, 85%	37.46^{j}	50.00^{efg}	50.51^{defg}	50.99^{defg}
		10°C, 90%	38.50^{j}	49.49^{efg}	50.00^{efg}	51.01^{defg}
		AT, ARH	38.40^{j}	46.50^{ghi}	50.75^{defg}	59.33^{abc}
	NP	5°C, 85%	37.19^{j}	40.15^{ij}	43.08^{hi}	54.87^{cd}
		5°C, 90%	37.99^{j}	50.00^{efg}	50.76^{defg}	51.14^{defg}
		10°C, 85%	37.46^{j}	50.26^{efg}	50.78^{defg}	56.57^{bc}
		10 °C, 90 %	38.50^{j}	49.90^{efg}	51.50^{defg}	56.06^{bcd}
		AT, ARH	38.40^{j}	55.05^{cd}	52.70^{def}	59.39^{abc}
Avoshine®	LDPE	5 °C, 85 %	37.19^{j}	37.30^{l}	42.45^{hi}	42.90^{hi}
		5 °C, 90 %	37.99^{j}	39.59^{ij}	40.61^{hij}	40.70^{hij}
		10°C, 85%	37.46^{j}	46.89^{fgh}	49.58^{efg}	50.00^{efg}
		10°C, 90%	38.50^{j}	46.26^{ghi}	48.50^{efgh}	52.24^{def}
		AT, ARH	38.40^{j}	44.72^{ghi}	51.33^{defg}	52.26^{def}
	Bio	5°C, 85%	37.19^{j}	43.20^{hi}	45.32^{ghi}	45.18^{ghi}
		5°C, 90%	37.99^{j}	46.80^{fgh}	48.73^{efgh}	50.50^{defg}
		10°C, 85%	37.46^{j}	43.30^{hi}	53.09^{def}	53.42^{de}
		10°C, 90%	38.50^{j}	52.35^{def}	52.94^{def}	52.12^{def}
		AT, ARH	38.40^{j}	54.82^{cd}	55.61^{bcd}	56.78^{bc}
	NP	5 °C, 85 %	37.19^{j}	45.22^{ghi}	45.23^{ghi}	45.96^{ghi}
		5 °C, 90 %	37.99^{j}	48.78^{efgh}	50.00^{efg}	51.53^{defg}
		10 °C, 85 %	37.46^{j}	52.00^{def}	51.55d^{efg}	53.83^{de}
		10 °C, 90 %	38.50^{j}	46.45^{ghi}	51.06^{defg}	53.81^{de}
		AT, ARH	38.40^{j}	48.53^{efgh}	54.31^{cd}	55.28^{cd}
	LDPE	5°C, 85%	37.19^{j}	41.84^{hij}	43.10^{hi}	50.93^{defg}
		5°C, 90%	37.99^{j}	50.25^{efg}	50.15^{efg}	50.37^{defg}
		10°C, 85%	37.46^{j}	50.85^{defg}	53.50^{de}	55.56^{bcd}
		10°C, 90%	38.50^{j}	48.90^{efgh}	52.98^{def}	57.99^{abc}
		AT, ARH	38.40^{j}	52.58^{def}	53.28^{de}	57.00^{bc}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
NPP	Bio	5°C, 85%	37.19^{j}	39.36^{ij}	48.02^{fgh}	49.75^{efg}
		5°C, 90%	37.99^{j}	47.82^{fgh}	48.08^{fgh}	48.29^{efgh}
		10°C, 85%	37.46^{j}	55.86^{bcd}	59.36^{abc}	60.65^{abc}
		10°C, 90%	38.50^{j}	49.61^{efg}	52.85^{def}	61.38^{ab}
		AT, ARH	38.40^{j}	47.68^{fgh}	50.00^{efg}	58.16^{abc}
	NP	5°C, 85%	37.19^{j}	46.20^{ghi}	50.38^{defg}	52.81^{def}
		5°C, 90%	37.99^{j}	51.60^{defg}	52.93^{def}	53.20^{de}
		10°C, 85%	37.46^{j}	50.25^{efg}	54.08^{cd}	57.90^{abc}
		10°C, 90%	38.50^{j}	47.15^{fgh}	47.62^{fgh}	53.55^{de}
		AT, ARH	38.40^{j}	55.47^{bcd}	59.60^{abc}	62.60^{a}
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		**				
AC		**				
BC		**				
AD		NS				
BD		NS				
CD		**				
ABC		**				
ABD		*				
ACD		*				
BCD		**				
ABCD		NS				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

As the avocado matures and ripens, the water content within the mesocarp of the fruit declines, with an increase in the oil content by a similar amount (Lee *et al*., 1983; Bower and Cutting, 1988; Chen *et al.,* 2009). Lee *et al*. (1983) and Chen *et al*. (2009) observed a close correlation between the percent oil content and percent DM as in the case of the oil content and MC. Therefore, a rise in the oil content is concomitant with the rise in the DM. Similar beneficial results in this experiment, due to the combination effect of Avoshine® and LDPE films, were observed for PWL, respiration rate, firmness and DM. The results obtained for DM indicate that the storage of avocado samples at lower temperatures and higher RH limits the DM accumulation by delaying the ripening process. In addition, the integrated use of Avoshine® wax with LDPE packaging was found to be beneficial in delaying the ripening of avocado samples, as observed in the analysis of the MC in this experiment.

3.11.11 pH value

Table 3.15 presents the pH values of the avocado pulp subjected to different pre-packaging and packaging treatments and storage conditions. The storage conditions and the storage period had a highly significant ($P \leq 0.001$) influence on the avocado pH. A general decline in the pH was observed for all treatments. Avocado samples exposed to low temperature storage exhibited a slower rate in the pH reduction, compared to avocado samples subjected to ambient storage conditions. Storage at (a) 5°C + 85% RH and (b) 5°C + 90% resulted in the lowest decrease in the pH. Pre-packaging and packaging films were found to have a significant ($P \leq 0.05$) effect on the pH of the avocado puree.

Pre-treated avocado samples with Avoshine® wax, or with hot water treatment, exhibited higher pH values than the pH values of avocado control samples. Similarly, packaged avocado samples had higher pH values than unpackaged control avocados. Maftoonazad and Ramaswamy (2008) deduced that wax-coated avocados exhibited higher pH values than the pH value of uncoated avocado samples, with a slower reduction in the pH with the progression of storage time. The study also showed that samples subjected to higher storage temperatures exhibited a faster and greater decrease in the pH value with increasing temperature. Jacobo-Velazquez and Hernandez-Brenes (2011) attributed a decline in the pH to the movement of organic acids from intercellular locations to the avocado matrix. In addition, the increase in acidity could be due to the increased concentration of free fatty acids as a result of trygliceride lipolysis (Jacobo-Velazquez and Hernandez-Brenes, 2011). Maftoonazad and Ramaswamy (2008) attributed the decline in pH value to the utilization of excess organic acids stored within the vacuoles as a respiratory substrate. An increase in acidity can be one of the changes associated with avocado deterioration, which is undesirable (Jacobo-Velazquez and Hernandez-Brenes, 2011).

Table 3.15 The pH of the avocado pulp subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		5°C, 85%	7.00^{a}	6.79^{def}	6.79^{def}	6.69^{fg}
		5°C, 90%	7.21^{a}	6.74^{f}	6.65^{gh}	6.65^{gh}
	LDPE	10°C, 85%	7.05^{a}	6.67^{fgh}	6.59^{i}	6.50^{j}
		10°C, 90%	6.99^{a}	6.60^{i}	6.59^{i}	6.58^{i}
		AT, ARH	7.16^{a}	6.63^{gh}	6.63^{gh}	6.48^{jk}
		5°C, 85%	7.00^{a}	6.77^{ef}	6.74^{f}	6.60^{i}
		5°C, 90%	7.21^{a}	6.77^{ef}	6.74^{f}	6.64^{gh}
HWT	Bio	10°C, 85%	7.05^{a}	6.77^{ef}	6.84^{cd}	6.62^{h}
		10°C, 90%	6.99^{a}	6.65^{gh}	6.58^{i}	6.59^{i}
		AT, ARH	7.16^{a}	6.44^{k}	6.43^{k}	6.41^{kl}
		5°C, 85%	7.00^{a}	6.63^{gh}	6.63^{gh}	6.59^{i}
		5°C, 90%	7.21^{a}	6.71^{fg}	6.61^{hi}	6.63^{gh}
	NP	10°C, 85%	7.05^{a}	6.39^{lm}	6.38^{m}	6.37^{m}
		10°C, 90%	6.99^{a}	6.62^{h}	6.52^{j}	6.51^{j}
		AT, ARH	7.16^{a}	6.85^{cd}	6.80^{def}	6.29^{p}
		5°C, 85%	7.00^{a}	6.89^{bc}	6.82^{de}	6.82^{de}
		5°C, 90%	7.21^{a}	6.77^{ef}	6.71^{fg}	6.73^{f}
	LDPE	10°C, 85%	7.05^{a}	6.77^{ef}	6.76^{ef}	6.65^{gh}
		10°C, 90%	6.99^{a}	6.98^{ab}	6.82^{de}	6.63^{gh}
		AT, ARH	7.16^{a}	6.38^{m}	6.30^{op}	6.46^{jk}
		5°C, 85%	7.00^{a}	6.84cd	6.83de	6.75ef
		5°C, 90%	7.21^{a}	6.75ef	6.74f	6.68fg
Avoshine®	Bio	10°C, 85%	7.05^{a}	6.82de	6.82de	6.62h
		10°C, 90%	6.99^{a}	6.51j	6.64gh	6.56ij
		AT, ARH	7.16^{a}	6.71fg	6.47jk	6.45k
		5°C, 85%	7.00^{a}	6.76^{ef}	6.78^{def}	6.61^{hi}
		5°C, 90%	7.21^{a}	6.71^{fg}	6.69^{fg}	6.67^{fgh}
	NP	10°C, 85%	6.99^{a}	6.44^{k}	6.39^{lm}	6.38^{m}
		10°C, 90%	7.16^{a}	6.57^{ij}	6.59^{i}	6.51^{j}
		AT, ARH	7.05^{a}	6.67^{fgh}	6.70^{fg}	6.32^{o}
		5°C, 85%	7.00^{a}	6.80^{def}	6.73^{f}	6.77^{ef}
		5°C, 90%	7.21^{a}	6.81^{de}	6.80^{def}	6.67^{fgh}
	LDPE	10°C, 85%	7.05^{a}	6.72^{fg}	6.72f^{g}	6.56^{ij}
		10°C, 90%	6.99^{a}	6.63^{gh}	6.61^{hi}	6.55^{ij}
		AT, ARH	7.16^{a}	6.38^{m}	6.35^{n}	6.33^{no}
		5°C, 85%	7.00^{a}	6.93^{abc}	6.95^{abc}	6.73^{f}
		5°C, 90%	7.21^{a}	6.75^{ef}	6.74^{f}	6.67^{fgh}
NPP	Bio	10°C, 85%	7.05^{a}	6.82^{de}	6.76^{ef}	6.55^{ij}

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		10°C, 90%	6.99^{a}	6.67^{fgh}	6.69^{fg}	6.57^{ij}
		AT, ARH	7.16^{a}	6.37^{m}	6.34^{n}	6.29^{p}
	NP	5°C, 85%	7.00^{a}	6.84^{cd}	6.83^{de}	6.67^{fgh}
		5°C, 90%	7.21^{a}	6.79^{def}	6.75^{ef}	6.61^{hi}
		10°C, 85%	7.05^{a}	6.32^{o}	6.28^{pq}	6.28^{pq}
		10°C, 90%	6.99^{a}	6.59^{i}	6.59^{i}	6.53^{j}
		AT, ARH	7.16^{a}	7.00^{a}	6.94^{abc}	6.16^{r}
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		**				
AC		**				
BC		**				
AD		**				
BD		**				
CD		**				
ABC		**				
ABD		**				
ACD		**				
BCD		**				
ABCD		**				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

All two-way, three-way and four-way interactions between pre-packaging, packaging, storage conditions and storage period were found to be highly significant (P≤0.001) with regard to the pH value after 12 days of storage, as indicated in Table 3.15. The greatest decrease in pH of 14.0% was observed for control samples devoid of pre-packaging, packaging and subjected to ambient conditions. In comparison, the treatment combination of Avoshine® wax, LDPE films and storage at 5°C + 85% RH resulted in the least pH reduction of 2.6%. In this experiment, the separation between vacuolar and cytosolic pH was not considered. However, studies by Lange and Kader (1997) indicated that exposure of the avocado fruit to increased concentrations of carbon dioxide resulted in acidification, in which the cytosolic pH experienced a greater reduction than the vacuolar pH. The rationale presented by Lange and Kader (1997) for the intracellular acidification of plant tissue as a result of increased levels of carbon dioxide, could be due to the dissociation of carbonic acid into bicarbonate and hydrogen ions.

The results indicate that low temperature storage is essential in maintaining better quality fruit in terms of decreasing the rate of pH reduction. The combined postharvest treatments of pre-packaging, packaging and low temperature are beneficial, compared to avocado control samples in maintaining or reducing the rate of decrease of pH by hindering the ripening process and the associated biochemical processes, which culminate in a pH reduction. As with the other quality parameters analyzed within the scope of this experiment, avocados coated with Avoshine®, packaged in LDPE and subjected to refrigerated conditions (5°C + 85%) exhibited the least decline in the pH values, which is desirable.

3.11.12 Total soluble solids

Table 3.16 presents the total soluble solids (TSS) of avocados subjected to pre-packaging, packaging and storage conditions over 12 days. The storage conditions and the storage period were found to significantly ($P \leq 0.001$) influence the TSS. A general increasing trend in the TSS was observed for all postharvest treatments, but was most evident at ambient conditions, compared to the refrigerated storage conditions. Similar findings were observed by Tefera *et al*. (2007) and Maftoonazad and Ramaswamy (2008). At low temperature and high RH storage conditions, the rate of increase was slower, compared to storage at ambient conditions. The increased temperature and reduced RH at ambient conditions may have contributed to the increased hydrolysis of carbohydrates stored within the avocados into soluble sugars. This, therefore, resulted in a higher TSS content and a reduced avocado shelf life, which is undesirable.

Packaging was found to be more significant ($P \leq 0.001$) than pre-packaging treatments ($P \leq 0.05$) with regard to the TSS. The TSS content of avocado fruit subjected to (a) HWT and LDPE, (b) HWT and Bio, (c) HWT only, (d) Avoshine® and LDPE, (e) Avoshine® and Bio, (f) Avoshine® only, (g) NP and LDPE, (h) NP and Bio and (i) NP and NPP ranged from (a) 2.45-4.80, (b) 2.45-5.00, (c) 2.45-5.27, (d) 2.45-4.80, (e) 2.45-5.10, (f) 2.45-5.10, (g) 2.45-5.00, (h) 2.45-5.30 and (i) 5.45-5.30 °Brix, respectively, during the storage period. The lower TSS content occurred at the lower temperature of 5°C + 85% or 90% RH and the highest TSS content was observed in avocado samples stored at ambient conditions. Avocados are similar to mangos in that both are characterized as being climacteric. Tefera *et al*. (2007) also observed an increase in the TSS of mangos. However, the rate of increase depends largely on the applied treatments.

The two-way interactions between (a) pre-packaging and the storage conditions, (b) packaging and the storage conditions, (c) packaging and the storage period, and (d) storage conditions and storage period were found to significantly ($P \leq 0.001$) influence the TSS accumulation. Avoshine® coated avocado samples displayed the least increase in the TSS at all storage conditions, compared to hot water treated samples without any pre-packaging treatments. The least rise in the TSS was observed for the 5°C + 90% and 5°C + 85% storage condition. LDPE films were found to be effective in reducing the increase in the TSS, specifically at low temperature storage conditions of 5°C + 90% and 5°C + 85%, as in the case of the Avoshine® pre-packaging treatment. The LDPE packaging is able to create a low vapour pressure deficit within the packaging and the additional benefit of the wax prevents excessive moisture loss from the avocado. The MC is, therefore, higher and as a result the TSS concentration remains low. The low temperature further provides the advantage of slowing down the biochemical processes associated with ripening.

The three-way interactions between (a) pre-packaging, packaging and storage conditions, and (b) packaging, storage conditions and storage period, were found to have a highly significant ($P \leq 0.001$) influence on the TSS content of avocados. The combination treatments of Avoshine®, LDPE and storage at 5°C + 90% resulted in the lowest TSS accumulation of 3.20 °Brix on Day 12, equating to a rise of 25.5%, compared to an increase of 124.5% for control samples stored at ambient conditions.

The four-way interaction between pre-packaging, packaging, storage conditions and the storage period had a significant ($P \leq 0.001$) influence on the TSS of avocados. The results obtained for the avocado TSS content indicated that the integration of pre-packaging (Avoshine®), packaging (LDPE films) and low temperature storage conditions, are effective in reducing the TSS, which may result in an increased shelf life. These results are in agreement with findings by Tefera *et al*. (2007) for mangos, Maftoonazad and Ramaswamy (2008) for avocados and Workneh *et al*. (2011) for carrots.

Table 3.16 The total soluble solids (°Brix) of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
HWT	LDPE	5°C, 85%	2.50^{j}	3.33^{g}	3.40^{g}	3.45^{g}
		5°C, 90%	2.55^{j}	2.80^{hij}	3.19^{h}	3.25^{gh}
		10°C, 85%	2.68^{j}	4.05^{ef}	4.10^{ef}	4.45^{de}
		10°C, 90%	2.57^{j}	3.35^{g}	3.53^{fg}	3.97^{hi}
		AT, ARH	2.45^{j}	3.20^{h}	4.60^{cd}	4.80^{bc}
	Bio	5°C, 85%	2.50^{j}	3.30^{gh}	3.40^{g}	3.50^{fg}
		5°C, 90%	2.55^{j}	3.15^{h}	3.30^{gh}	3.40^{g}
		10°C, 85%	2.68^{j}	4.23^{def}	4.40^{de}	4.60^{cd}
		10°C, 90%	2.57^{j}	2.77^{hij}	4.20^{def}	4.20^{def}
		AT, ARH	2.45^{j}	3.20^{h}	4.20^{def}	5.00^{abc}
	NP	5°C, 85%	2.50^{j}	3.40^{g}	3.55^{fg}	3.65^{f}
		5°C, 90%	2.55^{j}	3.30^{gh}	3.40^{g}	$3.60f^{g}$
		10°C, 85%	2.68^{j}	3.50^{fg}	3.80^{ef}	4.70^{c}
		10°C, 90%	2.57^{j}	2.70^{ij}	4.10^{ef}	4.20^{def}
		AT, ARH	2.45^{j}	3.60^{fg}	4.20^{def}	5.27^{ab}
Avoshine®	LDPE	5°C, 85%	2.50^{j}	2.65^{j}	2.70^{ij}	3.50^{fg}
		5°C, 90%	2.55^{j}	3.10^{hi}	3.17^{h}	3.20^{h}
		10°C, 85%	2.68^{j}	3.35^{g}	3.65^{f}	3.70^{f}
		10°C, 90%	2.57^{j}	2.90^{hi}	3.35^{g}	3.70^{f}
		AT, ARH	2.45^{j}	4.70^{c}	4.80^{bc}	4.80^{bc}
	Bio	5°C, 85%	2.50^{j}	3.43^{g}	3.37^{g}	3.50^{fg}
		5°C, 90%	2.55^{j}	3.10^{hi}	3.17^{h}	3.30^{gh}
		10°C, 85%	2.68^{j}	3.97^{ef}	4.30^{def}	4.40^{de}
		10°C, 90%	2.57^{j}	2.70^{ij}	2.80^{hij}	4.20^{def}
		AT, ARH	2.45j	4.60^{cd}	4.90^{abc}	5.10^{abc}
	NP	5°C, 85%	2.50^{j}	3.45^{g}	3.50^{fg}	3.60^{fg}
		5°C, 90%	2.55^{j}	3.17^{h}	3.20^{h}	3.45^{g}
		10°C, 85%	2.68^{j}	4.40^{de}	4.47^{de}	4.67^{c}
		10°C, 90%	2.57^{j}	3.07^{hi}	3.35^{g}	4.23^{def}
		AT, ARH	2.45^{j}	4.80^{bc}	4.93^{abc}	5.10^{abc}
NPP	LDPE	5°C, 85%	2.50^{j}	3.40^{g}	3.40^{g}	3.60^{fg}
		5°C, 90%	2.55^{j}	3.15^{h}	3.25^{gh}	3.30^{gh}
		10°C, 85%	2.68^{j}	4.35^{def}	4.40^{de}	4.55^{cd}
		10°C, 90%	2.57^{j}	3.90^{ef}	3.90^{ef}	4.00^{ef}
		AT, ARH	2.45^{j}	5.00^{abc}	5.10^{abc}	5.00^{abc}
	Bio	5°C, 85%	2.50^{j}	3.43^{g}	3.53^{fg}	3.60^{fg}
		5°C, 90%	2.55^{j}	3.30^{gh}	3.30^{gh}	3.70^{f}
		10°C, 85%	2.68^{j}	4.55^{cd}	4.75^{bc}	4.80^{bc}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		10°C, 90%	2.57^{j}	4.10^{ef}	4.20^{def}	4.33^{def}
		AT, ARH	2.45^{j}	5.10^{abc}	5.00^{abc}	5.30^{ab}
	NP	5°C, 85%	2.50^{j}	3.17^{h}	3.33^{g}	3.80^{ef}
		5°C, 90%	2.55^{j}	3.40^{g}	4.70^{c}	4.30^{def}
		10°C, 85%	2.68^{j}	4.27^{def}	4.47^{de}	4.87^{bc}
		10°C, 90%	2.57^{j}	4.03^{ef}	4.00^{ef}	4.70^{c}
		AT, ARH	2.45^{j}	4.75^{bc}	5.07^{abc}	5.50^{a}
Significance						
Pre-packaging (A)		*				
Packaging (B)		**				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		**				
BC		**				
AD		*				
BD		**				
CD		**				
ABC		**				
ABD		NS				
ACD		*				
BCD		**				
ABCD		**				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.11.13 Total titratable acidity

According to Table 3.17, the storage conditions and the storage period were found to be the most significant (P≤0.001) factors affecting the avocado TTA. A general increasing trend of the total titratable acidity (TTA) was observed in all avocado samples subjected to pre-packaging, packaging and storage conditions over 12 days of storage. Russo *et al.* (2013) found comparable results with an increase in the TTA of avocados during storage. However, Chitarra and Chitarra (2005; sited by Russo *et al.*, 2013) reported that many fruits exhibit a decrease in the TTA as a result of ripening. This can usually be attributed to the conversion of acid into sugar. Avocado samples subjected to ambient storage conditions exhibited a rapid increase in the TTA over the 12-day storage period, compared to the TTA of avocados stored at controlled temperature and RH conditions.

Pre-packaging and packaging treatments were found to have a significant ($P \leq 0.05$) effect on the avocado TTA. Samples that had been pre-treated with Avoshine® wax and those subjected to hot water treatment showed a slower rise in the TTA when compared to the increase in the TTA of control avocado samples. However, hot water treated samples showed slightly higher TTA at ambient conditions, compared to Avoshine® coated avocado samples. Similarly, packaged samples displayed a reduced rate of increase in TTA, compared to unpackaged avocado samples. Biodegradable packaging proved to be slightly more effective in maintaining the TTA content throughout the storage period. Low temperature controlled storage further reduced the rate of increase, as can be observed in Table 3.17 for 5°C + 85% and 5°C + 90%. An increase of only 21.1% in the TTA was found for each of the treatment combinations of (a) HWT, LDPE and 5°C + 85% and (b) Avoshine®, LDPE, 5°C + 85%, while control samples without any pre-packaging, packaging and stored at ambient conditions exhibited an increase of 350.0% in the TTA content.

These findings compare with the results obtained for pH in Section 3.11.11, in which a reduction in the pH was observed for all treatments and a similar general increase in the TTA was observed for all treatments. The treatment combination of Avoshine®, LDPE, 5°C + 85% resulted in the least reduction in the pH as well as the least rise in the TTA in the current experiment. An increase in the TTA is, therefore, synonymous with a decrease in the pH.

Table 3.17 The total titratable acidity of avocados subjected to pre-packaging, packaging and different storage conditions for a 12-day period

Treatment			Storage Period (Days)			
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**4**	**8**	**12**
		5°C, 85%	0.19^{h}	0.19^{h}	0.25^{fg}	0.25^{fg}
		5°C, 90%	0.17^{h}	0.23^{g}	0.24^{fg}	0.26^{fg}
	LDPE	10°C, 85%	0.19^{h}	0.19^{h}	0.22^{gh}	0.28^{efg}
		10°C, 90%	0.21^{h}	0.25^{fg}	0.26^{fg}	0.29^{efg}
		AT, ARH	0.20^{h}	0.23^{g}	0.34^{ef}	0.50^{cd}
		5°C, 85%	0.19^{h}	0.21^{h}	0.21^{h}	0.23^{g}
		5°C, 90%	0.17^{h}	0.22^{gh}	0.23^{g}	0.23^{g}
HWT	Bio	10°C, 85%	0.19^{h}	0.22^{gh}	0.22^{gh}	0.27^{efg}
		10°C, 90%	0.21^{h}	0.28^{efg}	0.28^{efg}	0.30^{ef}
		AT, ARH	0.20^{h}	0.26^{fg}	0.26^{fg}	0.59^{c}
		5°C, 85%	0.19^{h}	0.22^{gh}	0.28^{efg}	0.31^{ef}
		5°C, 90%	0.17^{h}	0.25^{fg}	0.28^{efg}	0.32^{ef}
	NP	10°C, 85%	0.19^{h}	0.22^{gh}	0.32^{ef}	0.33^{ef}
		10°C, 90%	0.21^{h}	0.22^{gh}	0.31^{ef}	0.35^{ef}
		AT, ARH	0.20^{h}	0.34^{ef}	0.44^{cd}	0.81^{ab}
		5°C, 85%	0.19^{h}	0.25^{fg}	0.26^{fg}	0.28^{efg}
		5°C, 90%	0.17^{h}	0.25^{fg}	0.26^{fg}	0.28^{efg}
	LDPE	10°C, 85%	0.19^{h}	0.21^{h}	0.28^{efg}	0.28^{efg}
		10°C, 90%	0.21^{h}	0.23^{g}	0.25^{fg}	0.28^{efg}
		AT, ARH	0.20^{h}	0.23^{g}	0.28^{efg}	0.51^{cd}
		5°C, 85%	0.19^{h}	0.19^{h}	0.22^{gh}	0.23^{g}
		5°C, 90%	0.17^{h}	0.20^{h}	0.22^{gh}	0.23^{g}
Avoshine®	Bio	10°C, 85%	0.19^{h}	0.25^{fg}	0.27^{efg}	0.32^{ef}
		10°C, 90%	0.21^{h}	0.26^{fg}	0.23^{g}	0.33^{ef}
		AT, ARH	0.20^{h}	0.23^{g}	0.33^{ef}	0.44^{cd}
		5°C, 85%	0.19^{h}	0.19^{h}	0.28^{efg}	0.29^{efg}
		5°C, 90%	0.17^{h}	0.20^{h}	0.24^{fg}	0.24^{fg}
	NP	10°C, 85%	0.19^{h}	0.24^{fg}	0.28^{efg}	0.33^{ef}
		10°C, 90%	0.21^{h}	0.26^{fg}	0.28^{efg}	0.33^{ef}
		AT, ARH	0.20^{h}	0.25^{fg}	0.42^{de}	0.56^{c}
		5°C, 85%	0.19^{h}	0.21^{h}	0.22^{gh}	0.25^{fg}
		5°C, 90%	0.17^{h}	0.22^{gh}	0.26^{fg}	0.28^{efg}
	LDPE	10°C, 85%	0.19^{h}	0.23^{g}	0.26^{fg}	0.31^{ef}
		10°C, 90%	0.21^{h}	0.22^{gh}	0.28^{efg}	0.40^{de}
		AT, ARH	0.20^{h}	0.31^{ef}	0.31^{ef}	0.68^{b}
		5°C, 85%	0.19^{h}	0.28^{efg}	0.28^{efg}	0.28^{efg}
		5°C, 90%	0.17^{h}	0.22^{gh}	0.23^{g}	0.25^{fg}
NPP	Bio	10°C, 85%	0.19^{h}	0.26^{fg}	0.29^{efg}	0.33^{ef}
		10°C, 90%	0.21^{h}	0.30^{ef}	0.34^{ef}	0.40^{de}

Treatment			Storage Period (Days)			
Pre-packaging	Packaging	Storage Conditions	0	4	8	12
		AT, ARH	0.20^{h}	0.23^{g}	0.39^{de}	0.65^{bc}
	NP	5°C, 85%	0.19^{h}	0.20^{h}	0.25^{fg}	0.30^{ef}
		5°C, 90%	0.17^{h}	0.25^{fg}	0.28^{efg}	0.28^{efg}
		10°C, 85%	0.19^{h}	0.20^{h}	0.28^{efg}	0.39^{de}
		10°C, 90%	0.21^{h}	0.26^{fg}	0.33^{ef}	0.42^{de}
		AT, ARH	0.20^{h}	0.28^{efg}	0.39^{de}	0.90^{a}
Significance						
Pre-packaging (A)		*				
Packaging (B)		*				
Storage Condition (C)		**				
Storage Period (D)		**				
AB		NS				
AC		*				
BC		NS				
AD		NS				
BD		NS				
CD		**				
ABC		NS				
ABD		NS				
ACD		NS				
BCD		NS				
ABCD		NS				

NS, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.001$, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.11.14 Subjective quality attributes

The subjective quality attributes of the avocado are presented in Table 3.18. There was a substantial difference in the exterior quality of avocado samples subjected to refrigerated storage conditions and those under ambient storage conditions. The colour change of the skin from a green to purple/black was most pronounced at ambient conditions, due to the excessively higher temperatures, compared to refrigerated storage conditions (5°C + 85%, 5°C + 90%, 10°C + 85%, 10°C + 90%). More so, the control avocado samples devoid of pre-packaging and packaging treatments exhibited the darkest skin colour. In addition to the increased in darkening of the skin, these avocados showed extreme softening.

No decay or mould development was observed on the surface of the avocados. Droplets of moisture began to collect in packaged samples at ambient conditions, indicating a loss in moisture from the avocado, resulting in excessively higher PWL. In general, avocado samples coated with Avoshine® and packaged displayed aesthetically appealing fruit with a glossy exterior, particularly at low temperature storage conditions.

Table 3.18 The descriptive quality attributes of avocados subjected to different postharvest treatments

Treatment			Final state of avocado	Rating
HWT	LDPE	5°C, 85%	Dull exterior, remained firm and green	Very good
		5°C, 90%	Dull exterior, remained firm and green	Very good
		10°C, 85%	Dull exterior, small degree of softening	Good
		10°C, 90%	Dull exterior, small degree of softening	Good
		AT + ARH	Dull exterior, darkening of the skin, slightly soft	Fair
	Bio	5°C, 85%	Dull exterior, remained firm and green	Very good
		5°C, 90%	Dull exterior, remained firm and green	Very good
		10°C, 85%	Dull exterior, small degree of softening	Good
		10°C, 90%	Dull exterior, small degree of softening	Good
		AT + ARH	Dull exterior, darkening of the skin, slightly soft	Fair
	NP	5°C, 85%	Dull exterior, remained firm and green	Very good
		5°C, 90%	Dull exterior, remained firm and green	Very good
		10°C, 85%	Dull exterior, small degree of softening	Good
		10°C, 90%	Dull exterior, small degree of softening	Good
		AT + ARH	Dull exterior, darkening of the skin, slightly soft	Fair
Avoshine®	LDPE	5°C, 85%	Glossy exterior, remained firm and green	Excellent
		5°C, 90%	Glossy exterior, remained firm and green	Excellent
		10°C, 85%	Glossy exterior, remained firm and green	Very good
		10°C, 90%	Glossy exterior, remained firm and green	Very good
		AT + ARH	Glossy exterior, darkening of the skin, slightly soft	Fair
	Bio	5°C, 85%	Glossy exterior, remained firm and green	Excellent
		5°C, 90%	Glossy exterior, remained firm and green	Excellent
		10°C, 85%	Glossy exterior, remained firm and green	Very good
		10°C, 90%	Glossy exterior, remained firm and green	Very good
		AT + ARH	Glossy exterior, darkening of the skin, slightly soft	Fair
	NP	5°C, 85%	Glossy exterior, remained firm and green	Very good
		5°C, 90%	Glossy exterior, remained firm and green	Very good
		10°C, 85%	Glossy exterior, remained firm and green	Very good
		10°C, 90%	Glossy exterior, remained firm and green	Very good
		AT + ARH	Glossy exterior, darkening of the skin, slightly soft	Fair

Treatment			Final state of avocado	Rating
	LDPE	5°C, 85%	Dull exterior, remained firm and green	Good
		5°C, 90%	Dull exterior, remained firm and green	Good
		10°C, 85%	Dull exterior, small degree of softening	Fair
		10°C, 90%	Dull exterior, small degree of softening	Fair
		AT + ARH	Dull exterior, high degree of skin darkening and softening	Poor
	Bio	5°C, 85%	Dull exterior, remained firm and green	Good
NPP		5°C, 90%	Dull exterior, remained firm and green	Good
		10°C, 85%	Dull exterior, small degree of softening	Fair
		10°C, 90%	Dull exterior, small degree of softening	Fair
		AT + ARH	Dull exterior, high degree of skin darkening and softening	Poor
	NP	5°C, 85%	Dull exterior, remained firm and green	Good
		5°C, 90%	Dull exterior, remained firm and green	Good
		10°C, 85%	Dull exterior, small degree of softening	Fair
		10°C, 90%	Dull exterior, small degree of softening	Fair
		AT + ARH	Dull exterior, high degree of skin darkening and softening	Poor

HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; AT, ambient temperature; ARH, ambient relative humidity.

3.12 Conclusion

This experiment revealed that the physical, chemical and subjective sensory quality parameters of the avocado are largely dependent on the postharvest handling, including pre-packaging, packaging and storage conditions and more so the integrated application of these postharvest treatments. The storage conditions and the storage period were consistently found to be the most significant factors in all of the avocado quality parameters that were analysed. The storage temperatures had a greater influence on the changes in quality, compared to the relative humidity. It was found that the lower temperature of 5°C had a positive effect on the quality parameters, which could extend the shelf life, compared to storage under ambient conditions. The higher temperatures and lower relative humidity at ambient conditions accelerated the avocado ripening process, which was most evident in the conversion of the skin colour from green to purple/black and the reduction in firmness, especially in control samples devoid of pre-packaging and packaging. Other changes associated with ripening as a result of ambient conditions were increased PWL, reduced marketability, increased peak in respiration, reduced viscosity with time, the darkening of the pulp puree, reduced MC with a corresponding increase in DM, increased TSS, reduced pH with a synonymous rise in the TTA. The change in the quality parameters of the avocado samples stored at controlled temperature and relative humidity conditions, specifically, at 5°C + 85% and 5°C + 90%, were relatively unchanged during the 12 days of storage. The storage period of 12 days was sufficient to demonstrate the variation in the avocado quality parameters, particularly at ambient conditions. Lower temperatures are often adopted to reduce enzyme activity and, hence, the physiological and biochemical processes that contribute to ripening and senescence. Low temperatures have been proven to reduce enzyme activity, including fruit-ripening enzymes. The effect of relative humidity on the quality parameters was not distinctly different between 85% and 90%.

Pre-packaged samples generally preserved the avocado quality, compared to control avocado samples. However, Avoshine® coated samples displayed better results, compared to hot water treatments in terms of reduced PWL, significantly lower respiration rates and the delayed onset of respiration, a higher percent of marketable fruit, improved skin colour, firmer fruit, improved puree colour, lower TSS and TTA levels. Despite literature indicating the beneficial use of hot water treatments in improving avocado skin colour, in this experiment, hot water treatments may have resulted in external and internal tissue damage. This was evident in the increased PWL, significantly high respiration rates, reduced firmness and darkening of the skin. Packaging maintained the quality parameters of avocado, compared to control unpackaged samples. LDPE films were found to be more beneficial than biodegradable films in producing a higher percent of marketable fruit.

The combined use of pre-packaging, packaging and low temperature storage conditions was found to be beneficial in maintaining the quality of the avocado fruit throughout the storage period. Avoshine® coated samples packaged in LDPE films produced fruit of the highest comparable quality. The combined treatment of Avoshine®, LDPE films and 5°C + 90% resulted in reduced PWL of only 1.94%, compared to 19.23% in control samples. The combination treatment also resulted in 100% marketable avocados throughout the storage period, under both refrigerated and ambient storage conditions. Delaying and reducing the climacteric peak is desirable in extending shelf life, which was achieved by using Avoshine®, LDPE films and 5°C + 85%. The peak respiration was delayed by four days and reduced to 415 $ml.kg^{-1}.h^{-1}$, compared to a peak respiration rate of 2307 $ml.kg^{-1}.h^{-1}$ in control samples. The exterior firmness of the avocado fruit remained relatively unchanged from Day 4 to Day

12 at 5°C + 85%. Storage at 5°C + 90% resulted in the firmest fruit, with a mere 6.0% reduction in firmness. Hot water treated avocados resulted in increased pulp firmness at 5°C + 85%, which could be attributed to cell wall secondary lignification. The change in colour in both the avocado skin and puree was most pronounced in control samples at ambient temperature and relative humidity conditions, with a reduction in the L*, b* and an increase in a* values, indicative of darkening. Storage at refrigerated conditions (5°C + 85% and 5°C + 90%), Avoshine® coated and LDPE packaged avocados remained green throughout the storage period. No significant changes in the avocado puree were observed between different pre-packaging and packaging treatments at refrigerated conditions. However, at ambient storage conditions there was an increase in the viscosity, except for samples treated with Avoshine® and LDPE films. The MC and DM were closely correlated, as an increase in the DM was concomitant with a decrease in the MC. Avocado samples that had been subjected to storage at 5°C + 90% and treated with Avoshine® and LDPE, resulted in a 4.4 % reduction in the MC, compared to a reduction of 39.3% in control samples. Similar findings were obtained for the DM, where control samples resulted in a higher increase of 38.7%, compared to only 6.7% increase in Avoshine® and LDPE treated samples at 5°C + 90%. A reduction in the pH value of the avocado puree could possibly be related to a reduction in the TTA, based on the results. The treatment combination of Avoshine®, LDPE and 5°C + 85% resulted in the least reduction in the pH (2.6%), as well as the least rise in the TTA (21.1%). A reduction in the pH value of the avocado puree could possibly be related to a reduction in the TTA, based on the results. The increase in the TSS of the avocado occurred at a slower rate when subjected to storage at 5°C + 90% and treated with Avoshine® and LDPE (25.5%).

Based on the results obtained, it can be deduced that the integrated application of suitable pre-packaging, packaging and low temperature storage conditions was effective in impeding the ripening process of avocado fruit and extending the shelf life. The combined use of Avoshine® wax coating as a pre-packaging treatment, perforated LDPE films as a packaging technique and storage at either 5°C + 85% or 5°C + 90%, preserved the quality of the avocado, compared to the other treatments investigated within the scope of this experiment.

This investigation compared the combined effect of different postharvest handling techniques and fixed temperature and relative humidity storage conditions for a period of 12 days. To further determine the prolonged effect of refrigerated storage conditions, combined with pre-packaging and packaging on the quality of avocado fruit, Experiment II was designed.

3.13 References

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4. THE EFFECT OF POSTHARVEST HANDLING AND TEMPERATURE-VARYING STORAGE CONDITIONS ON THE AVOCADO QUALITY – EXPERIMENT II

4.1 Abstract

To maintain avocado fruit quality proper integrated postharvest technologies should be required. Thus, the primary aim of this experiment was to evaluate the effects of combined postharvest treatments on the physical, chemical and subjective sensory quality parameters of 'Hass' avocados. The experiment focused on varying temperature over 28 days of storage, to simulate a realistic avocado cold chain in South Africa. A randomised complete block design with pre-packaging (hot water and Avoshine® wax coating), packaging (low density polyethylene (LDPE) and corn starch biodegradable films) and storage conditions (5.5°C ± 0.01°C for two days followed by storage at 5°C ± 0.01°C for six days and 4.5°C ± 0.01°C for 20 days and 95% relative humidity) with three replications, was used. The quality parameters that were evaluated included physiological weight loss (PWL), marketability, skin colour, firmness (exterior and pulp), puree colour, puree viscosity, moisture content (MC), dry matter (DM), pH, total soluble solids (TSS) and total titratable acidity (TTA). Storage conditions and the storage period significantly ($P \leq 0.001$) affected the PWL, marketability, skin colour, firmness (exterior and pulp), puree colour, MC, DM, pH, TSS and TTA. The cold chain conditions offered the greatest benefit in maintaining higher fruit marketability, compared to ambient conditions. Control samples that were subjected to ambient conditions without any pre-packaging or packaging treatments, exhibited increased rates of ripening, which were evident in increased PWL, reduced firmness (exterior and pulp), darkened skin colour, decline in pH, increased TSS and TTA. The combination of Avoshine® coating, LDPE packaging and cold chain conditions was beneficial in maintaining better fruit marketability, MC and DM, indicative of delayed ripening by approximately two weeks. Hot water treatment promoted a darkening of the skin, decreased pulp firmness and lower fruit marketability. The results show that cold storage is essential in improving the shelf life and maintaining the quality of avocado fruit during export. To further improve the quality and shelf life, Avoshine® wax applied to the avocado surface and thereafter packaged in micro-perforated LDPE films, can be employed.

4.2 Introduction

Perishable commodities such as avocados pose a challenge in their supply chain with regard to their qualitative and quantitative perishability. This is of great ethical, environmental and financial concern. The deterioration of perishable commodities, therefore, warrants the complexity of the cold chain management, with great emphasis being placed on controlling and regulating the desired storage conditions (Aiello *et al*., 2011). The avocado fruit (*Persea americana* Mill.) has long been known as a subsistent and marketable fruit and has been traded, both locally and internationally. The South African avocado industry is primarily based on export, making fruit quality an essential factor (Vorster *et al*., 1990; DAFF, 2010). In recent years there has been a deficiency in the quality of South African export avocados, compared to competitors such as Peru and Chile (Nelson, 2012). This creates an undesirable negative perception of the South African avocado quality standards. Due to the distant export markets, proper postharvest handling must be implemented so as to maintain the avocado quality throughout the export process.

Cold chain management is crucial in producing superior quality fruit from the point at which the avocados are harvested, till the final market destination is reached (Blakey and Bower, 2009; DAFF, 2010). Current technologies employ controlled atmosphere storage and/or 1-methylcyclopropene, which act as an ethylene action inhibitor during these extended shipping periods (Kok *et al*., 2010; Nelson, 2010). Blakey and Bower (2009) detected a significant reduction in the quality of avocados represented by unmarketable and shriveled fruit emanating from breaks in the cold chain. This was comparable to studies by Kok *et al*. (2010). This necessitates the implementation of effective postharvest handling and efficient transport systems in South Africa. Fruit softening and chilling injuries are the primary causes related to poor quality avocado fruit (Toerien, 1986; Nelson 2010; Nelson, 2012), inferring inadequate ventilation and an improper temperature regime (Toerien, 1986). Other physiological disorders associated with poor fruit quality are lenticel damage, grey pulp and pathological infections, such as cercospora, sooty mould and pepperspot (Nelson, 2010).

Low temperature is fundamental in extending the shelf life of avocados, by retarding the metabolism through reduced respiration rates, ethylene evolution, softening and colour change (Perez *et al*., 2004). A deviation of 1°C in the holding temperature can adversely affect the avocado quality (Milne, 1998). Therefore, strict adherence to the specified cold chain management regime is crucial, to maintain an acceptable quality of avocados. The application of a step-down temperature regime, which exposes the avocado to a series of temperatures in a decreasing order, has also proven to be beneficial (Milne, 1998). This is often practiced in industry. The interaction of time and temperature is a vital aspect in quality control of avocados (Vorster *et al*., 1990). The use of slightly higher temperatures during the early storage period, and slightly lower temperatures later in the storage period, were found to reduce the onset of physiological disorders in avocados, compared to a constant temperature throughout the storage period (Vorster *et al*., 1990).

The type of packaging that is used also contributes to the final fruit quality, as efficient packaging will allow for cool air to move uniformly around the fruit horizontally and vertically (Dodd *et al*., 2007). Studies have shown that the use of low temperature storage, the application of wax, 1-methylcyclopropene and preventing breaks in the cold chain, were all beneficial in postharvest handling of avocados (Lutge *et al*., 2012).

Based on literature, it is apparent that numerous studies have been conducted in determining the quality of avocados during the supply chain, with a focus on storage temperature. However, a comparison of the integration of pre-packaging, packaging and storage conditions on the physical, chemical and subjective sensory quality of avocados is deficient. The aim of this experiment is, therefore, to investigate the combined effect of postharvest treatments on the quality of avocados and to provide practical recommendations during avocado postharvest handling in South Africa. The specific objective formulated for this experiment was to investigate the effects of pre-packaging treatments, packaging films in combination with an avocado cold chain simulation on the physical, chemical and subjective sensory quality of avocados in South Africa.

4.3 Experimental Design

A factorial design consisting of the same pre-packaging treatments, packaging and replications as Experiment I was used (Section 3.5). However, two temperature and relative humidity (RH) storage conditions (cold chain and ambient) were required in this experiment, which were all arranged in a Randomised Complete Block Design.

4.4 Sample Preparation and Treatments

The same procedure as in Experiment I was used (Section 3.4 and Section 3.6), however, a total of 222 avocados were acquired for Experiment II. A total of 111 avocados were treated and tested per storage regime with three samples tested on Day 0 and 27 samples on Days 7, 14, 21 and 28.

4.5 Pre-packaging Treatments

The same pre-treatment procedure for Experiment I (Section 3.7) was applied to Experiment II. However, the number of fruit that were treated differed due to the different storage conditions. Thirty-six fruit were coated with Avoshine® wax, thirty-six other fruit were hot water treated and thirty-six different fruit were not pre-treated for storage at cold chain conditions. A similar procedure was followed for storage under ambient conditions.

4.6 Packaging Treatments

The same packaging films that were used in Experiment I (Section 3.8) were applied to Experiment II. However, the number of fruit that were packaged differed. Twelve wax-coated fruit, twelve hot water treated fruit and twelve non-treated fruit were each individually placed in the LDPE and biodegradable bags for the cold chain simulation. A similar packaging arrangement was used for storage under ambient conditions. A summary of the different treatment combinations are presented in Table 6.2 in Appendix A.

4.7 Temperature and Relative Humidity

One hundred and eight avocado samples were stored at 5.5°C ± 0.01°C for two days followed by 5°C ± 0.01°C for six days and 4.5°C ± 0.01°C for 20 days and at 95% relative humidity in the CTS Climate Test Chamber. This was to simulate a realistic avocado cold chain. The duration of this experiment was 28 days. Due to the variation in the cold chain temperature, which depends on the time of harvest and cultivar, an appropriate regime was obtained from the Everdon Estate packhouse. This regime coincided with the time at which the avocados were harvested, so as to depict a realistic cold chain. One hundred and eight control fruit were placed in six corrugated cardboard boxes in a single layer and placed in an undisturbed area of the Food Science and Agricultural Engineering laboratory, exposed to ambient temperature (± 25.14°C) and relative humidity (± 52.67%) conditions (Figures 7.3 and 7.4 in Appendix B). This trial represented the control conditions for a duration of 28 days. Two HOBO® data loggers, one placed on the avocado samples and the other placed in close proximity, were used to record the environmental conditions. Once the storage period had concluded, the BoxCar Pro 4.3 software was used to retrieve the environmental data from the data loggers for analysis.

4.8 Data Collection and Analysis

Data was collected on Days 0, 7, 14, 21 and 28 during storage. The differences between treatments were determined in a similar manner as Experiment II (Section 3.10). The quality parameters that were analyzed included the physiological weight loss, percent marketability, skin colour, firmness (exterior and pulp), puree colour, puree viscosity, moisture content, dry matter content, pH, total soluble solids and total titratable acidity. The same data collection and analysis methods as in Experiment I has been utilized in Experiment II (Section 3.10.1 - Section 3.10.12).

4.9 Results and Discussion

This section presents the results and discusses the changes in the physical, chemical and subjective sensory quality parameters of avocado fruit as a result of the treatment combinations.

4.9.1 Physiological weight loss

The physiological weight loss (PWL) of avocados subjected to the different pre-packaging, packaging and storage conditions for various durations are presented in Table 4.1. In this experiment, the storage conditions and the storage period were found to be highly significant ($P \leq 0.001$) with regard to the avocado PWL. The highest PWL was observed in avocados stored under ambient conditions due to the considerably higher temperatures (± 25.14°C) and lower relative humidity (± 52.6%), compared to the cold chain storage conditions. The higher temperature induced a larger vapour pressure deficit between the avocado and the surrounding external environment, thereby creating a driving force for moisture loss from the fruit (Vorster *et al*., 1990; Maftoonazad and Ramaswamy, 2005; Getinet *et al*., 2008; Li *et al*., 2010). The rate at which the moisture was lost by the avocado samples occurred at a faster rate, when subjected to storage under ambient conditions. The loss in moisture consequently contributed to an increase in the PWL. This implies that senescence may occur earlier and, therefore, result in a shorter shelf life. Avocado samples stored at cold chain conditions, remained marketable for the entire duration of the 28 days. However, avocados subjected to ambient storage conditions only remained marketable for 14 days before succumbing to excessive softening and shrivelling as a result of moisture loss, which is one of the factors leading to the PWL.

Pre-packaging and packaging treatments were found to have no significance ($P \leq 0.05$) on the avocado PWL when compared to the storage conditions and storage period. Avocado samples coated with Avoshine® wax had the lowest weight loss, which is in agreement with the findings reported by Bhaskaran *et al*. (2002), Jeong *et al.* (2003) and Maftoonazad and Ramswamy (2008). Similar observations regarding the application of Avoshine® wax were obtained in Experiment I of this study with regard to lower levels of the PWL (Section 3.11.1). Avocado samples treated with hot water only resulted in the highest PWL of 23.87% by Day 14, at ambient conditions. This substantial increase in the PWL can be attributed to heat-induced tissue damage, which has the potential to hasten the ripening process (Lurie, 1998). The use of LDPE and biodegradable films reduced the PWL, compared to the unpackaged avocado samples. This can be due to the increase in relative humidity of air created within the packaging micro-atmosphere leading to a lower vapour pressure deficit, due to the moisture barrier effect of the packaging (Aguila-Mendez *et al*., 2008; Mangaraj *et al*., 2009).

Table 4.1 The physiological weight loss (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

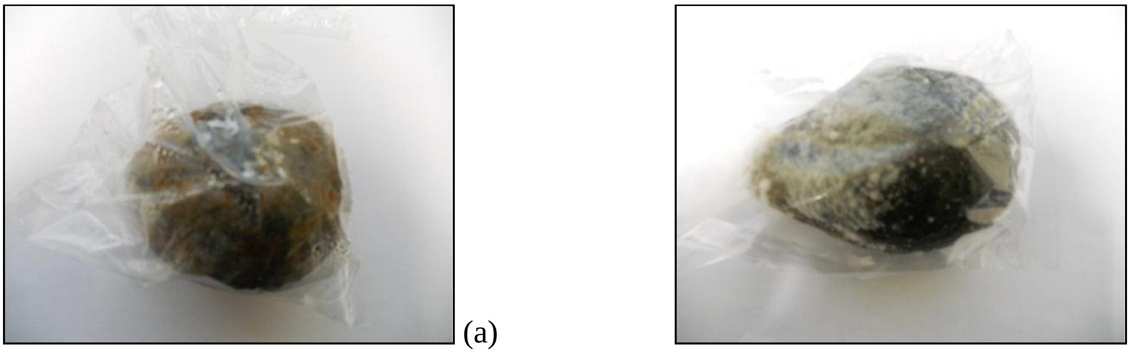
Treatment			**Storage Period (Days)**				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	0^{i}	2.75^{gh}	5.88^{fg}	7.46^{efg}	10.09^{de}
		AT, ARH	0^{i}	2.71^{gh}	3.61^{g}	-	-
	Bio	CC	0^{i}	1.22^{hi}	5.68^{fg}	7.09^{efg}	8.72^{ef}
		AT, ARH	0^{i}	8.25^{ef}	9.39^{def}	-	-
	NP	CC	0^{i}	2.75^{gh}	4.72^{fg}	4.72^{fg}	11.86^{bcd}
		AT, ARH	0^{i}	8.00^{ef}	23.87^{a}	-	-
Avoshine®	LDPE	CC	0^{i}	1.43^{ghi}	4.39^{fg}	4.49^{fg}	6.91^{fg}
		AT, ARH	0^{i}	5.94^{fg}	5.94^{fg}	-	-
	Bio	CC	0^{i}	1.55^{ghi}	2.26^{gh}	4.29^{fg}	5.99^{fg}
		AT, ARH	0^{i}	5.08^{fg}	8.79^{ef}	-	-
	NP	CC	0^{i}	0.97^{hi}	5.54^{fg}	6.34^{fg}	9.01^{ef}
		AT, ARH	0^{i}	7.08^{efg}	11.15^{cd}	-	-
NPP	LDPE	CC	0^{i}	1.59^{ghi}	3.88^{fg}	6.21^{fg}	6.85^{fg}
		AT, ARH	0^{i}	3.15^{g}	4.22^{fg}	-	-
	Bio	CC	0^{i}	5.15^{fg}	5.15^{fg}	6.16^{fg}	6.85^{fg}
		AT, ARH	0^{i}	7.47^{efg}	7.47^{efg}	-	-
	NP	CC	0^{i}	5.17^{fg}	6.39^{fg}	10.92^{d}	14.81^{bc}
		AT, ARH	0^{i}	8.87^{ef}	15.16^{b}	-	-

Significance	
Pre-packaging (A)	NS
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	**
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	NS
BCD	*
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interactions between (a) pre-packaging treatments and storage conditions, (b) packaging treatments and storage conditions, and (c) pre-packaging and storage period were found to be significant at P≤0.05, P≤0.001 and P≤0.05 levels, respectively. Avoshine® coated avocado samples stored under cold chain conditions resulted in the lowest PWL of 5.99% by Day 28. The reason for this is attributed to the coating behaving as a barrier film to moisture transfer to the surroundings. In comparison, control avocado samples devoid of any pre-packaging and/or packaging treatments resulted in the highest PWL for cold chain conditions of 14.81% by Day 28. However, the PWL for control avocado samples stored at ambient conditions was 15.16% at Day 14, which is still greater than the loss experienced by control avocado samples stored under cold chain conditions by Day 28. This indicates that the lower temperature played a vital role in reducing the rate and amount of PWL of avocado fruit by inactivating the enzymes responsible for the ripening process.

The three-way interaction between packaging, storage conditions and storage period were found to have a significant (P≤0.05) effect on the avocado PWL. Avocados packaged in LDPE or biodegradable films and stored at cold chain conditions exhibited the lowest PWL. Avocado samples were unable to endure the full storage duration of 28 days at ambient conditions as previously mentioned. At Day 14, most of the samples had succumbed to mould development, decay and a poor overall appearance, especially packaged avocados samples and had to, therefore, be discarded. This can be attributed to the build-up of heat and moisture within the packaging which created suitable conditions for the proliferation of micro-organisms when stored at relatively higher temperatures as indicated in Figure 4.1.



(a) (b)

Figure 4.1 Mould development, shrivelling and decay of avocados exposed to ambient conditions and packaged in (a) LDPE films, and (b) biodegradable films at 28 days of storage

Getinet *et al*. (2008) reported that a 10% PWL corresponds to the threshold level for the termination of shelf life. The PWL for avocados treated with (a) HWT and Bio, (b) Avoshine® and LDPE, (c) Avoshine® and Bio, (d) Avoshine® and NP, (e) NPP and LDPE, and (f) NPP and Bio remained below 10% for the entire storage duration of 28 days. From the results, it can be deduced that the combined effect of the cold chain conditions, Avoshine® and LDPE or biodegradable films were the most effective treatments in inhibiting excessive PWL throughout the 28-day storage period.

4.9.2 Percent marketability

The storage conditions and the storage period significantly ($P \leq 0.001$) influenced the marketability. All samples were initially at 100% marketability on Day 0 and remained so at cold chain conditions for the entire 28-day storage period for all treatments except for storage at AT and ARH. The preserved marketability can be attributed to the low temperature storage conditions of the cold chain (Getinet *et al*., 2008; Awole *et al*., 2011). A similar trend was obtained for marketability in Experiment I, Section 3.11.3. Table 4.2 illustrates the difference in the percentage of marketable avocados between cold chain and ambient storage conditions, with the cold chain conditions displaying a higher percentage of marketable fruit as desired by consumers and industries.

Marketability drastically decreased at ambient conditions from 100% to 0% by Day 14. Avocado samples subjected to ambient conditions experienced decay, shrivelling and extreme softness, which made the handling of the avocado samples almost impossible and they were, therefore, discarded, resulting in a marketability of 0% on Day 14. The visual appeal of avocados stored in controlled cold chain conditions was found to be higher, compared to those avocados stored under ambient conditions, which is explained further in Section 4.9.13.

Table 4.2 The marketability (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	Packaging	Storage Conditions	0	7	14	21	28
HWT	LDPE	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	33.33[c]	-	-
	Bio	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	0[d]	-	-
	NP	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	0[d]	-	-
Avoshine®	LDPE	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	100[a]	33.33[c]	-	-
	Bio	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	33.33[c]	-	-
	NP	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	33.33[c]	-	-
NPP	LDPE	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	66.67[b]	0[d]	-	-
	Bio	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	33.33[c]	0[d]	-	-
	NP	CC	100[a]	100[a]	100[a]	100[a]	100[a]
		AT, ARH	100[a]	33.33[c]	0[d]	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	NS
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	*
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Pre-packaging was found to have a significant (P≤0.05) effect on the marketability while packaging was found to be insignificant (P>0.05) with regard to storage under ambient conditions. Avocado samples coated with Avoshine® proved to have a glossy and healthy exterior (Johnston and Banks, 1998). In addition, to contributing to the aesthetic appeal, it can be noted that the Avoshine® coated avocados produced a higher percentage of marketable fruit (33%), compared to the hot water treatment and control samples on Day 14 at ambient conditions. The ability of a consumer to select fresh commodities for purchase and consumption is largely based on the physical appeal (Storey *et al*., 1974; Toivonen and Brummell, 2008; Gamble *et al*., 2010). This, therefore, necessitates the need to apply suitable treatments to maintain the quality of avocado fruit. In the analysis of fruit marketability, it can be deduced that the use of pre-packaging treatments such as Avoshine® have been proven to improve the aesthetics of the fruit, which are in agreement to the studies conducted by Maftoonanzad and Ramaswamy (2005; 2008).

4.9.3 Skin colour

The storage conditions and storage period had a significant (P≤0.001) influence on the avocado skin lightness (L*) colour parameter. Table 4.3 shows the change in the lightness of the avocado skin due to different pre-packaging, packaging and storage conditions. A similar trend was observed in Experiment I (Section 3.11.4), where the L* decreased with the progression of storage time. The reduction in the L* was more rapid at ambient conditions, compared to the changes in avocados stored using cold chain conditions. The darkening of the skin colour of 'Hass' is a natural process associated with ripening (Lurie, 1998; Osuna-Garcia *et al*., 2010), hence leading to a decrease in the L* values. Lower temperatures are often adopted to reduce enzyme activity and, hence, the biochemical processes that contribute to ripening such as the degradation of chlorophyll, resulting in the loss of the green colour. The lower temperature regime adopted in the cold chain simulation proved to be advantageous in suppressing the darkening of the skin colour.

Table 4.3 The lightness (L*) of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	SC	35.12[a]	34.58[ab]	33.88[ab]	33.11[bc]	30.29[de]
		AT, ARH	34.90[a]	34.45[ab]	24.23[g]	-	-
	Bio	SC	35.12[a]	34.91[a]	34.84[ab]	33.41[bc]	28.73[de]
		AT, ARH	34.90[a]	30.04[de]	23.81[h]	-	-
	NP	SC	35.12[a]	34.07[ab]	32.40[bc]	32.08[c]	28.96[de]
		AT, ARH	34.90[a]	25.75[efg]	24.07[gh]	-	-
Avoshine®	LDPE	SC	35.12[a]	34.74[ab]	33.75[ab]	33.39[bc]	34.15[ab]
		AT, ARH	34.90[a]	32.28[bc]	24.61[fg]	-	-
	Bio	SC	35.12[a]	32.58[bc]	34.13[ab]	31.77[cd]	32.39[bc]
		AT, ARH	34.90[a]	30.93[de]	24.37[g]	-	-
	NP	SC	35.12[a]	34.13[ab]	33.45[ab]	33.18[bc]	30.98[de]
		AT, ARH	34.90[a]	33.80[ab]	24.56[fg]	-	-
NPP	LDPE	SC	35.12[a]	33.21[bc]	33.20[bc]	31.53[cde]	27.89[de]
		AT, ARH	34.90[a]	27.95[de]	15.69[i]	-	-
	Bio	SC	35.12[a]	31.72[cd]	31.55[cde]	31.34[cde]	26.97[ef]
		AT, ARH	34.90[a]	32.03[c]	8.73[j]	-	-
	NP	SC	35.12[a]	34.01[ab]	33.56[ab]	32.48[bc]	27.19[e]
		AT, ARH	34.90[a]	29.18[de]	8.45[j]	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	NS
AD	*
BD	NS
CD	**
ABC	*
ABD	NS
ACD	*
BCD	*
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Pre-packaging was found to be significant ($P \leq 0.05$) in the lightness of the avocado samples. Avoshine® coated avocado samples showed the lowest intensity of darkening, compared to hot water treated and control avocado samples. The wax created a modified internal environment by increasing the carbon dioxide content within the fruit. This has been associated with preventing chlorophyll degradation. Similar findings were presented by Maftoonazad and Ramaswamy (2005; 2008) and Aguilar-Mendez *et al*. (2008). After Day 14, all avocado samples stored under ambient conditions were terminated from further sampling, as they were affected by mould development, extreme softening and decay.

The two-way interactions between (a) pre-packaging and the storage conditions, and (b) pre-packaging and the storage period were both found to have a significant ($P \leq 0.05$) influence on the avocado lightness. Pre-packaged avocado samples exhibited higher L* values, compared to unpackaged control avocado samples stored under both cold chain and ambient conditions. Higher L* values usually imply that the fruit has not yet undergone the physiological changes associated with the darkening of the avocado skin, which is one of the primary indicators of ripening.

The three-way interactions between (a) pre-packaging, packaging and the storage conditions, (b) pre-packaging, the storage conditions and the storage period, and (c) packaging, the storage conditions and the storage period, were all found to significantly ($P \leq 0.05$) influence the L* colour index. Avoshine® coated avocado samples, packaged in LDPE films and stored at cold chain conditions experienced a decrease of 2.8% in lightness from Day 0 to Day 28. In comparison, control avocado samples devoid of pre-packaging and packaging treatments subjected to cold chain conditions resulted in a 22.6% decrease in lightness by Day 28. The hot water treatment also produced fruit with higher L* values, compared to non-pre-packaged samples. This indicates the useful attributes of the integrated use of pre-packaging and packaging treatments in retarding the onset of ripening, which is evident in the delayed skin colour change from green to purple/black. Despite ambient conditions, resulting in a storage period of just 14 days, control samples still accounted for the highest loss in lightness of 75.8% from Day 0 to Day 14. This indicates the advantage of storing avocados at cold chain conditions, which improves the quality and increases the shelf life, compared to higher temperature storage.

Table 4.4 illustrates the a* colour index of avocados subject to different pre-packaging, packaging and storage conditions. The storage conditions and the storage period were highly significant ($P \leq 0.001$) with regard to the a* colour index of the avocado skin. The storage of avocados at cold chain conditions allowed the samples to remain within storage for the entire 28-day duration, compared to storage at ambient conditions, which could only withstand a 14-day storage period. Ambient conditions accelerated the increase in a* values, resulting in a colour-shift from green toward red, which was most pronounced in control avocado samples. Pre-packaging and packaging were found to be significant ($P \leq 0.05$) on the avocado a* values. Pre-packaging and packaging proved to be more effective in reducing the rate of increase of the a* values, when compared to control samples.

The three-way interaction of pre-packaging, the storage condition and the storage period were found to significantly ($P \leq 0.001$) influence the a* colour index. Avoshine® coated avocado samples stored at cold chain conditions demonstrated a glossy exterior during the 28-day storage period. The increase in the a* value for Avoshine® coated samples, packaged in LDPE films and subjected to cold chain conditions, was 28.0%.

Table 4.4 The a* colour parameter of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	SC	-7.65^{i}	-7.55^{i}	-7.41^{i}	-6.88^{hi}	-1.30^{cde}
		AT, ARH	-7.58^{i}	-1.93^{de}	1.03^{abc}	-	-
	Bio	SC	-7.65^{i}	-7.16^{i}	-5.47^{h}	-4.67^{gh}	-0.48^{cd}
		AT, ARH	-7.58^{i}	-3.03^{f}	0.98^{abc}	-	-
	NP	SC	-7.65^{i}	-6.08^{h}	-5.92^{h}	-4.67^{gh}	1.43^{abc}
		AT, ARH	-7.58^{i}	-3.47^{fg}	1.80^{ab}	-	-
Avoshine®	LDPE	SC	-7.65^{i}	-7.01^{i}	-6.79^{hi}	-5.35^{h}	-5.51^{h}
		AT, ARH	-7.58^{i}	-4.06^{g}	1.15^{abc}	-	-
	Bio	SC	-7.65^{i}	-6.38^{h}	-6.65^{h}	-6.08^{h}	-2.51^{e}
		AT, ARH	-7.58^{i}	-3.30^{f}	0.98^{abc}	-	-
	NP	SC	-7.65^{i}	-7.00^{i}	-6.08^{h}	-6.17^{h}	-2.63^{ef}
		AT, ARH	-7.58^{i}	-6.08^{h}	1.14^{abc}	-	-
NPP	LDPE	SC	-7.65^{i}	-7.26^{i}	-7.02^{i}	-6.27^{h}	1.11^{abc}
		AT, ARH	-7.58^{i}	-5.63^{h}	1.33^{abc}	-	-
	Bio	SC	-7.65^{i}	-6.34^{h}	-6.20^{h}	-5.91^{h}	2.26^{a}
		AT, ARH	-7.58^{i}	2.56^{a}	1.34^{abc}	-	-
	NP	SC	-7.65^{i}	-7.02^{i}	-7.26^{i}	-6.08^{h}	1.91^{ab}
		AT, ARH	-7.58^{i}	0.10^{c}	2.92^{a}	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	**
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Table 4.5 presents the b* values of avocados subjected to different pre-packaging, packaging and storage conditions. As with L* and a*, the storage conditions and the storage period were found to be highly significant ($P \leq 0.001$) with regard to the changes in the b* colour index during the 28-day storage period. As with the lightness, a general decrease in the b* values was observed, especially in avocado samples stored at ambient conditions, compared to changes associated with avocados stored under cold chain conditions. This is indicative of a colour change form yellow to blue. It can therefore, be assumed that ripening occurs at a faster rate at higher storage temperatures than at lower storage temperatures (Maftoonazad and Ramaswamy, 2008). Packaging was found to be of more significance ($P \leq 0.05$) than pre-packaging treatments on the changes of b* values. The packaged avocado samples showed higher b* values by Day 28, compared to the b* values of unpackaged avocado samples.

The three-way interactions between (a) pre-packaging, storage conditions and the storage period, and (b) packaging, storage conditions and the storage period were found to have a significant ($P \leq 0.001$) and ($P \leq 0.05$) effect on the b* colour index, respectively. The four-way interaction between pre-packaging, packaging, the storage conditions and the storage period was also found to be significant ($P \leq 0.05$) with regard to the changes associated in the b* colour index. The combination treatment of Avoshine® and LDPE packaging subjected to cold chain storage conditions resulted in the lowest rate of decrease in b* of 13.8% and was, therefore, favourable in reducing the rate at which the colour change of the skin occurred. Similar results were reported by Bhaskaran *et al*. (2002).

It can be deduced that pre-packaging and/or packaging treatments were more beneficial than no treatments, in retaining the colour of the avocado skin. The effectiveness of the cold chain regime, in combination with Avoshine® and LDPE films, contributed greatly in maintaining the green colour and preventing excessive skin darkening of avocados throughout the storage period.

Table 4.5 The b* colour parameter of the avocado skin subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	Packaging	Storage Conditions	0	7	14	21	28
HWT	LDPE	SC	19.15[a]	19.00[ab]	18.36[bc]	18.14[bc]	10.96[g]
		AT, ARH	19.75[a]	11.22[g]	0.28[n]	-	-
	Bio	SC	19.15[a]	18.51[bc]	16.75[cde]	16.26[cde]	7.58[ij]
		AT, ARH	19.75[a]	10.55[h]	0.35[n]	-	-
	NP	SC	19.15[a]	17.87[c]	16.07[cde]	15.48[de]	7.90[hi]
		AT, ARH	19.75[a]	12.99[fg]	3.72[kl]	-	-
Avoshine®	LDPE	SC	19.15[a]	18.47[bc]	17.02[cd]	15.52[de]	16.50[cde]
		AT, ARH	19.75[a]	12.26[fg]	0.82[n]	-	-
	Bio	SC	19.15[a]	16.00[cde]	16.48[cde]	15.23[de]	13.45[efg]
		AT, ARH	19.75[a]	10.82[g]	16.48[cde]	-	-
	NP	SC	19.15[a]	16.48[cde]	16.00[cde]	14.62[e]	12.04[fg]
		AT, ARH	19.75[a]	15.20[de]	0.80[n]	-	-
NPP	LDPE	SC	19.15[a]	17.66[c]	19.59[a]	17.20[c]	6.71[j]
		AT, ARH	19.75[a]	17.43[c]	0.96[m]	-	-
	Bio	SC	19.15[a]	17.51[c]	16.03[cde]	14.03[ef]	5.42[k]
		AT, ARH	19.75[a]	3.26[l]	1.04[m]	-	-
	NP	SC	19.15[a]	17.43[c]	17.67[c]	17.09[cd]	4.61[kl]
		AT, ARH	19.75[a]	6.11[jk]	1.98[lm]	-	-

Significance	
Pre-packaging (A)	NS
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	**
BCD	*
ABCD	*

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

4.9.4 Exterior firmness (puncture force)

The storage conditions and the storage period were found to be highly significant ($P \leq 0.001$) with regard to the exterior avocado firmness. A decrease in the firmness was observed in avocados subjected to all experimental treatments throughout the 28-day storage period (Table 4.6). However, the reduction in the firmness of avocado samples stored under ambient conditions occurred at a faster rate, compared to cold chain conditions. The increased softening can be attributed to enzyme activity promoting cell wall degradation and a consequential loss in turgidity (Arzate-Vazquez *et al.*, 2011), which occurs at a faster rate at higher temperatures. This diminution in firmness was most pronounced in control avocado samples devoid of pre-packaging and packaging treatments of 92.3% from Day 0 to Day 14. This compares to studies by Aguilar-Mendez *et al*. (2008) and Maftoonazad and Ramaswamy (2008).

Pre-packaging had a higher significance ($P \leq 0.05$), compared to packaging treatments, which was not found to be significant ($P > 0.05$). Avoshine® coated avocado samples were more firm under both ambient and cold chain storage conditions, with the latter producing more firm avocados. Samples without any pre-packaging treatments offered no benefit in terms of firmness and were especially detrimental at higher temperatures (Maftoonazad and Ramaswamy, 2005; 2008). Low oxygen and high carbon dioxide concentrations within the packaging reduces the pectin-esterase and polygalactronase enzyme activities, which are responsible for the depolymerization of pectin substances during ripening, leading to firmer fruit (Maftoonazad and Ramaswamy, 2008).

The two-way interaction between pre-packaging treatments and the storage period was found to be significant ($P \leq 0.05$) with regard to the exterior firmness of avocados. Avocado samples subjected to ambient conditions were only able to remain in storage for two weeks (14 days) before being discarded as a result of mould development and decay, caused by the high temperatures. This contributed to the avocados being unmarketable. The two-way interaction between the storage conditions and the storage period was found to be highly significant ($P \leq 0.001$) on the exterior avocado firmness.

The three-way interaction between pre-packaging, storage conditions and storage period was found to be highly significant ($P \leq 0.001$) with regard to the exterior avocado firmness. The combination of Avoshine® coated samples packaged in LDPE film produced the most firm fruit at cold chain conditions with a reduction of only 16.6% in firmness from Day 0 to Day 28. Control avocado samples stored at cold chain conditions resulted in a 68.0% loss in firmness by Day 28. However, this reduction was found to be low when compared to that encountered by control samples at ambient conditions of 92.3% by Day 14.

It has been suggested that an array of cell wall hydrolytic enzymes such as cellulose, polygalacturonase, xylanase and xylosidase have been associated with cell wall breakdown and ultimate softening (Sakurai and Nevins, 1997). Several studies have indicated that avocado firmness can be correlated with fruit maturity and the expected storage period (Mizrach and Flitsanov, 1999, Flitsanov *et al*., 2000; Mizrach *et al*., 2000). Firmness is an important indicator of avocado ripening (Flitsanov *et al*., 2000, Li *et al*., 2010). The storage temperature and actual storage period have been found to have a profound influence of the firmness of avocados (Flitsanov *et al*., 2000). The declination of firmness is ultimately due to cell wall degradation and reduction in intercellular adhesion, resulting in a loss of cell structure (Aguilar-Mendez *et al*., 2008; Li *et al*., 2010; Arzate-Vazquez *et al*., 2011).

The results obtained for the firmness in this experiment indicated that low temperature storage is essential in reducing softening in avocados. Furthermore, the integrated use of Avoshine® and LDPE films has also proven to be beneficial. Mans *et al*. (1995) found that the different step down temperature regimes had no effect on the ‘Fuerte’ avocado fruit firmness. However, in this experiment, the benefits offered by stepping down the temperature (cold chain regime) greatly surpass storage at ambient conditions for ‘Hass’. Further research can be done to determine the effect of multiple temperature regimes in combination with pre-packaging and packaging treatments on the quality of avocado fruit.

Table 4.6 The firmness (N) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	18.30[a]	17.68[bc]	17.28[c]	15.73[cd]	11.53[gh]
		AT, ARH	18.25[a]	12.22[fg]	2.42[lm]	-	-
	Bio	CC	18.30[a]	17.51[c]	17.25[c]	17.59[c]	9.62[h]
		AT, ARH	18.25[a]	11.42[gh]	4.85[kl]	-	-
	NP	CC	18.30[a]	17.99[ab]	17.66[bc]	15.65[cd]	9.09[hi]
		AT, ARH	18.25[a]	13.72[f]	1.88[m]	-	-
Avoshine®	LDPE	CC	18.30[a]	17.78[abc]	17.84[abc]	16.41[c]	15.26[cde]
		AT, ARH	18.25[a]	13.90[def]	3.37[lm]	-	-
	Bio	CC	18.30[a]	17.71[abc]	16.03[c]	16.84[c]	11.77[g]
		AT, ARH	18.25[a]	14.10[de]	4.89[kl]	-	-
	NP	CC	18.30[a]	17.92[ab]	17.09[c]	15.85[cd]	9.95[h]
		AT, ARH	18.25[a]	15.78[cd]	4.17[l]	-	-
NPP	LDPE	CC	18.30[a]	17.18[c]	17.36[c]	16.94[c]	8.97[hi]
		AT, ARH	18.25[a]	13.77[ef]	7.01[j]	-	-
	Bio	CC	18.30[a]	18.22[a]	16.32[c]	16.54[c]	8.22[hij]
		AT, ARH	18.25[a]	5.77[k]	4.24[l]	-	-
	NP	CC	18.30[a]	18.00[ab]	17.51[c]	17.42[c]	5.85[k]
		AT, ARH	18.25[a]	8.09[ij]	1.40[m]	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	**
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

4.9.5 Pulp firmness (Kramer shear)

Table 4.7 illustrates the firmness of the avocado pulp subjected to different pre-packaging, packaging treatments and storage conditions. As with exterior avocado firmness in Section 4.9.4, the storage conditions and the storage period were found to be highly significant ($P \leq 0.001$) on the pulp firmness by shear. The avocado pulp firmness declined considerably during the 14 days of storage at ambient conditions and had to, thereafter, be discarded due to excessive softening and mould development. This can be attributed to the higher biochemical changes associated with ripening that occur when stored at the higher temperatures under ambient conditions. Pre-packaging and packaging treatments were found to be significant ($P \leq 0.05$) with regard to the avocado pulp firmness. Hot water treated avocado samples and those coated with Avoshine® wax resulted in higher pulp firmness, compared to the control avocado samples. However, Avoshine® coated samples proved to be slightly more beneficial in terms of maintaining the avocado pulp firmness, compared to hot water treatments.

The two-way interaction between pre-packaging treatments and the storage period was found to be significant ($P \leq 0.05$) in terms of the avocado pulp firmness. In Experiment I (Section 3.11.6), avocado samples stored at 5°C and 85% RH, experienced an increase in the firmness during the Kramer shear cell test subjected to all treatments. This increase in firmness was observed between Day 8 and 12, of which Day 12 represented the greatest firmness. A similar trend was also observed in Experiment II, in which an increase in pulp firmness was observed on day 14 for all avocado samples subjected to the different treatments and exposed to cold chain conditions, after which a decrease in the pulp firmness ensued. The increase in the pulp firmness can be attributed to cell wall secondary lignification in the flesh due to the exposure to low temperature which may result in firm flesh, dry pulp, peel adhesion and ultimate deterioration (Li *et al*., 2010). However, an inspection of the pulp revealed a buttery smooth texture and no dryness between Days 14 to 28 for samples stored at cold chain conditions.

Table 4.7 The pulp firmness ($N.g^{-1}$) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			**Storage Period (Days)**				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	180.7^{bc}	161.6^{cd}	211.2^{ab}	194.6^{abc}	42.7^{h}
		AT, ARH	181.9^{bc}	99.36^{f}	1.2^{i}	-	-
	Bio	CC	180.7^{bc}	149.1^{d}	239.5^{a}	205.3^{abc}	16.00^{i}
		AT, ARH	181.9^{bc}	90.30^{fg}	2.1^{i}	-	-
	NP	CC	180.7^{bc}	160.2^{cd}	177.6^{bcd}	165.9^{bcd}	10.9^{i}
		AT, ARH	181.9^{bc}	107.4^{ef}	2.2^{i}	-	-
Avoshine®	LDPE	CC	180.7^{bc}	173.6^{bcd}	210.8^{ab}	156.6^{cd}	153.3^{cd}
		AT, ARH	181.9^{bc}	121.3^{def}	15.34^{i}	-	-
	Bio	CC	180.7^{bc}	174.3^{bcd}	187.1^{abc}	165.4^{bcd}	99.5^{f}
		AT, ARH	181.9^{bc}	106.4^{ef}	3.07^{i}	-	-
	NP	CC	180.7^{bc}	185.3^{abc}	196.4^{abc}	183.3^{abc}	77.4^{fgh}
		AT, ARH	181.9^{bc}	159.3^{cd}	2.0^{i}	-	-
NPP	LDPE	CC	180.7^{bc}	192.6^{abc}	194.3^{abc}	176.5^{bcd}	27.2^{hi}
		AT, ARH	181.9^{bc}	148.8^{d}	0.7^{i}	-	-
	Bio	CC	180.7^{bc}	186.9^{abc}	185.5^{abc}	154.8^{cd}	11.9^{i}
		AT, ARH	181.9^{bc}	2.2^{i}	0^{i}	-	-
	NP	CC	180.7^{bc}	200.6^{abc}	210.7^{ab}	170.7^{bcd}	3.2^{i}
		AT, ARH	181.9^{bc}	49.61^{gh}	0.7^{i}	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	*
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	**
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interaction between pre-packaging treatments, the storage conditions and the storage period significantly ($P \leq 0.001$) influenced the pulp firmness. Avoshine® coated avocado samples, packaged in LDPE films and subjected to cold chain conditions resulted in the lowest rate of firmness reduction of approximately 15.2% from Day 0 to Day 28. These treatments had a synergistic effect in suppressing the physiological activities that promote avocado tissue degradation, resulting in more firm fruit. In comparison, a loss in firmness of 98.2% occurred in control avocado samples without pre-packaging or packaging treatments at cold chain conditions. The results obtained in this experiment for the pulp firmness shows that varying temperature conditions representative of the cold chain conditions are beneficial in preventing soft fruit, which is one of the primary indicators of avocado ripening. The use of Avoshine®, in combination with cold chain conditions further reduced the softening rate, compared to avocados that had not been subjected to either pre-packaging or packaging treatments.

4.9.6 Puree colour

Storage conditions and the storage period were found to be highly significant ($P \leq 0.001$) on the lightness (L*) of the avocado puree colour, as indicated in Table 4.8. A decrease in the lightness (L*) of the avocado puree was observed under all treatments. However, this decrease was most apparent in samples subjected to ambient conditions. Fabi *et al*. (2007) observed a decrease in the lightness of papaya pulp, which is also characterized as being a climacteric fruit like the avocado. Avocado samples subjected to cold chain conditions were capable of being stored for the full 28-day duration, whereas those avocados under ambient conditions were only able to be stored for half the storage period of 14 days. Pre-packaging and packaging treatments were found to be significant on the puree lightness at $P \leq 0.001$ and $P \leq 0.05$ levels, respectively. Hot water treated and Avoshine® coated avocados subjected to cold chain conditions displayed the lighter puree, compared to those without any pre-packaging treatments. Pre-packaging and packaging seemed to have reduced the rate of pulp discolouration and darkening, which is synonymous with the biochemical processes associated with ripening. This is desirable as a reduction in the ripening maintains the quality of the avocado and improves the shelf life. Similar results were obtained for the lightness of the avocado puree in Experiment I, Section 3.11.7.

The two-way interactions between (a) pre-packaging and storage conditions, (b) pre-packaging and storage period, (c) packaging and storage period, and (d) storage conditions and storage period were found to be significant at $P \leq 0.05$, $P \leq 0.05$, $P \leq 0.001$ and $P \leq 0.001$ levels of significance, respectively, on the puree lightness. The pre-packaging and packaging treatments alleviated excessive pulp darkening, which is an indication of better quality maintenance. It is interesting to note that hot water treated samples without any packaging at ambient conditions displayed an increase in the lightness between Day 0 and 7 and thereafter, a decrease between Day 7 and 14. This could be possible as individual avocado samples were used on sampling days and this particular avocado sample may have had a lighter puree colour, compared to other samples.

The three-way interactions between (a) pre-packaging, storage conditions and storage period, and (b) packaging, storage conditions and storage period were found to be highly significant ($P \leq 0.001$) with regard to the avocado puree lightness. Control avocado samples that were not subjected to pre-packaging and packaging treatments and stored under ambient conditions succumbed to the greatest rate of decrease in L* of 30.3% over a 14-day period. Control avocado samples subjected to the same treatment but stored at cold chain conditions resulted in a reduction of 46.9% over a 28-day period. In comparison, the rate of reduction in lightness for control avocado samples at ambient conditions is greater than the reduction, when stored at cold chain conditions. This indicates the advantage of cold chain conditions for the storage of avocados for prolonged periods of time.

Physiological disorders during storage for extended periods or undesirable temperatures can result in discolouration of the flesh, stringy vascular tissue, vascular leaching and a darkening of the outer flesh (Woolf *et al.*, 2005). Avocados stored at cold chain conditions did not display any of these qualities. Zauberman and Jobin-Decor (1995) observed similar results in pulp discolouration and darkening, which became more pronounced at higher temperature. Adams and Brown (2007) have associated the brown discolouration of the avocado mesocarp with increased peroxidase and polyphenol oxidase activities.

Table 4.8 The lightness (L*) of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	67.63^{ab}	67.09^{abc}	66.03^{bc}	65.07^{cd}	65.94^{cd}
		AT, ARH	67.74^{ab}	66.42^{abc}	65.68^{cd}	-	-
	Bio	CC	67.63^{ab}	67.65^{ab}	67.18^{abc}	66.06^{bc}	62.08^{ef}
		AT, ARH	67.74^{ab}	66.41^{abc}	61.71^{f}	-	-
	NP	CC	67.63^{ab}	67.57^{ab}	66.32^{bc}	65.58^{cd}	64.87^{cd}
		AT, ARH	67.74^{ab}	68.69^{a}	67.72^{ab}	-	-
Avoshine®	LDPE	CC	67.63^{ab}	67.02^{abc}	66.93^{abc}	65.35^{cd}	64.18^{cde}
		AT, ARH	67.74^{ab}	65.82^{cd}	63.95^{cde}	-	-
	Bio	CC	67.63^{ab}	66.73^{abc}	65.83^{cd}	65.71^{cd}	64.90^{cd}
		AT, ARH	67.74^{ab}	66.45^{abc}	65.80^{cd}	-	-
	NP	CC	67.63^{ab}	66.93^{abc}	67.13^{abc}	65.68^{cd}	64.68^{cd}
		AT, ARH	67.74^{ab}	67.00^{abc}	62.97^{def}	-	-
NPP	LDPE	CC	67.63^{ab}	64.79^{cd}	65.17^{cd}	65.49^{cd}	64.37^{cd}
		AT, ARH	67.74^{ab}	65.38^{cd}	64.40^{cd}	-	-
	Bio	CC	67.63^{ab}	65.05^{cd}	64.07^{cde}	63.59^{cde}	62.90^{def}
		AT, ARH	67.74^{ab}	64.54^{cd}	52.85^{g}	-	-
	NP	CC	67.63^{ab}	65.60^{cd}	63.56^{cde}	55.04^{g}	35.94^{i}
		AT, ARH	67.74^{ab}	64.67^{cd}	47.21^{h}	-	-

Significance	
Pre-packaging (A)	**
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	NS
AD	*
BD	**
CD	**
ABC	NS
ABD	NS
ACD	**
BCD	**
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Table 4.9 presents the a* colour index of the avocado puree due to different pre-packaging, packaging and storage conditions. Statistical analysis indicated the storage conditions and the storage period to be highly significant ($P \leq 0.001$) with regard to the a* colour index of the avocado puree. Storage under ambient conditions resulted in the greatest increase of a* values, particularly in control avocado samples implying a darkening of the puree. A general increase in the a* colour values, was observed for all avocado samples subjected to the different treatment combinations. This increase is synonymous with a colour conversion from green to red. Pre-packaging treatments were found to be more significant ($P \leq 0.001$) on the a* values, compared to packaging treatments. Hot water and Avoshine® pre-treatments demonstrated lower a* values than control samples. Adams and Brown (2007) have mentioned the use of hot water treatments at 38°C to reduce the ethylene production in avocados, which in effect reduces the biochemical processes responsible for ripening such as the discolouration or darkening of the pulp.

The two-way interactions between (a) pre-packaging and packaging, (b) packaging and storage conditions, (c) pre-packaging and storage period, (d) packaging and storage period, and (e) storage conditions and storage period were found to be highly significant at $P \leq 0.05$, $P \leq 0.001$, $P \leq 0.001$, $P \leq 0.05$ and $P \leq 0.001$ levels of significance, respectively. Avocado samples pre-packaged with either Avoshine® or hot water treatments and packaged in LDPE or biodegradable films and stored under cold chain conditions, exhibited a greener puree colour.

Table 4.9 The a* colour parameter of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	-7.00 m	-6.68 kl	-6.14 j	-4.40 fg	-4.40 fg
		AT, ARH	-7.11 m	-5.95 j	-5.66 j	-	-
	Bio	CC	-7.00 m	-6.80 kl	-6.72 kl	-5.30 ij	-5.30 ij
		AT, ARH	-7.11 m	-5.44 ij	-0.96 c	-	-
	NP	CC	-7.00 m	-6.29 k	-5.14 hi	-5.36 ij	-4.72 h
		AT, ARH	-7.11 m	-5.08 hi	-5.86 j	-	-
Avoshine®	LDPE	CC	-7.00 m	-6.69 kl	-5.55 j	-4.53 gh	-4.46 fg
		AT, ARH	-7.11 m	-5.32 ij	-5.16 hij	-	-
	Bio	CC	-7.00 m	-6.87 lm	-6.25 k	-6.11 j	-6.64 kl
		AT, ARH	-7.11 m	-6.19 jk	-3.01 de	-	-
	NP	CC	-7.00 m	-6.59 kl	-6.18 j	-5.82 j	-5.16 hij
		AT, ARH	-7.11 m	-5.88 j	-2.63 d	-	-
NPP	LDPE	CC	-7.00 m	-6.95 m	-5.30 ij	-5.12 hi	-3.38 def
		AT, ARH	-7.11 m	-5.76 j	-2.33 d	-	-
	Bio	CC	-7.00 m	-5.76 j	-5.62 j	-4.53 gh	-4.53 gh
		AT, ARH	-7.11 m	-5.39 ij	2.95 b	-	-
	NP	CC	-7.00 m	-4.26 efg	-2.63 d	-4.40 fg	-4.40 fg
		AT, ARH	-7.11 m	-4.32 efg	5.32 a	-	-

Significance	
Pre-packaging (A)	**
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	*
AC	NS
BC	**
AD	**
BD	*
CD	**
ABC	**
ABD	**
ACD	**
BCD	**
ABCD	**

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Control avocado samples subjected to ambient storage conditions produced a darker, almost brown pulp colour. Figure 4.2 shows the difference in colour of the avocado puree subjected to different postharvest treatments. The significance of these combinations indicates the advantage offered by pre-packaging and packaging treatments in alleviating excessive pulp darkening.


(a)


(b)

Figure 4.2 Colour of avocado puree treated with Avoshine®, LDPE and stored at cold chain conditions (a), and control stored at ambient conditions (b)

All three-way interactions significantly (P≤0.001) influenced a* values of the avocado puree colour (Table 4.9). Similarly, the four-way interaction between pre-packaging, packaging, storage conditions and storage period was found to be highly significant (P≤0.001) with regard to the a* colour values. Control avocado samples that were not subjected to pre-packaging and packaging treatments and stored under ambient conditions, had higher increase in a* of -7.11 to 5.32 over a 14-day period. In comparison, Avoshine® coated samples packaged in biodegradable bags and stored under cold chain conditions demonstrated the least increase in a* from -7.00 to -6.64 over a 28-day period. The reason for this could be due to the low temperature reducing the enzymatic activity responsible for ripening. In addition the low vapour pressure deficit atmosphere created by the packaging and the partially permeable barrier offered by the wax contribute to a reduction in the ripening, hence less darkening. This indicates the advantage of cold chain conditions, pre-packaging and packaging treatments for storage of avocado fruit for prolonged periods of time in ensuring a greener aesthetically appealing pulp colour.

Table 4.10 illustrates the b* values of the avocado puree subjected to different pre-packaging, packaging and storage conditions for a 28-day storage period. Statistical analysis indicated the storage conditions and the storage period to significantly ($P \leq 0.001$) influence the b* colour values. A general decrease in the b* values for avocados subjected to all treatments occurred throughout the storage period. This decrease in the b* values is synonymous with a colour change from yellow to blue. Avocados treated with (a) HWT and LDPE, (b) HWT and Bio, (c) HWT and NP, (d) Avoshine® and LDPE, (e) Avoshine® and Bio, (f) Avoshine® and NP, and (g) NPP and LDPE demonstrated no significant changes in the b* value under both cold chain and ambient storage conditions after Day 7. Avocado samples stored, using biodegradable films only and control avocado samples devoid of any pre-packaging or packaging, exhibited a substantial decrease in the b* values of 27.2% and 71.1%, respectively. Pre-packaging treatments were found to have a higher significance ($P \leq 0.05$) with regard to the b* values, compared to packaging treatments, which had no significant ($P > 0.05$) influence on b* values during storage.

A decrease in the L* and b* values and an increase in a* values imply a darkening of the flesh, hence ripening. Polyphenol oxidase (PPO) is an enzyme containing copper. In the presence of oxygen, PPO catalyses the oxidation of phenolic substrates in quinones, which are then polymerized to form black, brown or red pigments (Bower and Cutting, 1988; Soliva *et al*., 2001). Organic acids, such as citric, malic, tartaric, phosphoric and ascorbic acid, are added to fresh avocado purees as they are able to inhibit PPO activity by lowering the pH (Soliva *et al*., 2001). Maintaining the internal appearance of the avocado pulp is of importance to both customers and the commercial avocado industry, as it is essentially the pulp that is either consumed either directly, or processed into purees or pastes.

Table 4.10 The b* colour parameter of the avocado puree subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	47.34^{a}	46.09^{ab}	45.65^{ab}	45.01^{ab}	44.17^{ab}
		AT, ARH	46.71^{a}	45.37^{ab}	43.75^{ab}	-	-
	Bio	CC	47.34^{a}	46.56^{ab}	44.70^{ab}	43.03^{ab}	42.88^{ab}
		AT, ARH	46.71^{a}	45.10^{ab}	42.31^{ab}	-	-
	NP	CC	47.34^{a}	47.28^{a}	45.60^{ab}	43.56^{ab}	43.58^{ab}
		AT, ARH	46.71^{a}	43.28^{ab}	43.28^{ab}	-	-
Avoshine®	LDPE	CC	47.34^{a}	49.66^{a}	45.21^{ab}	41.51^{b}	42.19^{ab}
		AT, ARH	46.71^{a}	46.80^{a}	43.64^{ab}	-	-
	Bio	CC	47.34^{a}	46.57^{ab}	46.86^{a}	46.64^{ab}	43.38^{ab}
		AT, ARH	46.71^{a}	46.39^{ab}	45.24^{ab}	-	-
	NP	CC	47.34^{a}	45.54^{ab}	44.25^{ab}	43.39^{ab}	43.08^{ab}
		AT, ARH	46.71^{a}	45.84^{ab}	45.15^{ab}	-	-
NPP	LDPE	CC	47.34^{a}	44.62^{ab}	46.36^{ab}	42.56^{ab}	42.67^{ab}
		AT, ARH	46.71^{a}	44.27^{ab}	41.84^{ab}	-	-
	Bio	CC	47.34^{a}	44.06^{ab}	45.81^{ab}	43.46^{ab}	42.37^{ab}
		AT, ARH	46.71^{a}	42.58^{ab}	34.02^{c}	-	-
	NP	CC	47.34^{a}	45.31^{ab}	41.95^{ab}	41.93^{ab}	41.84^{ab}
		AT, ARH	46.71^{a}	46.26^{ab}	13.51^{d}	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	NS
BD	NS
CD	NS
ABC	NS
ABD	NS
ACD	NS
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

4.9.7 Puree viscosity

The viscosity of avocado purees was evaluated based on the postharvest treatments that were applied, as indicated in Table 4.11. The storage conditions and the storage period were found to have a significant ($P \leq 0.05$) and highly significant ($P \leq 0.001$) effect on the avocado puree viscosity, respectively. A substantial increase in the viscosity was observed for avocado samples subjected to ambient storage conditions between Days 7 and 14. Storage at cold chain conditions resulted in no change in the viscosity for 21 days of storage for all treatments. A similar trend was observed in Experiment I (Section 3.11.8). This could be due to the lower temperature suppressing the enzyme activity responsible for cell wall degradation as a result of ripening.

Pre-packaging and packaging treatments were found to have a significant ($P \leq 0.05$ and $P \leq 0.001$) effect on the avocado puree viscosity, respectively. Avoshine® coated avocado samples showed the lowest change in viscosity, compared to hot water treated and control samples. Control avocado samples exhibited the highest increase in viscosity. Packaged avocado samples also proved to have smaller changes in the viscosity, compared to unpackaged samples. It has been found that changes in the viscosity and elasticity of avocado tissue are a result of fruit ripening (Sakurai and Nevins, 1997). A decrease in the elasticity of the cell walls was observed during the ripening of avocados, mediated by endo-type hydrolytic enzymes in the breakdown of xyloglucan molecules (Sakurai and Nevins, 1997). This decrease in the elasticity could account for the increase in the viscosity of the puree avocado pulp as result of ripening. This, therefore, indicates the benefit offered by pre-packaging and packaging treatments in reducing the rate of ripening, which is evident in a fairly unchanging viscosity of the avocado puree.

All two-way and three-way interactions were found to not be significant ($P > 0.05$), as presented in Table 4.11. However, the four-way interaction between pre-packaging, packaging, storage conditions and storage period had a significant ($P \leq 0.05$) influence on the puree viscosity. Control avocado samples devoid of pre-packaging, packaging and exposed to ambient storage conditions contributed to the greatest increase in the viscosity from 0.03 Pa.s on Day 0 to 7.11 Pa.s by Day 14. The combination treatment of Avoshine® wax and LDPE films with storage at cold chain conditions maintained the viscosity throughout the storage period.

Table 4.11 The puree viscosity (Pa.s) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	0.02^{m}	0.03^{m}	0.03^{m}	0.153^{m}	3.71^{efg}
		AT, ARH	0.03^{m}	0.05^{m}	2.29^{jk}	-	-
	Bio	CC	0.02^{m}	0.06^{m}	0.10^{m}	0.06^{m}	4.68^{cd}
		AT, ARH	0.03^{m}	1.27^{l}	2.66^{ij}	-	-
	NP	CC	0.02^{m}	0.06^{m}	0.02^{m}	0.04^{m}	3.06^{h}
		AT, ARH	0.03^{m}	4.91^{cd}	6.08^{b}	-	-
Avoshine®	LDPE	CC	0.02^{m}	0.06^{m}	0.04^{m}	0.06^{m}	0.02^{m}
		AT, ARH	0.03^{m}	0.01^{m}	2.50^{jk}	-	-
	Bio	CC	0.02^{m}	0.06^{m}	0.05^{m}	0.03^{m}	3.50^{gh}
		AT, ARH	0.03^{m}	0.02^{m}	2.63^{ij}	-	-
	NP	CC	0.02^{m}	0.08^{m}	0.04^{m}	0.04^{m}	2.70^{hi}
		AT, ARH	0.03^{m}	0.03^{m}	4.09^{def}	-	-
NPP	LDPE	CC	0.02^{m}	0.03^{m}	0.04^{m}	0.03^{m}	4.40^{cde}
		AT, ARH	0.03^{m}	0.09^{m}	2.01^{kl}	-	-
	Bio	CC	0.02^{m}	0.03^{m}	0.04^{m}	0.06^{m}	3.62^{fgh}
		AT, ARH	0.03^{m}	3.01^{hi}	2.52^{jk}	-	-
	NP	CC	0.02^{m}	0.06^{m}	0.08^{m}	0.06^{m}	5.15^{cd}
		AT, ARH	0.03^{m}	2.37^{jk}	7.11^{a}	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	**
Storage Condition (C)	*
Storage Period (D)	**
AB	NS
AC	NS
BC	NS
AD	NS
BD	NS
CD	NS
ABC	NS
ABD	NS
ACD	NS
BCD	NS
ABCD	*

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

Unripened avocados have been proven to have high moisture content according to Table 4.12. Therefore, when passing the unripened puree though the muslin, the fluid was more easily separated from the solid components of the puree, which could also be attributed to propectin. Propectin has the ability to hold the cells together resulting in larger particles, as opposed to the ripe avocado puree, which had a smooth buttery texture. A higher viscosity is, therefore, synonymous with fruit ripening. Sanchez *et al*. (2009) stated that unripened white guava had a greater amount of propectin, compared to ripened guava. It can be implied that the rapid increase in viscosity at ambient conditions is as a result of ripening. The results presented in this experiment indicated the benefit obtained from using pre-packaging (Avoshine®) and packaging (LDPE) treatments in conjunction with low temperature storage techniques in preserving the quality and delaying the onset of ripening of the avocado fruit for a prolonged storage period.

4.9.8 Moisture content

Table 4.12 displays the MC of avocados subjected to different pre-packaging, packaging and storage conditions. In this experiment, the influence of storage conditions and storage period were found to be highly significant ($P \leq 0.001$) on the changes in the avocado moisture content (MC). The MC was observed to decrease in both ambient and cold chain conditions. The loss in moisture was more apparent at ambient conditions, compared to the loss in avocado moisture when stored at cold chain conditions. Avocado samples at ambient conditions were unable to endure the higher temperatures and were, therefore, discarded after 14 days of storage, as the higher temperatures contribute to a greater loss in moisture (Ozdemir and Topuz, 2004). Higher temperatures contribute to a greater loss in MC (Ozdemir and Topuz, 2004). The higher temperatures promote excessive respiration, which results in the loss of moisture as a by-product. This in turn accelerates the fruit ripening leading to a shorter shelf life. Packaging films were found to be significant ($P \leq 0.05$), with LDPE and biodegradable packaged avocados having greater MC than unpackaged samples at both cold chain conditions and ambient conditions. Under cold chain conditions, the avocado packaged in LDPE had a slightly higher MC, compared to biodegradable films. The packaging creates a low vapour pressure deficit between the avocado sample and the surroundings due to the build-up of moisture initially released from the fruit. This atmosphere is then capable of reducing the moisture loss from the avocado, which is desirable.

Table 4.12 The moisture content (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
	LDPE	CC	54.05^{a}	52.88^{cd}	51.00^{ef}	48.91^{f}	48.18^{gh}
		AT, ARH	55.00^{a}	52.24^{de}	48.02^{gh}	-	-
HWT	Bio	CC	54.05^{a}	53.58^{ab}	50.00^{f}	48.09^{gh}	46.04^{hij}
		AT, ARH	55.00^{a}	51.00^{ef}	48.51^{fg}	-	-
	NP	CC	54.05^{a}	53.10^{abc}	51.15^{ef}	48.28^{gh}	45.40^{j}
		AT, ARH	55.00^{a}	47.02^{h}	43.78^{lm}	-	-
	LDPE	CC	54.05^{a}	54.80^{a}	50.12^{f}	49.85^{f}	48.92^{f}
		AT, ARH	55.00^{a}	50.74^{ef}	47.74^{gh}	-	-
Avoshine®	Bio	CC	54.05^{a}	50.75^{ef}	50.81^{ef}	49.24^{f}	47.79^{gh}
		AT, ARH	55.00^{a}	49.55^{f}	49.23^{f}	-	-
	NP	CC	54.05^{a}	51.62^{ef}	49.02^{f}	48.51^{fg}	45.23^{jk}
		AT, ARH	55.00^{a}	50.83^{ef}	47.21^{gh}	-	-
	LDPE	CC	54.05^{a}	51.04^{ef}	48.47^{fg}	48.48^{fg}	45.74^{ij}
		AT, ARH	55.00^{a}	48.59^{fg}	45.00^{kl}	-	-
NPP	Bio	CC	54.05^{a}	50.43^{f}	49.77^{f}	48.57^{fg}	45.79^{ij}
		AT, ARH	55.00^{a}	51.47^{ef}	48.67^{fg}	-	-
	NP	CC	54.05^{a}	46.76^{h}	46.42^{h}	41.79^{no}	39.00^{op}
		AT, ARH	55.00^{a}	52.48^{cde}	42.00^{mn}	-	-

Significance	
Pre-packaging (A)	NS
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	NS
AD	NS
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	NS
BCD	*
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interaction between pre-packaging treatments and the storage condition significantly ($P \leq 0.05$) influenced the avocado MC. Samples that had been pre-packaged had a higher MC, compared to control samples without any pre-packaging. Control avocado samples devoid of pre-packaging and packaging treatments resulted in the highest MC loss of 27.8% on Day 28 and 23.6% on Day 14, respectively, at both cold chain and ambient storage conditions.

The three-way interaction involving packaging films, the storage conditions and the storage period were found to be significant ($P \leq 0.05$) on the avocado MC. The lowest reduction in the MC throughout the storage period of 9.5% at Day 28 was recorded for avocado samples pre-treated with Avoshine® wax, packaged in LDPE films and stored at cold chain conditions. The results obtained for the MC analysis emphasises the beneficial use of pre-packaging and packaging in reducing the MC, with the added advantage of low temperature storage. Hofman and Jobin-Decor (1999), Ozdemir and Topuz (2004), Landahl *et al*. (2009) and Obenland *et al*. (2012) have found the MC, dry matter and oil content of avocado fruit to be well correlated. A delay in the loss of the MC, therefore, implies a delay in the accumulation of oil and dry matter within the avocado, hence a delay in the ripening process.

4.9.9 Dry matter

The storage conditions and the storage period were found to be significant ($P \leq 0.001$) on the avocado DM (Table 4.13). The percentage DM in avocado samples increased in both ambient and cold chain storage conditions. Due to the close correlation between the MC and DM on a percentage basis, a reduction in the MC, as a result of the increased vapour pressure deficit at ambient conditions, will result in an increase in the DM by a similar amount. An increase in the DM has been observed with the progression of avocado ripening (Hofman and Jobin-Decor, 1999). Hofman and Jobin-Decor (1999) found that the storage of avocados at lower relative humidities of 40% and 60%, as opposed to 80% and 98%, resulted in an increase in the DM content by approximately 1% towards the end of the storage period at 22°C. An increase in dry matter is synonymous with avocado fruit maturation and the ripening process (Zauberman and Jobin-Decor, 1995). These findings are in agreement with the results of this experiment, as the relative humidity (RH) of the cold chain conditions was higher (95%) than at ambient conditions (± 52.67%). Packaging films were found to be significant ($P \leq 0.05$) with LDPE and biodegradable packaged avocados having lower DM than unpackaged avocado samples, at both cold chain conditions and ambient conditions.

The two-way interaction between pre-packaging treatments and the storage conditions was found to be significant at a $P \leq 0.05$ level of significance. Pre-packaged avocados displayed a lower DM, compared to control avocado samples without any pre-packaging. Control avocado samples devoid of any pre-packaging and packaging treatments resulted in the highest DM accumulation at both cold chain and ambient storage conditions of 32.8% at Day 28 and 28.9% at Day 14, respectively. A similar trend is observed for the avocado MC in Section 4.9.8.

The three-way interaction involving packaging films, the storage conditions and the storage period were found to be significant ($P \leq 0.05$) on the DM. The least increase in the DM throughout the storage period of 11.2% at Day 28, was observed for samples pre-treated with Avoshine® wax, packaged in LDPE films and stored at cold chain conditions. These results demonstrate the advantage of pre-packaging and packaging treatments, in combination with cold chain conditions for avocado DM. No distinct relation could be found between the effect of varying temperatures and relative humidity on the percent DM and oil contents of avocados during storage, thus motivating research to be conducted in this field.

Table 4.13 The dry matter (%) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	Packaging	Storage Conditions	0	7	14	21	28
HWT	LDPE	CC	45.95^{no}	47.12^{kl}	49.00^{ij}	51.09^{i}	51.82^{gh}
		AT, ARH	45.00^{no}	47.76^{jk}	51.98^{gh}	-	-
	Bio	CC	45.95^{no}	46.42^{mn}	50.00^{i}	51.91^{gh}	53.96^{efg}
		AT, ARH	45.00^{no}	49.00^{ij}	51.49^{hi}	-	-
	NP	CC	45.95^{no}	46.90^{lm}	48.85^{ij}	51.72^{gh}	54.60^{e}
		AT, ARH	45.00^{no}	52.98^{g}	56.22^{bc}	-	-
Avoshine®	LDPE	CC	45.95^{no}	45.20^{no}	49.88^{i}	50.15^{i}	51.08^{i}
		AT, ARH	45.00^{no}	49.26^{ij}	52.26^{gh}	-	-
	Bio	CC	45.95^{no}	49.25^{ij}	49.19^{ij}	50.76^{i}	52.21^{gh}
		AT, ARH	45.00^{no}	50.45^{i}	50.77^{i}	-	-
	NP	CC	45.95^{no}	48.38^{ij}	50.98^{i}	$51.49h^{i}$	54.77^{de}
		AT, ARH	45.00^{no}	47.52^{jkl}	52.79^{gh}	-	-
NPP	LDPE	CC	45.95^{no}	48.96^{ij}	51.53^{hi}	51.51^{hi}	54.26^{ef}
		AT, ARH	45.00^{no}	51.41^{hi}	53.58^{fg}	-	-
	Bio	CC	45.95^{no}	49.57^{i}	50.23^{i}	51.43^{hi}	54.21^{ef}
		AT, ARH	45.00^{no}	48.53^{ij}	51.33^{hi}	-	-
	NP	CC	45.95^{no}	53.24^{g}	55.00^{cd}	58.21^{ab}	61.00^{a}
		AT, ARH	45.00^{no}	49.17^{ij}	58.00^{abc}	-	-

Significance	
Pre-packaging (A)	NS
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	NS
AC	*
BC	NS
AD	NS
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	NS
BCD	*
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

4.9.10 pH value

Storage conditions and storage period significantly ($P \leq 0.001$) influenced the avocado pH value (Table 4.14). A general decline in the pH value was observed for avocado samples subjected to the different postharvest treatments, specifically those stored under ambient conditions, which is in agreement with the results of Experiment I (Section 3.11.11). The trend in the pH values of the avocados are also in agreement with the findings of Maftoonazad and Ramswamy (2008) and Jacobo-Velazquez and Hernandez-Brenes (2011). Pre-packaging treatments had a higher significance ($P \leq 0.05$) than packaging films on the avocado pH values. Avoshine® coated avocado samples had higher pH values when stored under both cold chain and ambient conditions. Jacobo-Velazquez and Hernandez-Brenes (2011) reported the decline in the pH value to be attributed to the movement of organic acids from intercellular locations to the avocado puree. In addition, the increase in acidity could be due to the increase in concentration of free fatty acids as a result of trygliceride lipolysis (Jacobo-Velazquez and Hernandez-Brenes, 2011). An increase in the acidity can be one of the changes associated with avocado deterioration. The rate at which the organic acids are liberated into the avocado puree can be affected by the moisture content, micro-viscosity and storage temperature (Jacobo-Velazquez and Hernandez-Brenes, 2011).

The two-way interactions between (a) pre-packaging and packaging treatments, and (b) packaging and the storage conditions were found to be significant ($P \leq 0.05$). It would be expected that avocado samples devoid of pre-packaging and packaging treatments display the lowest pH values, which is in agreement with the results of Experiment I. However, hot water pre-treated samples, packaged in biodegradable films and stored under ambient conditions, exhibited a decrease in the pH value over a 14-day storage period by 10.8%, compared to a decrease by 9.9% in control avocado samples. The lower pH values of hot water treated avocado samples can possibly be attributed to tissue damage. The pH values of hot water treated samples without packaging and stored under cold chain conditions fluctuated during the 28-day storage.

Table 4.14 The pH of the avocado pulp subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	6.74[a]	6.60[cde]	6.58[de]	6.55[e]	6.53[fg]
		AT, ARH	6.76[a]	6.50[gh]	6.44[hi]	-	-
	Bio	CC	6.74[a]	6.68[c]	6.62[cde]	6.59[cde]	6.46[h]
		AT, ARH	6.76[a]	6.52[g]	6.03[k]	-	-
	NP	CC	6.74[a]	6.55[e]	6.58[de]	6.54[ef]	6.58[de]
		AT, ARH	6.76[a]	6.60[cde]	6.19[j]	-	-
Avoshine®	LDPE	CC	6.74[a]	6.73[ab]	6.66[c]	6.61[cde]	6.58[de]
		AT, ARH	6.76[a]	6.76[a]	6.59[cde]	-	-
	Bio	CC	6.74[a]	6.72[abc]	6.71[abc]	6.69[bc]	6.65[c]
		AT, ARH	6.76[a]	6.65[c]	6.52[g]	-	-
	NP	CC	6.74[a]	6.67[c]	6.69[bc]	6.66[c]	6.61[cde]
		AT, ARH	6.76[a]	6.69[bc]	6.61[cde]	-	-
NPP	LDPE	CC	6.74[a]	6.63[cd]	6.58[de]	6.57[de]	6.53[fg]
		AT, ARH	6.76[a]	6.73[ab]	6.12[jk]	-	-
	Bio	CC	6.74[a]	6.63[cd]	6.65[c]	6.63[cd]	6.60[cde]
		AT, ARH	6.76[a]	6.53[fg]	6.41[i]	-	-
	NP	CC	6.74[a]	6.63[cd]	6.61[cde]	6.58[de]	6.58[de]
		AT, ARH	6.76[a]	6.61[cde]	6.09[jk]	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	NS
Storage Condition (C)	**
Storage Period (D)	**
AB	*
AC	NS
BC	*
AD	NS
BD	NS
CD	**
ABC	*
ABD	*
ACD	NS
BCD	NS
ABCD	*

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The three-way interactions of (a) pre-packaging, packaging and storage conditions, and (b) pre-packaging, packaging and storage period were significant ($P \leq 0.05$) with regard to the avocado pH values. The four-way interaction of pre-packaging, packaging, storage conditions and storage period was significant ($P \leq 0.05$). Avoshine® coated avocados packaged in biodegradable films and subjected to cold chain storage conditions, displayed the lowest rate of reduction of 1.3% in the pH value from Day 0 to Day 28. This could be attributed to the lower temperature inactivating the enzymes responsible for ripening and senescence, thereby maintaining the pH level. Cold storage proved to be highly beneficial for avocados. Avoshine® coated samples displayed the least reduction in the pH at both cold chain and ambient conditions. The use of hot water treatments appeared to have reduced the pH more than in the control samples, which is not desirable. The results clearly indicated that the integrated use of pre-packaging, packaging and cold chain conditions were effective in preserving the avocado pH for a prolonged storage period of 28 days.

4.9.11 Total soluble solids

Table 4.15 displays the avocado total soluble solids (TSS), based on the different pre-packaging, packaging and storage conditions. The storage conditions and the storage period significantly ($P \leq 0.001$) affected the TSS of avocados stored for 28 days. A general increase in the TSS with an increase in the storage duration was observed in avocados subjected to the different treatments throughout the storage period. The increase in the TSS occurred at a faster rate at ambient conditions, compared to cold chain storage conditions. The increased temperature and reduced RH under ambient storage conditions may have contributed to the increased hydrolysis of carbohydrates stored within the avocado fruit into soluble sugars. This results in a higher TSS and a reduction in the avocado shelf life, which is undesirable. Packaging treatments displayed a higher significance ($P \leq 0.05$), compared to pre-packaging on the avocado TSS. Packaged avocado samples, generally demonstrated lower TSS values, compared to unpackaged control samples at both cold chain conditions and ambient conditions. This is in agreement with the Tefera *et al.* (2007) and Workneh *et al*. (2011).

Table 4.15 The total soluble solids (°Brix) of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	2.90^{h}	3.20^{g}	3.27^{fg}	3.20^{g}	4.20^{bcd}
		AT, ARH	2.95^{h}	3.07^{gh}	4.40^{bc}	-	-
	Bio	CC	2.90^{h}	2.95^{h}	3.00^{h}	3.10^{gh}	3.87^{d}
		AT, ARH	2.95^{h}	3.07^{gh}	4.30^{bc}	-	-
	NP	CC	2.90^{h}	3.47^{ef}	3.80^{de}	3.80^{de}	4.47^{bc}
		AT, ARH	2.95^{h}	4.70^{ab}	5.00^{a}	-	-
Avoshine®	LDPE	CC	2.90^{h}	3.00^{h}	3.27^{fg}	3.30^{efg}	3.45^{ef}
		AT, ARH	2.95^{h}	3.10^{gh}	3.95^{d}	-	-
	Bio	CC	2.90^{h}	3.13^{gh}	3.53^{ef}	3.40^{efg}	3.70^{e}
		AT, ARH	2.95^{h}	3.10^{gh}	4.57^{abc}	-	-
	NP	CC	2.90^{h}	3.13^{gh}	3.27^{fg}	3.30^{efg}	3.90^{d}
		AT, ARH	2.95^{h}	2.63^{hi}	$4.30b^{c}$	-	-
NPP	LDPE	CC	2.90^{h}	3.20^{g}	3.45^{ef}	3.50^{ef}	3.50^{ef}
		AT, ARH	2.95^{h}	2.90^{h}	3.70^{e}	-	-
	Bio	CC	2.90^{h}	3.13^{gh}	3.30^{efg}	3.37^{efg}	3.83^{de}
		AT, ARH	2.95^{h}	4.15^{cd}	4.00^{d}	-	-
	NP	CC	2.90^{h}	3.00^{h}	3.10^{gh}	3.27^{fg}	4.30^{bc}
		AT, ARH	2.95^{h}	3.67^{e}	4.80^{ab}	-	-

Significance	
Pre-packaging (A)	NS
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	**
AC	NS
BC	NS
AD	NS
BD	NS
CD	**
ABC	NS
ABD	NS
ACD	NS
BCD	NS
ABCD	NS

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interaction between pre-packaging and packaging treatments significantly (P≤0.001) influenced the TSS of avocados during the storage period. Hot water treated samples devoid of packaging and subjected to ambient conditions demonstrated the highest TSS value of 5.00 °Brix on Day 14, equivalent to an increase of 69.5%. Control avocado samples without any pre-packaging and packaging under ambient conditions exhibited an increase in the TSS of 62.7% from Day 0 to Day 14. Avocados treated with Avoshine®, LDPE and control storage conditions displayed the lowest rate of increase in the TSS of only 19.0% from Day 0 to Day 28. Tefera *et al*. (2007) attributed the increase in TSS of mangos to desiccation. By referring to Table 4.1, which illustrates the changes in the PWL, it can be observed that the greatest PWL was encountered on Day 14 for samples treated with hot water only at ambient conditions. Similarly the highest TSS level was encountered at the same time for the same treatment combination. This is in agreement with the findings of Workneh *et al*. (2011).

4.9.12 Total titratable acidity

Table 4.16 presents the avocado total titratable acidity (TTA) subject to different pre-packaging, packaging and storage conditions. The storage conditions and the storage period were highly significant (P≤0.001) in terms of the avocado TTA. A general increase in the TTA was observed for all treatment conditions. The increase in the TTA was higher under ambient conditions over a 14-day storage period when compared to cold chain conditions over a 28-day storage period. Maftoonazad and Ramaswamy (2008) observed a similar trend of a more rapid rise in the TTA of avocados stored at higher temperatures. This could be due to the higher temperatures promoting the biochemical production of acids within the fruit. Comparable results were found by Russo *et al*. (2013) with a rise in the avocado TTA during storage. However, Chitarra and Chitarra (2005; sited by Russo *et al*., 2013) reported that many fruit exhibit a decrease in the TTA as a result of ripening, which can usually be attributed to the conversion of acid into sugar. Pre-packaging and packaging were found to have a significant (P≤0.05) influence on the avocado TTA. Pre-package avocados displayed lower TTA values, compared to non-pre-packaged samples. Similarly packaged samples demonstrated lower TTA values than unpackaged samples.

Table 4.16 The total titratable acidity of avocados subjected to pre-packaging, packaging and different storage conditions for a 28-day period

Treatment			Storage Period (Days)				
Pre-packaging	**Packaging**	**Storage Conditions**	**0**	**7**	**14**	**21**	**28**
HWT	LDPE	CC	0.19^{k}	0.36^{hi}	0.39^{h}	0.37^{h}	0.50^{def}
		AT, ARH	0.17^{k}	0.49^{ef}	0.84^{bc}	-	-
	Bio	CC	0.19^{k}	0.31^{hij}	0.31^{hij}	0.23^{jk}	0.23^{jk}
		AT, ARH	0.17^{k}	0.38^{h}	0.81^{bcd}	-	-
	NP	CC	0.19^{k}	0.34^{hi}	0.41^{gh}	0.36^{hi}	0.42^{fgh}
		AT, ARH	0.17^{k}	0.20^{k}	0.84^{bc}	-	-
Avoshine®	LDPE	CC	0.19^{k}	0.31^{hij}	0.32^{hij}	0.34^{hi}	0.37^{h}
		AT, ARH	0.17^{k}	$0.14k^{l}$	0.51^{de}	-	-
	Bio	CC	0.19^{k}	0.34^{hi}	0.34^{hi}	0.44^{fg}	0.70^{bcd}
		AT, ARH	0.17^{k}	0.19^{k}	0.62^{d}	-	-
	NP	CC	0.19^{k}	0.31^{hij}	0.36^{hi}	0.44^{fg}	0.58^{d}
		AT, ARH	0.17^{k}	0.58^{d}	0.65^{cd}	-	-
NPP	LDPE	CC	0.19^{k}	0.22^{jk}	0.30^{hij}	0.44^{fg}	0.62^{d}
		AT, ARH	0.17^{k}	0.45^{fg}	0.53^{de}	-	-
	Bio	CC	0.19^{k}	0.26^{ij}	0.26^{ij}	0.87^{b}	0.81^{bcd}
		AT, ARH	0.17^{k}	0.45^{fg}	0.43^{fg}	-	-
	NP	CC	0.19^{k}	0.25^{j}	0.27^{ij}	0.84^{bc}	1.15^{a}
		AT, ARH	0.17^{k}	0.50^{def}	0.75^{bcd}	-	-

Significance	
Pre-packaging (A)	*
Packaging (B)	*
Storage Condition (C)	**
Storage Period (D)	**
AB	**
AC	**
BC	*
AD	**
BD	*
CD	**
ABC	*
ABD	*
ACD	**
BCD	*
ABCD	**

NS, *, **, - Non-significant or significant at P≤0.05 or significant at P≤0.001 or discarded, respectively. Means within a column followed by the same letter/s are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05). HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

The two-way interaction between (a) pre-packaging and packaging, (b) pre-packaging and storage conditions, (c) pre-packaging and storage period, and (d) storage condition and storage period were found to have a significant ($P \leq 0.001$) effect on the avocado TTA, as indicated in Table 4.16. The increase in TTA corresponds to a decrease in the pH (Section 4.9.10). The lowest increase in the TTA from 0.19 to 0.37 occurred in samples coated with Avoshine®, packaged in LDPE film and stored at cold chain conditions for a period of 28 days. In contrast, the highest rates of increase in the TTA from 0.19 to 1.15 and from 0.17 to 0.75 were observed for control samples stored at cold chain and ambient storage conditions, respectively. This, therefore, illustrates the benefit of pre-packaging and packaging treatments in maintaining the quality of avocado fruit. The four-way interaction between pre-packaging, packaging, storage conditions and the storage period was found to significantly ($P \leq 0.001$) influence the avocado TSS.

4.9.13 Subjective quality attributes

The subjective quality attributes of the avocado are presented in Table 4.17. Avocado samples stored at ambient condition succumbed to more mould development, specifically those that had been packaged. Droplets of moisture began to collect in packaged samples at ambient conditions, indicating a loss in moisture from the avocado, which resulted in excessively higher PWL. The micro-environment within the packaging was conducive for the proliferation of mould, due to the higher temperature and RH. The visual comparison between avocado samples stored under ambient conditions and cold chain conditions revealed a higher percentage of marketable fruit at the lower temperature, which is also evident in Table 4.2. The change in appearance of the skin from a green to purple/black was most pronounced at ambient conditions due to the excessively higher temperatures. Control avocado samples devoid of pre-packaging and packaging treatments exhibited the darkest skin colour. In addition to the increase in darkening of the skin, these avocados showed extreme softening. Avocado samples at ambient conditions were discarded after 14 days of storage due to excessive softening and decay. In general, avocado samples coated with Avoshine® and packaged in LDPE films displayed aesthetically appealing fruit, with a glossy exterior, particularly at cold chain conditions, and were able to remain in storage for the complete 28-day period. The mould development for samples stored at ambient conditions was more evident in samples that had been packaged. This can be attributed to the build-up of moisture within the film at ambient conditions leading to the proliferation of bacteria. Despite hot water treatments being used for the elimination of superficial bacteria, Avoshine® coated samples demonstrated a lower degree of mould formation from visual observations.

Table 4.17 The descriptive quality attributes of avocados subjected to different postharvest treatments

Treatment			Final state of avocado	Rating
HWT	LDPE	CC	Dull exterior, remained firm and green	Good
		AT, ARH	Dull exterior, soft, darkening of the skin, mould development, shrivelling	Poor
	Bio	CC	Dull exterior, remained firm and green	Good
		AT, ARH	Dull exterior, soft, darkening of the skin, mould development, shrivelling	Poor
	NP	CC	Dull exterior, remained firm and green	Fair
		AT, ARH	Dull exterior, soft, darkening of the skin, high degree of shrivelling	Poor
Avoshine®	LDPE	CC	Shiny exterior, slight softening and darkening of the skin	Excellent
		AT, ARH	Shiny exterior, softening and darkening of the skin, slight mould development	Fair
	Bio	CC	Shiny exterior, remained firm and green	Excellent
		AT, ARH	Shiny exterior, softening and darkening of the skin, slight mould development	Fair
	NP	CC	Shiny exterior, slight softening and darkening of the skin	Very good
		AT, ARH	Shiny exterior, softening and darkening of the skin, slight mould development	Poor
NPP	LDPE	CC	Dull exterior, slight softening and skin darkening	Fair
		AT, ARH	Dull exterior, excessive softening, darkening of the skin, mould development, shrivelling, condensation within packaging	Poor
	Bio	CC	Dull exterior, slight softening and skin darkening	Fair
		AT, ARH	Dull exterior, excessive softening, darkening of the skin, mould development, shrivelling, condensation within packaging	Poor
	NP	CC	Dull exterior, softening and skin darkening	Poor
		AT, ARH	Dull exterior, most excessive softening, darkening of the skin, mould development, shrivelling	Very bad

HWT, hot water treatment; NPP, no pre-packaging; Bio, biodegradable corn starch packaging; LDPE, low density polyethylene packaging; NP, no packaging; CC, cold chain; AT, ambient temperature; ARH, ambient relative humidity.

4.10 Conclusion

The storage conditions and the storage period were found to have the greatest influence on most of the avocado quality parameters analyzed within the scope of this experiment. The simulation of a realistic cold chain, incorporating stepping down the temperature from 5.5°C to 4.5°C over a 28-day period, has proven to preserve the postharvest quality of avocados, compared to storage at ambient conditions. The lower temperature was instrumental in reducing the PWL, increasing the quality of marketable fruit, reducing the rate of skin darkening, producing firmer fruit, reducing the rate of increase in DM and reduction in MC, lowering the viscosity, reducing the rate of pH reduction and the rate of increase in the TSS and TTA. It was also evident that avocado samples subjected to the low temperature storage conditions remained in storage for the entire 28-day duration. In comparison, avocado samples subjected to ambient conditions succumbed to mould development, excessive softening, shrivelling and ultimate decay after 14 days of storage. Avocado samples packaged in either LDPE or biodegradable films exhibited a higher degree of mould development at ambient conditions, due to the build-up of moisture within the packaging films, which created an environment conducive for microbial growth. A build-up of moisture was evident in the formation of droplets of water within the packaging. LDPE and biodegradable films were found to improve the quality of the avocados at cold chain conditions, compared to control samples, resulting in reduced PWL, higher MC, lower DM and lower TSS. These results indicate slower ripening, which led to an extended shelf life by up to two weeks, compared to at ambient conditions.

Avoshine® coated avocados demonstrated superior qualities, compared to hot water treated and control avocado samples. Avoshine® coated avocados appeared aesthetically more appealing, with a glossy exterior, lower PWL, a higher percent of marketable fruit, firmer fruit, minimal variation in the puree colour, higher pH and a lower TTA. Hot water treatments were not found to be as effective, but rather resulted in an excessive PWL of 23.87% at ambient conditions.

Similarly, in Experiment I, the combined use of pre-packaging, packaging and low temperature storage conditions were instrumental in delaying the ripening process and extending the shelf life. The greatest benefit was observed in combining Avoshine®, LDPE and cold chain conditions. This treatment combination resulted in a reduction in the PWL by

6.91%, compared to 15.16% in control avocado samples. The marketability was improved, with 100% of avocado fruit still marketable on Day 7, compared to other treatments. The conversion of the skin colour from green to purple/black was hastened under ambient conditions, with control samples exhibiting the most darkening. The exterior firmness was reduced by 16.6%, compared to a substantial reduction of 92.3% in control samples. An increase in the pulp firmness was recorded in avocado samples subjected to cold chain storage conditions, which could be attributed to cell wall secondary lignification. However, Avoshine® coated and LDPE treated avocado samples resulted in the least softening of 15.2%, compared to 98.2% in control avocado samples. A decrease in the MC was accompanied by an increase in the DM. Once again, the combination of Avoshine® coated and LDPE at cold chain storage conditions resulted in the least change in these quality parameters by 9.5% and 11.2%, respectively. Changes in the quality of pureed avocados were also improved with the combination of Avoshine®, LDPE and cold chain conditions. The puree viscosity remained relatively unchanged with lower discolouration. The lowest reduction in the pH of 1.3% was observed under this treatment, which can be associated with the lowest increase in the TTA under the same treatment. The lowest increase of 19.0% in the TSS was also observed for Avoshine® coated, LDPE and cold chain treatment. It can, therefore, be deduced that the combined use of Avoshine® coating, LDPE films and cold chain storage conditions is beneficial in preserving the postharvest quality of avocados, by delaying the ripening process and consequently extending the shelf life of avocados.

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5. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The beneficial effects of the integrated use of pre-packaging, packaging and low temperature storage was evident in both Experiment I and Experiment II. Avoshine® pre-packaged avocado fruit, in combination with LDPE film packaging at 5°C + 85% RH or 5°C + 90% RH, demonstrated superior quality in Experiment I. The following changes in the quality parameters of avocados subjected to the treatment combination of Avoshine®, LDPE film packaging and stored at 5°C + 85% RH or 5°C + 90% RH were found:

(a) lowest PWL of 1.94%,
(b) fruit marketability was maintained at 100%,
(c) delayed and lowest respiration peak (415 $ml.kg^{-1}.h^{-1}$),
(d) skin colour remained green,
(e) lowest firmness reduction of 6.0%,
(f) increased pulp firmness, which could be attributed to cell wall secondary lignification in the flesh,
(g) least pulp softening of 6.5%,
(h) reduced darkening of the avocado puree colour,
(i) no significant changes in the viscosity of the avocado puree,
(j) lowest loss in moisture of 4.4%,
(k) lowest increase in the DM of 6.7%,
(l) lowest decline in the pH value of 2.6%,
(m) lowest increase in the TSS of 25.5%,
(n) lowest increase in the TTA of 21.1%, and
(o) superior visual quality and aesthetic appeal.

The findings from the abovementioned experiment confirms that the integrated use of Avoshine®, LDPE films and the storage conditions of 5°C + 85% RH or 5°C + 90% RH resulted in the best postharvest quality of the avocado fruit and, therefore, a possibility for commercialization.

The following changes were found in the quality parameters of avocados subjected to the treatment combination of Avoshine®, LDPE film packaging and stored under cold chain conditions (5.5°C ± 0.01°C for two days, 5°C ± 0.01°C for six days and 4.5°C ± 0.01°C for 20 days and 95% relative humidity):

(a) lowest PWL of 6.91%,
(b) highest percentage of marketability,
(c) delayed conversion of skin colour change from green to purple/black,
(d) lowest reduction of firmness by 16.6%,
(e) increased pulp firmness (similar to Experiment I). However, by Day 28 the avocado pulp still remained firm (only 15.2% firmness reduction),
(f) reduced darkening of the avocado puree colour,
(g) no significant changes in the viscosity of the avocado puree,
(h) lowest reduction in moisture by 9.5%,
(i) lowest increase in the DM by 11.2%,
(j) lowest decline in the pH value of 1.3%,
(k) lowest increase in the TSS of 19.0%,
(l) lowest increase in the TTA, and
(m) superior visual quality and aesthetical appeal.

The findings from the abovementioned experiment confirms that the integrated use of Avoshine®, LDPE films and the cold chain storage conditions resulted in the best postharvest quality of the avocado fruit and, therefore, a possibility for commercialization.

Hot water treatments appeared to have hastened the ripening process in both experiments, which was evident in an increased respiration rate, skin darkening and increased PWL. The prolonged exposure of avocados to ambient conditions was found to be detrimental to the postharvest quality. The increased temperatures hastened the ripening process and resulted in increased proliferation of micro-organisms, as exhibited by avocados in Experiment II. Packaged avocados at ambient conditions showed the greatest development of micro-organisms, due to the increased RH within the packaging films as a result of fruit respiration. Ultimately, low temperature storage should be used to preserve the fruit quality, prevent deterioration and extend the shelf life.

A lower temperature is able to slow down the various biochemical processes responsible for the ripening of avocados. The greater the vapour pressure deficit between the fruit and the surrounding environment, the greater the amount of moisture that is lost by the fruit. This in turn, leads to increase PWL, reduced firmness and inferior marketability. The results obtained demonstrated the beneficial application of suitable integrated pre-packaging, packaging and storage conditions. It can, therefore, be deduced that the combined use of Avoshine® coating, LDPE film and low temperature storage conditions are beneficial in preserving the postharvest quality of avocados by delaying the ripening process and consequently, extending the avocado shelf life.

5.1 Future Research

There was an immense amount of data that had been collected for this study. The analysis focused mainly on evaluating the effect of the different pre-packaging, packaging and storage conditions on the postharvest avocado quality. However, this data can be used for further research into PhD to develop an avocado ripening model and to determine the correlation between the quality parameters under the different treatment combinations.

The incorporation of pre-harvest and harvesting techniques to the postharvest handling of avocados could be of interest to provide a holistic approach for a more comprehensive quality evaluation. This study focused predominantly on laboratory analysis. However, avocados are subject to breaks in the cold chain, which can compromise the final fruit quality. Therefore, a more practical analysis is required, involving the precise targeting of critical points at which breaks in the cold chain occur and determining suitable techniques to mitigate the losses that may be incurred. Research delving into more technologically advanced pre-packaging and packaging techniques could also prove to be beneficial for the South African Avocado Industry. In addition an investigative comparison based on multiple temperature-varying storage conditions could also be of significance in order to obtain the most suitable regime, which can be commercialized.

6. APPENDIX A: EXPERIMENTAL DESIGN

Table 6.1 Summary of treatments for Experiment I

Cultivar	Pre-packaging	Packaging	Temperature	Relative Humidity
‘Hass’	Hot Water Treatment	LDPE	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
		Biodegradable	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
		Control	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
	Avoshine®	LDPE	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
		Biodegradable	5 °C	85%
				90%
			10°C	85%
				90%
			AT	ARH
		Control	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
	Control	LDPE	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH
		Biodegradable	5 °C	85%
				90%
			10 °C	85%
				90%

Cultivar	Pre-packaging	Packaging	Temperature	Relative Humidity
			AT	ARH
		Control	5 °C	85%
				90%
			10 °C	85%
				90%
			AT	ARH

Table 6.2 Summary of treatments for Experiment II

Cultivar	Pre-packaging	Packaging	Temperature Regime
'Hass'	Hot Water Treatment	LDPE	Cold chain
			AT+ARH
		Biodegradable	Cold chain
			AT+ARH
		Control	Cold chain
			AT+ARH
	Avoshine®	LDPE	Cold chain
			AT+ARH
		Biodegradable	Cold chain
			AT+ARH
		Control	Cold chain
			AT+ARH
	Control	LDPE	Cold chain
			AT+ARH
		Biodegradable	Cold chain
			AT+ARH
		Control	Cold chain
			AT+ARH

7. APPENDIX B: AMBIENT TEMPERATURE AND RELATIVE HUMIDITY

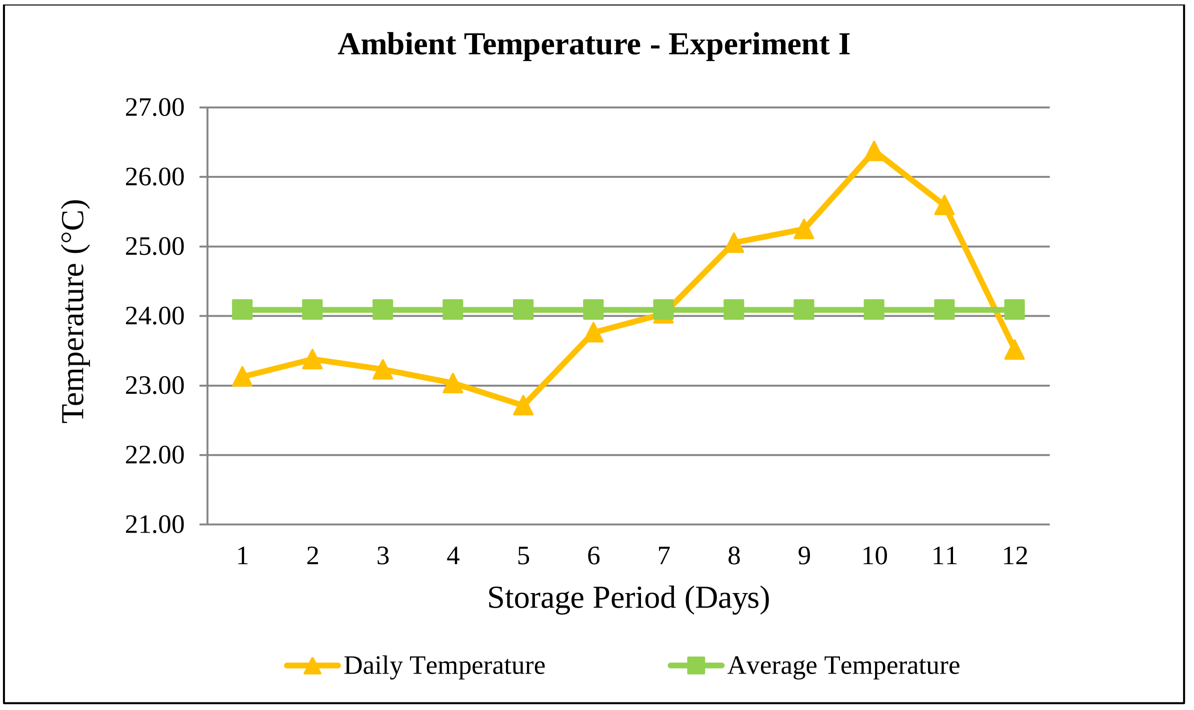

Figure 7.1 Ambient temperature fluctuation for Experiment I

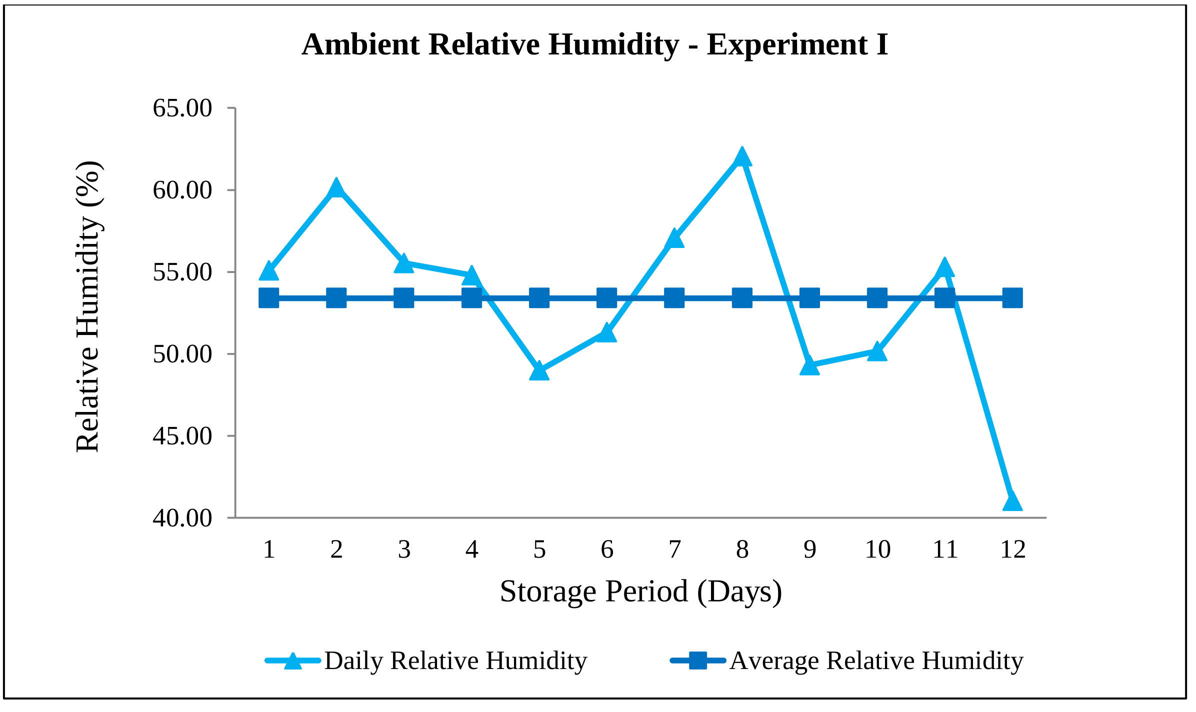

Figure 7.2 Ambient relative humidity fluctuation for Experiment I

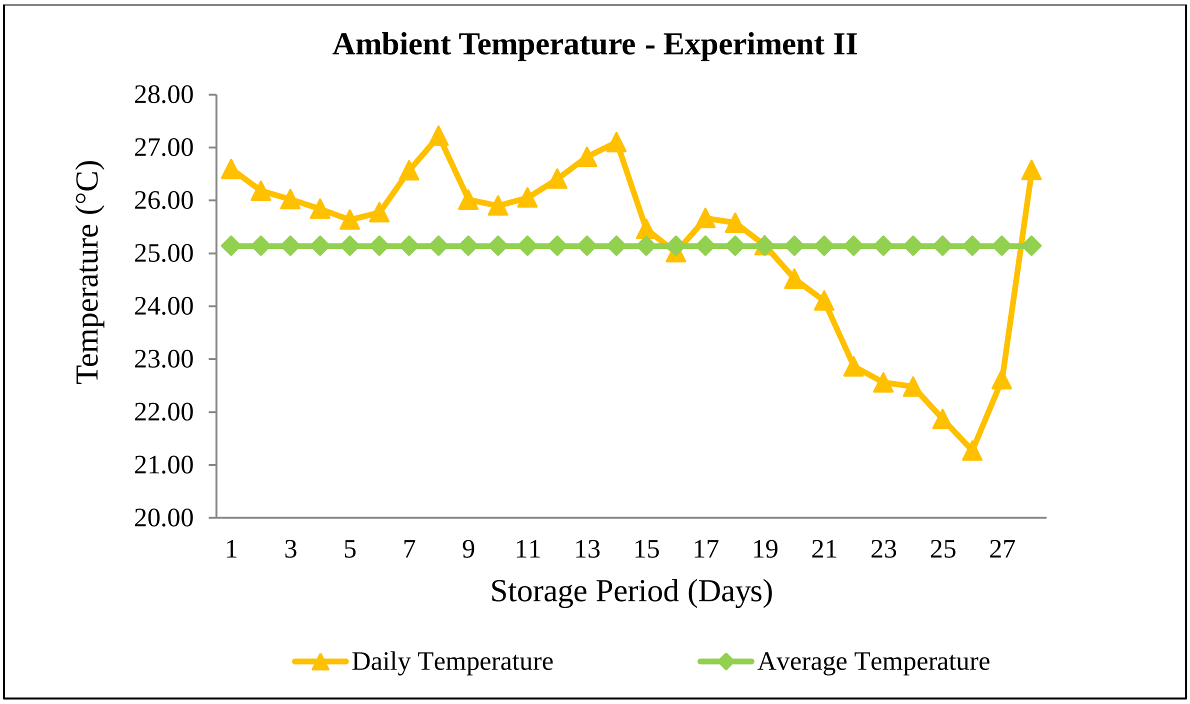

Figure 7.3 Ambient temperature fluctuation for Experiment II

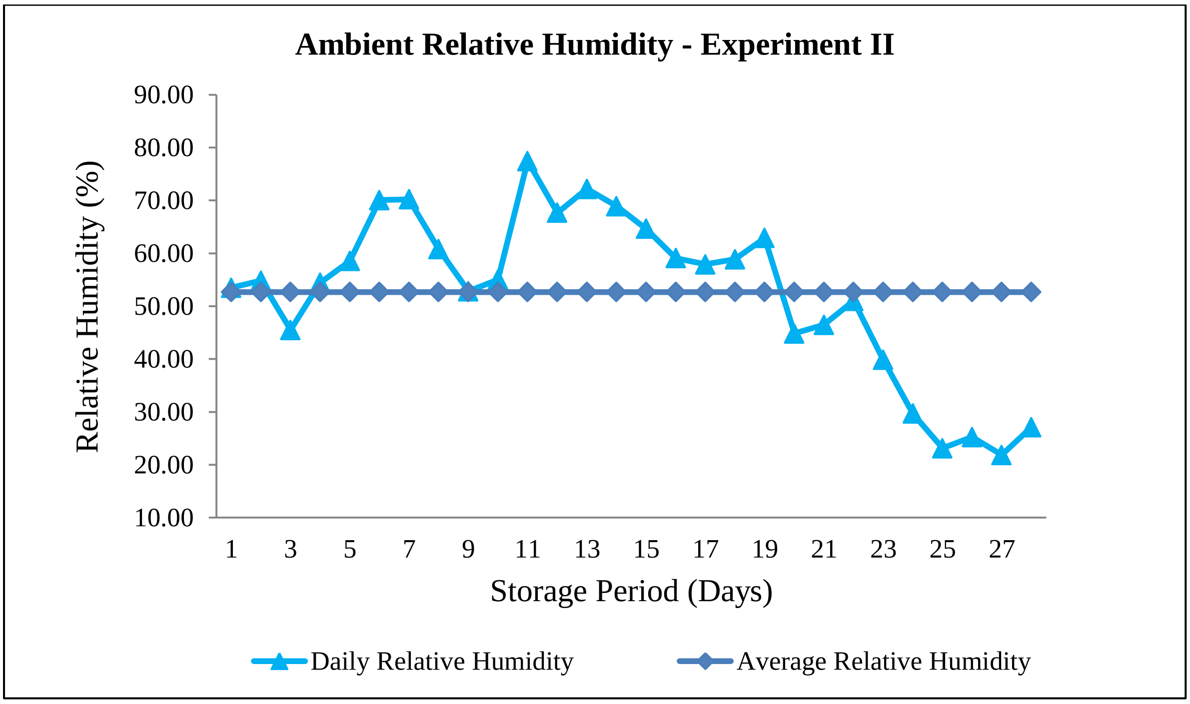

Figure 7.4 Ambient relative humidity fluctuation for Experiment II