EFFECT OF MODIFIED ATMOSPHERE ON STORAGE LIFE OF PURPLE PASSIONFRUIT AND RED TAMARILLO

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science in Horticultural Science

At Massey University, Palmerston North

New Zealand

Wattana Pongjaruvat

2007

ABSTRACT

This study investigates methods to improve storage life of purple passionfruit (*Passiflora edulis* Sims) and tamarillo (*Cyphomandra betacea* (Cav.) Sendt). For passionfruit, the main problem for export and storage is shrivelling whereas for tamarillo the quality of the stem is a key factor in export standards.

Eating quality of passionfruit was best described by the titratable acidity (TA) and the soluble solids content (SSC) with the optimal eating flavour found at an SSC/TA ratio between 10-11. Wax coating, ethylene scavenging, and modified atmosphere packaging (MAP) were assessed as tools to improve storage life. MAP with varying oxygen transmission rates (OTR at 5°C; 854, 1437, 2347 and 3089 ml m^{-2} day^{-1}) were compared to the standard packaging in a cardboard box during storage at the commercial temperature of 8°C. Fruit quality was measured after 20, 28, and 42 days of storage with and without seven days of shelf life at 20°C in the same packaging as during storage. Waxing did not improve the quality of the fruit. MAP prevented shrivelling but in the packaging with lower OTR (854 - 1437 ml m^{-2} day^{-1}) unacceptable external defects developed. Fruit quality in the packaging with the higher OTR (2347 - 3089 ml m^{-2} day^{-1}) was similar except for the development of off-flavours in the packaging with an OTR of 2347 ml m^{-2} day^{-1} during shelf life possibly due to the high ethylene accumulation since the addition of an ethylene scavenger in a second trial eliminated the off-flavour development. The highest OTR MAP is the best option for long term storage. The second highest OTR MAP could be used providing an ethylene scavenger is added.

To extend the storage life of tamarillo, two MAP options (OTR at 5°C; 1437 and 3089 ml m^{-2} day^{-1}) were compared to the standard packaging in a cardboard box with polyliner as well as the effect of adding clove oil releasing sachets. All fruit were stored at 4°C for 56 days and fruit and stem quality was measured fortnightly with and without three days of shelf life at 20°C. MAP delayed the development of stem yellowing, which was related to chlorophyll degradation, but did not improve fruit quality and increased stem blackening and bleeding in the locule, especially when clove oil was added. Blackening was related to

polyphenol oxidase activity and was aggravated by clove oil or by injury (e.g. disruption of cellular membranes) due to lower O_2, higher CO_2 and higher ethylene concentrations. Thus, for the two films tested, MAP with or without the addition of clove oil offered no advantages over conventional air storage.

ACKNOWLEDGEMENTS

I wish to express my appreciation to the following people who supported me and were involved in this thesis.

Dr. Wendy Schotsmans, Postharvest Physiologist and my supervisor, for her academic comments, support and encouragement, throughout my study and for her kindness to me. Thanks for offering me academic opportunities and checking and correcting the thesis.

Associate Professor John Mawson, Manager of Fresh Technologies and my supervisor, for his academic comments, suggestion in my experiment, and correction in this thesis.

I could thank all of my colleagues at Fresh Technologies at the Institute of Food, Nutrition and Human Health of Massey University one by one for the interesting time I spent as part of the group but that would take me too far. Special thanks goes out to Weerawate Utto, Packaging Technologist and Lecturer in Ubon Ratchathani University, for introducing me to postharvest studies. Thanks also for passing on your deep knowledge of postharvest and laboratory work, and to his wife and lovely daughter for being friends and family, and helping during my life in New Zealand.

Dr. Supranee Manushinakorn for lecturing on polyphenol oxidase and chemical analysis and laboratory work and her friendliness and kindness.

Dr. Narumol Matan for providing all information on eugenol measurement and related topics.

Dr. Bruce Mackay for his professional skill in statistics, discussing, and checking statistical analysis.

Sue Nicholson for her professional skills in lab guidance, advice, and discussion particularly on gas measurements. Sunil Pinnamaneni and Aziz AI-Harthy for laboratory work and being friends in the postharvest laboratory. Peter Jeffery for your computer expertise when I had problems with my computers.

Mark Bloxwich and Janette from PassionNZ for the passionfruit and the input of trade knowledge. Haydon Fisher Memorial Tamarillo Bursary for providing funds for the tamarillo research. The Craig and Robyn Watson Redwood orchard for supplying the tamarillo fruit. Andrew Sheerin from Convex plastics for supplying the packaging material and industry knowledge.

I also want to express my appreciation for all the help with administrative details and questions from Yvonne Parkes, and Kathy Hamilton.

Thai friends (Pattamawadee, Duljira, Aunkana, and Chanapha) for discussion, advises, and parties throughout my study life.

YouTube website for providing funny clips and stopping my homesickness. It was only one thing that could make me happy during writing thesis.

Finally, I would like to thank my 'Pongjaruvat' family (parents and brothers) for their support, mentally, and physically, during my lonely life in New Zealand. Thinking about seeing the most beautiful smile from my family after I got a master degree, I would forget and forgive my bad days and that really drove me to work harder and harder.

Wattana Pongjaruvat **July, 2007**

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LIST OF SYMBOLS AND ABBREVATIONS

a^*	redness/greenness	
A	the surface area of the fruit	m^2
A_{GC}	area of gas chromatogram according to injected volume of sample (area)	
A_{film}	the surface area of the plastic packaging film	m^2
b^*	yellowness/blueness	
C^*	colour intensity/chroma	
C	chlorophyll a or b	
C^{Eug}	eugenol concentration	mol m^{-3}
CA	controlled atmosphere	
Cl	clove oil	
h°	hue angle	
K_{GC}	detector response or slope of eugenol standard curve	
L^*	lightness	
L_{film}	the thickness of the plastic packaging film	m
MAP	modified atmosphere packaging	
$M_{initial}$	the initial weight of the fruit	g
M_{final}	the final weight of the fruit	g
M_f	the fruit mass	kg
NS	not significant	
OTR	oxygen transmission rate	
P	packaging	
PE	polyethylene	
ppm	parts per million	
PPO	polyphenol oxidase	
$P^f_{H_2O}$	the partial pressure of water vapour in the fruit	Pa
$P^e_{H_2O}$	the partial pressure of water vapour of the environment	Pa
$P^{sat}_{H_2O}(T_w)$	the saturated water vapour pressure at the wet bulb temperature	Pa

P'_{H_2O}	the fruit skin permeance to water vapour	mol s^{-1} m^{-2} Pa^{-1}
P_{net}	net partial pressure of gas *i* as the difference between the partial pressure quantified when the fruit was placed in the jar and a certain period after placing the fruit in the sealed jar	Pa
P'_{H_2O}	the film permeability to water vapour	mol m s^{-1} m^{-2} Pa^{-1}
ΔP_{H_2O}	the difference in partial pressure between the fruit and the environment/ the difference in partial pressure between the inside of the aluminium can and the environment	Pa
R	the universal gas constant (8.3145)	Pa m^3 mol^{-1} K^{-1}
RH	relative humidity	%
RQ	the respiratory quotient	
r_{CO_2}	the respiration rate at storage or room temperature	mol g^{-1} s^{-1}
r'_{H_2O}	the rate of water loss	mol s^{-1}
r_i	the specific rate of exchange of gas *i*	mol kg^{-1} s^{-1}
Sd	Storage duration	
Sl	Shelf life	
SSC	soluble solids content	°brix
t	time	s
T	temperature	K
TA	titratable acidity	
T_e	the air (dry bulb) temperature	°C
T_f	the fruit temperature directly under the skin of the fruit	°C
T_w	the wet bulb temperature	°C
V	the volume of 80% acetone	ml
V_{net}	the free volume in the jar calculated as the difference in volume between the fruit and the jar	m^3
V_f	the volume of the fruit	m^3
Vol_{inj}	Injected volume of sample	m^3
W	the weight of sample	g
W_w	the weight of displaced water	kg

WVP	water vapour permeance	mol s^{-1} m^{-2} Pa^{-1}
γ	the psychometric constant (67)	Pa $°C^{-1}$
ρ_w	the density of water at 20°C (998.20)	kg m^{-3}

CHAPTER 1

INTRODUCTION

1.1. Passionfruit and tamarillo in New Zealand

Passionfruit (*Passiflora edulis* Sims) and tamarillo or tree tomato (*Cyphomandra betacea* (Cav.) Sendt) are subtropical fruit that have been successfully grown for domestic and export sales in New Zealand (Janet, 2005). Passionfruit and tamarillo are exported fresh, packed in a cardboard box with each fruit sitting in an individual cup within a tray, and for tamarillo the tray is covered by polyliner. The main export markets for both passionfruit and tamarillo are shown in Table 1-1 in order of importance with the highest volume of exports to the United States. From 2004 to 2006 the export value for passionfruit is expected to almost double, while the domestic value is expected to be slightly lower. The export value for tamarillo has not increased as much but is higher than for passionfruit with the major market being the domestic market. The main growing areas of passionfruit and tamarillo are located in the North Island of New Zealand. The growing area for tamarillo is significantly larger than for passionfruit and the yield for tamarillo is higher.

Table 1-1. Features of New Zealand passionfruit and tamarillos (* from Janet (2005), ** from MAF (2006))

	Passionfruit	Tamarillo
Export Market*	USA, Australia, Japan, Pacific Islands, Singapore, Hong Kong, Indonesia, and Europe	USA, Japan, Australia, Hong Kong, Singapore, and Pacific Islands
Export value (NZ$FOB) for July 2003-June 2004**	384,962	738,235
Export value (NZ$FOB) (year ended March 2006)**	700,000	900,000
Domestic value (NZ$) (year ended June 2004)**	600,000	1,400,000
Growing area (ha)**	66	206
Growing regions**	Northland, Bay of Plenty, and Taranaki	Northland, Bay of Plenty, and Auckland
Yield in 2006 (tonnes/ha)**	5.5	7.0

Passionfruit is a native plant of Brazil (Pruthi, 1963) and is a member of the *Passifloraceae* family (Rodriguez-Amaya, 2003). Purple and yellow passionfruit are most popular for commercial use and have a similar round shape containing aromatic juicy pulp with black seeds in it. Passionfruit is high in vitamin C and carotene, and contains a lot of vitamins A, E, and B_6. Yellow passionfruit is larger, has little shrivelling, and yields more pulp and juice with a lower soluble solids content, less reducing and total sugars, but a higher acidity compared to purple passionfruit which is superior in aroma and flavour, but shrivels when the fruit is fully ripe. The peak harvest period is from February to May (MAF, 2006) and the harvest method is to gather fruit from the ground as it naturally drops when it ripens (Chavan & Kadam, 1995). Alternatively, the fruit is picked from the vine at 70 days after flowering when the colour changes from green to purple, the soluble solids content is at its maximum level, and the acidity starts to decrease (Shiomi *et al.*, 1996b).

Passionfruit has a short storage and shelf life due to the high respiration rate and ethylene production (Pruthi, 1963) as well as shrivelling caused by water loss from the peel as this affects consumer and market perception although it does not change the quality of the edible portion of the fruit. Pruthi (1963) noted that wax coating could enhance the appearance of purple passionfruit for five weeks contrary to findings by Dagama *et al.* (1991) where waxing did not effectively improve the fruit quality. Modified atmosphere (13% O_2 and 0.5% CO_2) packaging of yellow passionfruit slows down shrivelling and weight loss during storage (Arjona *et al.*, 1994). However, no studies have determined the effect of packaging on quality attributes of purple passionfruit.

Tamarillo is a native plant of South and Central America and is categorised in the same *Solanaceae* family as tomato, eggplant, and capsicum (Sale & Pringle, 1999). There are three types of tamarillo according to their skin colour: red, yellow, and purple (Prohens & Nuez, 2000). All red varieties were developed in New Zealand and are now the main varieties being planted commercially (Sale & Pringle, 1999). The tamarillo fruit has an egg shape and smooth skin containing a lot of small seeds in it. Tamarillo fruit is rich in iron, potassium, vitamins A, B_6, C and E. The fruit takes around 21 to 26 weeks from flowering to mature and its harvesting period lasts from April to November (Sale & Pringle, 1999). The best indicator to assess fruit maturity for red tamarillo is the skin colour as immature

fruit are green, mature fruit are purple, and ripe fruit turn a deep red. Purple coloured fruit are considered to be at optimum maturity for harvest and marketing and the fruit continues to develop the red colour after harvest with an increase in soluble solids content and decrease in acidity (El-Zeftawi *et al.*, 1988). If tamarillo fruit is harvested immature, it will have a shorter shelf life, shrivel quickly during storage, and not develop the full red colour (Pratt & Reid, 1976; Sale & Pringle, 1999).

Fungal infections dramatically reduce storage life of tamarillo fruit and have not been successfully prevented by preharvest treatments (Sale & Pringle, 1999). Hot water dip complemented with a postharvest fungicide dip and waxing have been introduced as a successful method to reduce the fungal infections of tamarillo (Yearsley *et al.*, 1987). Although hot water dip reduces fungal rots during storage, it cannot prevent stem discoloration, which affects customer acceptance. In cherry, a hot water dip at a temperature over 48°C increased stem browning (Feng *et al.*, 2004). Therefore, there is a need to develop an alternative method to maintain all tamarillo quality attributes during storage.

Stem discoloration has been associated with dehydration and physical damage (Sale & Pringle, 1999). After harvest, stems of tamarillo have been found to lose moisture quickly. For cherries, the loss of moisture from the stem reduces membrane integrity followed by decompartmentation, and subsequently allows polyphenol oxidase and phenolic substrates to mix causing stem discoloration (Schick & Toivonen, 2002). Hence, the reduction of moisture loss after harvest could be a reasonable approach to slow down the stem colour changes during storage.

1.2. Modified atmosphere packaging

Modified atmosphere packaging (MAP) refers to packaging with depleted O_2 and enriched CO_2 atmospheres with high relative humidity inside the packaging and has been used successfully to improve storage life of many products (Kader *et al.*, 1989). Passive MAP refers to the modification of the atmosphere inside the packaging due to fruit respiration and characteristics of packaging film, while active MAP is related to the addition of a precise amount of gases; O_2, CO_2, or ethylene absorber; or essential oil to the packaging.

As passionfruit has a high ethylene production, the addition of an ethylene scavenger to MAP may extend the postharvest life as found in Japanese pear (Szczerbanik *et al.*, 2005) and kiwifruit (Ben-Arie & Sonego, 1985) where the addition of an ethylene scavenger to MAP reduces ethylene and CO_2 concentrations, delays ripening, and reduces disorders.

The addition of pure essential oil, especially eugenol, in a separated package inside packaging enhanced the beneficial effect of MAP in terms of maintenance of cherry fruit quality for longer storage and delay of stem browning (Serrano *et al.*, 2005). Therefore, the use of MAP with the addition of essential oil could be an approach to maintain tamarillo fruit quality and stem colour.

1.3. Research structure and objective of the thesis

1.3.1. Aim and objectives of the thesis

The aim of this work is to improve storage life of two popular fruits in New Zealand using techniques like MAP, wax coating, ethylene scavenging, and essential oil release.

Specifically for passionfruit, the objectives are

- to obtain quantitative information regarding the physiological attributes determining quality and how they evolve during storage
- to investigate the effect of waxing, MAP, and ethylene scavenging on storage life during storage at 8°C and shelf life at 20°C

For tamarillo the objectives are

- to determine the mechanisms of stem discoloration of tamarillo
- to study the effects of MAP and addition of clove oil on storage life of the fruit and the stem during storage at 4°C and shelf life at 20°C

1.3.2. Research structure

Chapter 2 is a literature review covering the postharvest physiology of passionfruit and tamarillo, postharvest deterioration of the stem of tamarillo, and postharvest technologies to improve storage of passionfruit and tamarillo.

In Chapter 3 the materials and methods used in this study are described for the experiments with passionfruit and tamarillo including the experimental design and the data analysis methods used.

Chapter 4 discusses the results for the storage experiments performed for purple passionfruit. This includes investigations into the effects of wax coating, MAP, and ethylene scavenging on the quality attributes of purple passionfruit and their storage life.

In Chapter 5 the results and discussion of the experiments for red tamarillo fruit are outlined. Besides the effects of MAP and the addition of essential oil on the quality attributes and storage life of tamarillo, the chemical characteristics of the stem under these conditions are discussed.

Chapter 6 offers general conclusions from this body of work as well as recommendations for storage of passionfruit and tamarillo, and future work.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Passionfruit is a climacteric fruit and quickly respires and produces a high amount of ethylene after harvest leading to a shorter shelf life for storage and transportation (Pruthi, 1963). Also, shrivelling due to water loss from the peel affects consumer purchase decisions. Tamarillo is a non-climacteric fruit and slowly respires with only traces of ethylene produced after harvest causing a long shelf life (Sale & Pringle, 1999). However, fungal infections dramatically reduce storage life of tamarillo fruit and blackening of the stem develops quickly after harvest influencing consumer and market perceptions.

In this review, the postharvest behaviour of fruit is discussed, especially focussing on passionfruit and tamarillo. This includes the factors affecting changes in physiology and biochemistry of the fruits. This review also covers postharvest deterioration of the stem of tamarillo with reference to the development of tissue browning in other fruits and vegetables. The final focus will be on postharvest technologies to improve storage life.

2.2. Postharvest physiology

2.2.1. Weight loss

Weight loss mainly relates to water loss by transpiration and carbon loss through respiration (Maguire *et al.*, 2001). Transpiration is the loss of water through stomata, lenticels, or cuticle from the fruit to the surrounding atmosphere and respiration is a metabolic reaction to produce energy by metabolising substrates as carbohydrates, lipids, and organic acids into water and CO_2 (Wills *et al.*, 1998).

Weight loss increases with storage time as the fruit continues to transpire and respire after harvest (Maguire *et al.*, 2001). Although some weight loss will not affect the appearance and other quality aspects, 5% loss of water from the fruit causes shrivelling of apple due to

a decrease in cell turgor (Maguire *et al.*, 2001). Excessive water loss can also cause changes in texture as seen in toughening of asparagus (Albanese *et al.*, 2007) and firmness loss of tomato (Hertog *et al.*, 2004a).

The weight loss of passionfruit (Schotsmans *et al.*, 2007) and tamarillo (El-Zeftawi *et al.*, 1988) increased with storage duration. The weight loss of passionfruit was also related to the development of shrivelling (Schotsmans *et al.*, 2007).

Weight loss is associated with factors that influence transpiration and respiration rate: the ratio of fruit surface area to volume, permeance to water vapour of the fruit skin, relative humidity, gas atmosphere, and temperature (Maguire *et al.*, 2001).

2.2.1.1. The ratio of fruit surface area to volume

The fruit surface area/volume ratio directly influences the rate of water loss as a smaller fruit has a greater surface area to weight ratio, resulting in a faster loss of water compared to a bigger fruit (Maguire *et al.*, 2001). In passionfruit, the higher surface area/volume ratio of less mature fruit compared to mature fruit results in more weight loss in less mature fruit (Shiomi *et al.*, 1996b) and tamarillo fruit with lower surface area/volume ratio loses less water (Prohens *et al.*, 1996).

2.2.1.2. Permeance to water vapour

Water vapour permeance is a characteristic of the fruit describing the ease at which water vapour can move through the skin (Maguire *et al.*, 2001). Hence, the structure of the fruit skin directly affects the water loss of the fruit. The fruit skin contains four layers: epidermal hair, cuticle, epidermis, and hypodermis with the last two very permeable to water (Maguire *et al.*, 2001). The cuticle has a high resistance to water vapour and is a major barrier to water vapour diffusion; hence, a loss of cuticle causes a fast loss of water from the fruit peel (Wills *et al.*, 1998).

The cuticle is the main barrier to water diffusion from the rigid peel of purple passionfruit, which contains a white internal layer like the albedo of citrus fruit having a high permeance to water migration (Pruthi, 1963). Tamarillo fruit is categorised in the same

Solanaceae family as tomato and capsicum (Sale & Pringle, 1999), whose skin has a thick cuticle (Bargel & Neinhuis, 2005) and does not have stomata (Blanke & Holthe, 1997). Hence, the water loss of these fruit solely depends on the water permeance of the cuticle. The tamarillo fruit is harvested with closed calyx and stem as commercially required (Sale & Pringle, 1999) and the resistance of the stem scar to water vapour diffusion is very poor (Maguire *et al.*, 2001). Hence, the cut end of the tamarillo stem may increase water vapour permeance, and subsequently water loss from the fruit.

2.2.1.3. Relative humidity

Relative humidity refers to the amount of water vapour in the air (Maguire *et al.*, 2001). In general, the water vapour pressure of fruit is assumed to be a saturated level and higher than that of the surrounding air in storage. This creates a gradient of water movement due to the difference of partial pressure, called water vapour pressure deficit (Wills *et al.*, 1998), between the fruit and the surrounding air. An increase in relative humidity in storage then reduces the water vapour pressure deficit leading to a decrease in water diffusion, and subsequently weight loss (Wills *et al.*, 1998) as found in tomato (Hertog *et al.*, 2004a), apple (Tu *et al.*, 2000), and tamarillo (Schotsmans *et al.*, 2005). However, an increase in relative humidity up to a very high level (more than 95%) can induce fungal growth, which leads to fungal attack and decay (Wills *et al.*, 1998).

The amount of water vapour in the air also depends on temperature. A lower temperature generates a lower water vapour pressure deficit compared to a higher temperature resulting in a reduction of the driving force for water vapour movement from the fruit to the surrounding air (Hertog *et al.*, 2004a). During cold storage, the optimal relative humidity is 90% for fruits and 98-100% for vegetables (Wills *et al.*, 1998).

Modified atmosphere packaging film generally has a low permeability to water vapour and creates high relative humidity inside the packaging (Kader *et al.*, 1989). If fruit is inside the closed packaging, water vapour released by the fruit is not removed from the packaging quickly, and subsequently a high humidity environment develops. This decreases the driving force for water vapour transfer from the fruit to the surrounding environment, thus resulting in a decreased water loss.

2.2.1.4. Temperature

Temperature is an important factor affecting transpiration and respiration. A decrease in temperature of the air surrounding the fruit reduces fruit temperature, resulting in a reduction in water vapour pressure at the fruit surface, and subsequently the driving force for water loss (Maguire *et al.*, 2001). A reduced fruit temperature also slows down fruit respiration leading to a lower CO_2 production and a lower weight loss (Hertog *et al.*, 1998).

Moreover, an increase in temperature between 0-30°C interferes with the structure of soluble cuticular lipids, which are a component in the cuticle and act as a main barrier of water transfer through the cuticle, resulting in an increase in water vapour permeance, and subsequently water loss (Maguire *et al.*, 2001).

In passionfruit (Arjona *et al.*, 1992) and tamarillo (Schotsmans *et al.*, 2005), an increase in temperature increased the weight loss of the fruits.

2.2.2. Respiration

Respiration is a major metabolic process to produce energy for fruit after harvest by metabolising substrates like starch, sugars, fats, or organic acids, to CO_2 and water (Wills *et al.*, 1998). A common substrate for respiration is glucose and the overall equation is as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

Respiration occurring in the presence of oxygen is called aerobic respiration, while in the absence of oxygen it is called anaerobic respiration or fermentation (Wills *et al.*, 1998). The pathway of aerobic respiration is classified into three steps: glycolysis, tricarboxylic acid (TCA) cycle, and electron transport system (Wills *et al.*, 1998). The glycolysis system occurs in the cytoplasm and converts respiratory substrates into pyruvic acid as a substrate for the TCA cycle, and also produces ATP and NADH (Salisbury & Ross, 1978). The TCA cycle occurs in the mitochondria and oxidises pyruvic acid or organic acids stored in the fruit tissues, such as citric acid, α-ketoglutatric acid, succinic acid, fumaric acid, malic

acid, and oxaioacetic acid, to CO_2 and produces electron donors like NADH and $FADH_2$ (Salisbury & Ross, 1978). Finally, NADH and $FADH_2$ are oxidised via the electron transport system to produce water and ATP (Wills *et al.*, 1998). However, in the absence of O_2 (anaerobic respiration), the electron transport stops and products of glycolysis (pyruvate and NADH) start to pile up. Pyruvic acid then converts into acetaldehyde, ethanol, and CO_2 (Wills *et al.*, 1998). The rate of CO_2 production or O_2 consumption is used to express the respiration rate. In general, a higher respiration rate indicates a shorter shelf life (Robertson, 1993).

In general, the respiration rate is high for immature fruit and increases during ripening, corresponding to the development of eating quality. This increase in respiration rate is called the climacteric rise and is found in all climacteric fruit (Wills *et al.*, 1998). Non-climacteric fruit do not exhibit the climacteric rise although fruit composition changes during ripening. The respiration rate of both climacteric and non-climacteric fruits declines during senescence (Wills *et al.*, 1998). The respiration rate depends on the availability of O_2/CO_2 inside the fruit and in the surrounding air, substrates stored in the fruit after harvest, maturity stage, and temperature.

The respiration rate of the climacteric passionfruit increases during ripening followed by a decrease during senescence (Pruthi, 1963; Shiomi *et al.*, 1996b). The respiration rate of non-climacteric tamarillo slowly decreases after harvest and starts to increase at the onset of fruit senescence (Pratt & Reid, 1976).

2.2.2.1. Available O_2/CO_2

The volumetric ratio of CO_2 production to O_2 consumption rate is called the respiratory quotient (RQ) and has been used to characterise the balance between aerobic and anaerobic respiration. RQ around 0.7-1.3 indicates aerobic respiration; RQ higher than 1.3 is generally indicative of a switch to anaerobic respiration (Salisbury & Ross, 1978; Robertson, 1993).

An increase in O_2 partial pressure up to 30-70 kPa increases respiration and accelerates ripening of climacteric fruits (Kader & Ben-Yehoshua, 2000). However, excessive O_2

partial pressure (more than 70 kPa) can cause the production of reactive O_2 (or O_2 toxicity), which damages the cytoplasm, inhibits metabolic activities, and finally leads to deterioration of fruit tissues (Kader & Ben-Yehoshua, 2000). A decrease in O_2 concentration below the O_2 tolerance limits induces fermentation (Hertog *et al.*, 1998), and increases pyruvic acid, acetaldehyde, and ethanol accumulation as in bell pepper stored in 0% O_2 (Imahori *et al.*, 2002) and tomato stored in 0.5 and 1% O_2 (Klieber *et al.*, 1996). Moreover, the excessive accumulation of acetaldehyde and ethanol during anaerobic respiration is related to the development of physiological disorders such as skin pitting, shrivelling, and discoloration of tomato stored in 0.5 and 1% O_2 (Klieber *et al.*, 1996).

Increasing the CO_2 partial pressure up to 60 kPa decreases the respiration of climacteric fruits such as peach, tomato, and banana, but it does not affect that of non-climacteric fruits such as citrus, grape, and Japanese pear (Kubo *et al.*, 1990). The elevated CO_2 atmosphere inhibits the respiratory activity through several steps of glycolysis and the TCA cycle resulting in a decrease in pyruvic acid and accumulation of succinic acid in banana exposed to 60% CO_2 (Liu *et al.*, 2004). The accumulation of succinic acid in the TCA cycle indicates that the elevated CO_2 inhibits the activity of the enzyme succinate oxidase to convert succinic acid into malic acid. Too high CO_2 levels can cause a similar injury as too low O_2 levels. Tomato exposed to 80% CO_2 developed skin pitting, shrivelling, and discoloration similar to fruit exposed to 0.5% and 1% O_2 (Klieber *et al.*, 1996). Moreover, the high CO_2 levels can also induce fermentation demonstrated by Fernandez-Trujillo *et al.* (2007) who reported an increase in acetaldehyde and ethanol in strawberry stored in 20% CO_2.

The tolerance limits to low O_2 and high CO_2 to maintain aerobic respiration vary among commodities (Kader *et al.*, 1989). Pruthi (1963) noted that passionfruit could be kept in 5% O_2 and 5% CO_2 for six weeks. There is no available information on optimal O_2 and CO_2 levels for storage of tamarillo. For tomato and bell pepper, which are related to tamarillo, the minimum O_2 and maximum CO_2 levels are 3% and 2%, respectively (Kader *et al.*, 1989).

2.2.2.2. Substrate availability

Climacteric fruits like avocado, banana, and passionfruit have high levels of starch and sugars and utilise those as substrates for the respiratory mechanism, while non-climacteric fruits like cherry, grape, citrus, and tamarillo have lower starch and sugar levels and respire by utilising organic acids (Salisbury & Ross, 1978; Wills *et al.*, 1998). During aerobic respiration, the RQ can be used as an indicator of the respiratory substrate (Wills *et al.*, 1998). The RQ of climacteric fruits is generally around 1 as aerobic respiration of the most common substrate, glucose ($C_6H_{12}O_6$), used up equivalent amounts of O_2 as it produces CO_2 on a molar or volumetric basis. The RQ of non-climacteric fruits is generally higher than 1 due to higher oxygen per carbon atom of organic acids substrate, as malic acid ($C_4H_6O_5$) and citric acid ($C_6H_8O_7$), resulting in a lower requirement of O_2 consumption for the CO_2 production (Wills *et al.*, 1998).

2.2.2.3. Maturity

Fruit harvested at different stages of maturity have different respiration rates, with fruits harvested at an early stage of maturity having a lower respiration rate than those harvested at a later stage of maturity as found for instance in pawpaw fruit (Archbold & Pomper, 2003), apple (Song & Bangerth, 1996), pepino (Ahumada & Cantwell, 1996), and passionfruit (Shiomi *et al.*, 1996b). The lower respiration rate of less mature fruit is related to a lower level of substrates for utilisation as less mature fruit have a lower soluble solids content as found in pepino (Ahumada & Cantwell, 1996) and passionfruit (Shiomi *et al.*, 1996b).

2.2.2.4. Temperature

In general, higher rates of O_2 consumption and CO_2 production are found with an increase in temperature (Hertog *et al.*, 1998). Hence, ripening of horticultural products will accelerate when stored at higher temperature, and slow down at lower temperature. The respiration rates reported in the literature for purple passionfruit and red tamarillo are reviewed in Table 2-1.

Table 2-1. Respiration rates of passionfruit and tamarillo under normal atmosphere

Cultivar	Temperature (°C)	Respiration rate (μmol CO_2 $kg^{-1}s^{-1}$)	References
Purple passionfruit	6.5	0.25-0.82	Pruthi (1963)
	10	0.64-1.72	"
	20	1.26-1.89	Shiomi *et al.* (1996b)
	10	0.21	Schotsmans *et al.* (2007)
	25	0.40	"
Red tamarillo	20	0.19-0.25	Pratt & Reid (1976)

The respiration rate of passionfruit is higher with higher temperatures; differences observed between studies may be due to differences in cultivar or production practices as well as measurement protocols.

The respiration rate of tamarillo is considerably lower compared to passionfruit at the same temperature. No data on the variation of tamarillo respiration rate with temperature are available.

2.2.3. Ethylene production

Ethylene is a plant hormone that affects plant growth and development, and storage life of many fruits (Saltveit, 1999). The response of fruits to applied ethylene is also used to classify them into climacteric and non-climacteric fruits (Wills *et al.*, 1998). Ethylene accelerates the respiration rate and ethylene production of climacteric fruit, whereas in non-climacteric fruit the respiration rate temporarily increases, but ethylene production does not. The most obvious response of non-climacteric fruit to applied ethylene is a change of peel colour and chlorophyll degradation. Both climacteric and non-climacteric fruits produce ethylene during development and ethylene production increases during ripening with a higher production for climacteric fruit (Wills *et al.*, 1998).

The ethylene biosynthesis pathway is shown in Figure 2-1. The amino acid methionine (MET) is converted into S-adenosyl-methionine (SAM) by the enzyme SAM synthase (Saltveit, 1999). SAM is then metabolised into 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase, which is thought to be a limiting factor in ethylene

biosynthesis. Its activity generally increases during fruit ripening. In the final step, the enzyme ACC oxidase requires O_2 to oxidise ACC to ethylene; hence, a reduction of O_2 level inhibits ethylene synthesis.

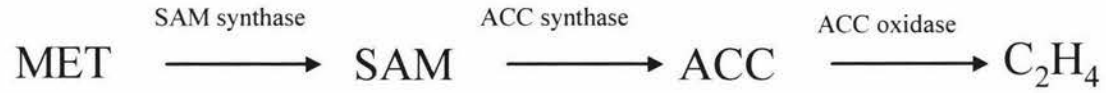

Figure 2-1. Pathway of ethylene biosynthesis (Saltveit, 1999)

The ethylene then binds to receptors and activates a signalling cascade resulting in enhanced transcription and translation of certain genes to produce the enzymes responsible for the actual ethylene responses such as cell wall hydrolysis, increased flavour and aroma volatiles, and loss of chlorophyll (Reid, 1992) and also inducing the ethylene synthesis (Yang, 1987). An increase in CO_2 level is thought to interfere in the ethylene activity. It may accumulate in the intercellular space and compete with ethylene for its binding site (Yang, 1985), resulting in fruit tissue being insensitive to ethylene (Zagory, 1995). High CO_2 also reduces the ACC content by inhibiting ACC synthase, and subsequently the ethylene production, as it did in tomato (de Wild *et al.*, 2005).

In purple passionfruit, the ACC content and ACC synthase activity are low during the initial stage of ripening with a low ethylene production and increase during ripening with an increase in ethylene production, especially in the juice sac and seed (Shiomi *et al.*, 1996a). The ethylene production of tamarillo is low during postharvest life, but dramatically increases when the fruit starts to senesce (Pratt & Reid, 1976). The ethylene production of passionfruit and tamarillo is reviewed in Table 2-2. The ethylene production of purple passionfruit increases with harvest maturity and is much higher than that of tamarillo.

Table 2-2. Ethylene production (C_2H_4) of purple passionfruit and red tamarillo

Cultivar	Conditions		C_2H_4 production rate (nmol $kg^{-1}s^{-1}$)	References
	Harvest	Storage		
Purple passionfruit	The initial stage of ripening		0.03	Shiomi *et al.* (1996a)
	60 DAF	10 days at 25°C	< 2.34	Shiomi *et al.* (1996b)
	70 DAF	10 days at 25°C	8.18	"
	80 DAF	10 days at 25°C	7.01	"
	90 DAF	10 days at 25°C	9.35	"
		20 days at 25°C	4.21	Pruthi (1963)
Red tamarillo		20°C	< 0.001	Pratt & Reid (1976)

DAF = days after flowering

Ethylene can have both beneficial and detrimental effects on the quality of horticultural produce (Saltveit, 1999). In citrus, the promotion of the yellow colour development is a wanted effect while the yellowing in vegetables is not (Saltveit, 1999). If fruit is exposed to an optimum level of ethylene, fruit ripening is induced; whereas, if exposed for too long or exceeding the tolerance limits, senescence is induced resulting in the development of physiological disorders.

Purple passionfruit is sensitive to exogenous ethylene as it accelerates endogenous ethylene production (Shiomi *et al.*, 1996a), and improves colour development of fruit harvested at the mature-green stage (Arjona & Matta, 1991). The response of tamarillo fruit to applied ethylene is an acceleration of fruit ripening as found through a higher respiration rate, a decrease in firmness, development of red skin colour and yellow flesh colour, and an increase in the ratio of soluble solids content to acidity (Pratt & Reid, 1976; Prohens *et al.*, 1996).

The reduction of ethylene action by removal of ethylene or inhibition of ethylene perception improves fruit quality and extends storage life as it decreases respiration rate, delays the loss of firmness, acidity, and weight, and the change of colour as found in avocado (Jeong *et al.*, 2003), tomato (Wills & Ku, 2002; Guillen *et al.*, 2007), and plum (Valero *et al.*, 2003).

2.2.4. Colour

The colour development is related to the changes of pigments, such as chlorophyll, carotenoids, and anthocyanins, situated in the fruit tissue (Wills *et al.*, 1998). Chlorophyll is a green pigment; carotenoids are yellow, orange, and red pigments; and anthocyanins are red, purple, and blue pigments. The change of fruit colour during ripening involves chlorophyll degradation unmasking other pigments which have been synthesised and exist in the fruit tissues, as found in a change from green to yellow/red colour in banana, paprika, and chilli (Kays, 1991; Krajayklang *et al.*, 2000). The development of fruit colour has been used as a maturity index in many fruits such as banana, tomato, and strawberry (Kays, 1991). The control of colour change during storage and transportation is necessary as the colour is used by consumers and markets to assess the general quality of fruits, even though the quality and colour in some fruits such as apple and citrus are not correlated (Kays, 1991). A number of factors affecting the change of colour are harvest time, cultivar, light, temperature, and O_2 concentration. The exposure of fruit to the light and an increase in temperature and O_2 concentration enhances the synthesis of carotenoids and anthocyanins, and the chlorophyll degradation (Kays, 1991).

The colour development of purple passionfruit can be used as a maturity index as immature fruit is yellowish green, turning partially purple for mature fruit, and fully purple for ripe fruit (Pruthi, 1963). Fruit can be harvested when the skin is partially purple (approximately 70 days after flowering) as it will then continue to develop the full purple colour after harvest (Shiomi *et al.*, 1996b). However, fruit will not develop the full purple colour if it is harvested at an immature stage (before 70 days after flowering). Additionally, consumers prefer a purple colour over at least 80-90% of the fruit surface, and less than 75% is unacceptable for markets (Arjona & Matta, 1991). During storage at 6.5°C, passionfruit gradually loses the purple colour and develops brown discolouration on the skin, being more pronounced at higher temperature, due to the degradation of anthocyanin. Below 6.5°C fruit is affected by chilling injury resulting in red discoloration on the surface (Pruthi, 1963).

The colour development of red tamarillo is considered as a maturity index: immature fruit is green, mature fruit is purple, and ripe fruit turns deep red (Sale & Pringle, 1999). Purple coloured fruit is considered to be at optimum maturity for harvest and marketing as the fruit will continue to develop the red colour after harvest (El-Zeftawi *et al.*, 1988). However, immature fruit fails to develop the full red skin colour after harvest (Pratt & Reid, 1976). During storage at 20°C, lightness and yellowness of the skin of tamarillo decreased, while redness increased with an increase in ripeness (Mwithiga *et al.*, 2007). This is different from findings in red tamarillo (Schotsmans *et al.*, 2005) where lightness, chroma value, and hue angle did not change during 54 days of storage and was not different for fruit stored at 1.5°C, 3.5°C, 7°C, and 20°C. Additionally, Mwithiga *et al.* (2007) noted that a gradual decrease in lightness during ripening correlated to a decrease in lightness of the pulp and an increase in the soluble solids content of the juice.

2.2.5. Firmness

Fruit firmness can be used to assess fruit quality and is related to the structure and composition of the cell wall (Hertog *et al.*, 2004a) as well as the water status or the turgor pressure in the cells (Wills *et al.*, 1998). The cell wall consists of three layers: the middle lamella (mainly pectin, cellulose, and hemicellulose), the primary wall (mainly pectin), and the secondary wall (mainly lignin) (Brett & Waldron, 1996). All the cell wall layers are cross-linked to each other through cellulose binding to pectin, hemicellulose, protein, or lignin. Cell wall breakdown during ripening involves two mechanisms (MacRae *et al.*, 1990). Firstly, the cross-linking of hemicellulose between cellulose and pectin decreases, causing a loss of integrity of the cell wall. Then, the pectin is degraded by the enzymes pectin methyl esterase (PME) and polygalacturonase (PG), and the cell wall becomes loosened and takes up more extracellular fluid, resulting in swelling of the cell wall and softening. Cell turgor pressure also contributes to fruit firmness (Wills *et al.*, 1998) and a decrease in cell turgor due to water loss through transpiration and respiration results in firmness loss (Hertog *et al.*, 2004a).

Fruit softening is related to endogenous and exogenous ethylene concentration (Johnston *et al.*, 2002) as well as environmental factors like temperature, relative humidity, and atmosphere composition.

Due to the different mechanisms of firmness loss, the selection of methods to measure firmness has to be considered. A destructive and invasive puncture test mainly measures firmness of the cell wall, which depends on temperature and the enzymatic cell-wall breakdown (Hertog *et al.*, 2004a). Non-destructive methods like acoustic impulse response and compression force are associated with temperature, the enzymatic cell-wall breakdown, and water status in the cell. In apple, the firmness measured by the invasive puncture during the last stage of fruit ripening remained constant although the fruit were transferred from 1°C to 20°C, whereas the stiffness measure dramatically decreased (Hertog *et al.*, 2004a). This indicates the limitation of the destructive measure since it does not assess the effect of water loss or water status in the cells on firmness when fruit become fully ripe.

The firmness using a puncture force dramatically decreased for tamarillo stored at 21°C at the onset of fruit ripening and the decrease slowed down thereafter (Mwithiga *et al.*, 2007), indicating that the firmness loss was related to a decrease in cell wall strength. Mwithiga *et al.* (2007) noted that firmness of tamarillo could also be used to predict the internal fruit quality as its decrease corresponded to an increase in juice yield during ripening. In purple passionfruit, compression firmness of packaged and non-packaged fruit remained constant during long term storage with a lower level for fruit in MAP packaging (Schotsmans *et al.*, 2007). The higher firmness of non-packaged fruit was possibly due to a higher water loss as it causes peel shrivelling and toughening (Pruthi, 1963; Arjona *et al.*, 1994).

2.2.5.1. Ethylene

Ethylene controls cell wall hydrolases, PME and PG, as an inhibition of ethylene action by binding the ethylene receptor with 1-methylcyclopropene (1-MCP) suppresses the PME and PG activities and delays firmness loss in avocado and persimmon (Jeong *et al.*, 2003; Luo, 2007). Exogenous ethylene application accelerated fruit softening in carambola (Miller & McDonald, 1997), papaya (An & Paull, 1990), and tamarillo (Pratt & Reid,

1976). The inhibition of ethylene action by 1-MCP or ethylene scavenging slowed down softening in tomato (Guillen *et al.*, 2007), plum (Valero *et al.*, 2003), Japanese pear (Szczerbanik *et al.*, 2005), and kiwifruit (Ben-Arie & Sonego, 1985).

2.2.5.2. Temperature

The effect of temperature on fruit softening is related to the effect of temperature on enzymatic cell-wall breakdown and the cell turgor. Enzyme reactions are temperature-dependent with an increase in temperature resulting in higher enzyme activity (Wills *et al.*, 1998). Hence, the activity of the cell-wall hydrolases increases with an increase in temperature resulting in faster cell-wall breakdown and an acceleration of fruit softening. Higher temperatures also increase water loss from the fruit resulting in a decrease in cell turgor, and subsequently lower firmness (Hertog *et al.*, 2004a).

In tamarillo, fruit softening measured as stiffness and compression firmness was slower at lower temperature (1.5 and 3.5°C) compared to a higher temperature at 7°C (Schotsmans *et al.*, 2005).

2.2.5.3. Relative humidity

Softening is faster at lower relative humidity compared to higher relative humidity as the lower relative humidity increases the water vapour pressure deficit resulting in an increase in water loss from the fruit to the surrounding air (Hertog *et al.*, 2004a). In tomato, fruit stored at a lower relative humidity of 30% at 11.8°C lost stiffness and compression firmness with the same rate as those stored at a higher relative humidity of 75% at 16.5°C. This shows that the decrease in relative humidity cancels out any beneficial effect of the decrease in temperature since the lower temperature reduces water movement from the fruit and decreases the activity of the enzymes involved in cell-wall breakdown but softening is still accelerated due to the higher water loss resulting from the lower relative humidity.

In tamarillo, relative humidity between 80% and 100% did not alter stiffness and compression firmness of fruit stored at a constant temperature of 7°C (Schotsmans *et al.*, 2005).

2.2.5.4. Atmosphere composition

Low O_2 and/or high CO_2 atmosphere delay softening through a reduction in ethylene production as found in apple (Jobling & McGlasson, 1995) and kiwifruit (McDonald & Harman, 1982), and an inhibition of the PME and PG activities as found in pepino fruit (Huyskens-Keil *et al.*, 2006) and carambola (Ali *et al.*, 2004). The effect of low O_2 and high CO_2 levels on PME and PG involves two different mechanisms as it reduces the synthesis of enzymes or inhibits the transcription of enzymes as shown by a low level of PG mRNA in nectarine stored at 3% O_2 and 10% CO_2 (Zhou *et al.*, 2000).

Moreover, low O_2 and high CO_2 levels decrease the respiration rate, leading to a decrease in the production of energy for oxidative reactions in the cells, and subsequently decreased softening as found in kiwifruit stored at 0-21% O_2 and 0-5% CO_2 (Hertog *et al.*, 2004b). However, if the low O_2 and high CO_2 levels exceed the tolerance limits, the energy from both oxidative and fermentative processes then drives softening.

2.2.6. Pulp and juice characteristics

The internal edible properties of fruit involve taste and odor which are determined by water, sugars, starch, organic acids, and volatile compounds (Kays, 1991). Changes in composition, especially of sugars and organic acids, have been used to assess the fruit quality and correlate it with aspects like colour and maturity. During ripening of fruit with starch reserves, sugars accumulate through the conversion of the starch reserves in the fruit tissue and organic acids generally decrease. However, some fruit, especially non-climacteric fruit, do not convert starch into sugars. Instead, the sugar level depends on its translocation from the plant prior to harvest. Hence, the consideration of harvest time must be careful to optimise the final taste of fruit. Because the amount of sugars and organic acids changes during development and ripening, the ratio of soluble solids content to titratable acidity is used to determine the maturity index and optimal eating quality for

many fruits such as strawberry within the range 10-12 (Kafkas *et al.*, 2007), tomato within the range 9-10 (Guillen *et al.*, 2007), and table grape within the range 55-60 (Valverde *et al.*, 2005).

The pulp of passionfruit is comprised of the juice aril with black seeds in it (Pruthi, 1963) and is aromatic and flavourful with high vitamin C, A, E, B_6, and carotene contents. Yellow passionfruit has a lower soluble solids content, less reducing and total sugars, but a higher acidity, and yields more pulp and juice compared to purple passionfruit which is superior in aroma and flavour (Rodriguez-Amaya, 2003). The soluble solids content in the juice of purple passionfruit increases with an increase in maturity, whereas the acidity increases during the first 60 days after flowering and decreases thereafter (Shiomi *et al.*, 1996b). During postharvest storage, both soluble solids content and acidity decrease with a faster decrease for acidity due to the utilisation as respiratory substrates. Shiomi *et al.* (1996a) noted that the eating quality of purple passionfruit improves mainly due to a decrease in acidity. Low temperature storage at 5°C and 10°C delayed the loss of soluble solids of yellow passionfruit (Arjona *et al.*, 1992), indicating that the low temperature slows down the respiration rate to convert sugars to carbon dioxide (Wills *et al.*, 1998). However, the use of packaging (polystyrene tray sealed in a plasticized poly vinyl chloride film) did not maintain the soluble solids content of yellow passionfruit compared to non-wrapped fruit during cold storage at 10°C (Arjona *et al.*, 1994).

Red tamarillo is rich in iron, potassium, vitamin A, B_6, C, and E (Sale & Pringle, 1999) and the juice content increases during ripening with an increase in pH and soluble solids content (Mwithiga *et al.*, 2007) and a decline in acidity (El-Zeftawi *et al.*, 1988). This is different from findings by Schotsmans *et al.* (2005) where soluble solids content and acidity did not change during 54 days of storage and were not affected by temperature as they were similar for fruit stored at 1.5, 3.5, 7, and 20°C.

2.2.7. Postharvest pathology

The current harvest method of passionfruit is to gather from the ground as the fruit naturally drops when it ripens (Chavan & Kadam, 1995). Hence, fruit is highly contaminated with soil-borne pathogens. The common fungal attacks are by *Alternaria*

passiflorae with circular, sunken, and brown spots on the fruit surface, and septoria blotch caused by *Septoria passiflorae* (Rodriguez-Amaya, 2003). Pruthi (1963) noted that during long term storage at 6.5°C, passionfruit was attacked by white (*Fusarium oxysporum*), blue (*Penicillium expansum*), and black (*Aspergillus niger* and *Rhyzopus nigricans*) fungus. The use of polyethylene bags to store passionfruit did not reduce fungal attack due to high relative humidity inside the bag (Pruthi, 1963). However, adding a 5% Lysol solution to the polyethylene bag successfully reduced fungal attack.

Field infections also dramatically reduce the storage life of tamarillo fruit and have not been successfully prevented by preharvest treatments (Sale & Pringle, 1999). The common fungal attacks are bitter rots, such as circular and brown-black spots caused by *Glomerella cingulata*, *Colletotrichum acutatum*, *Phoma exigua*, or *Diaporthe phaseolorum* (Sale & Pringle, 1999), and stem-end rots, such as a soft brown rot occurring around the base of the stem caused by *Botrytis* (Sutton & Strachan, 1971). The use of irradiation successfully inhibited the stem-end rots during storage, but this method caused tamarillo fruit tissue to be sensitive to low temperature injury resulting in tissue breakdown and discoloration during cold storage (Sutton & Strachan, 1971). Hot water dip (50°C for 8 min) complemented with a postharvest fungicide (imazalil) dip and waxing has been introduced as a successful method to reduce the fungal infections of tamarillo (Yearsley *et al.*, 1987). However, fruit treated with the fungicide could have detectable residues and this is unacceptable for export (Sale & Pringle, 1999).

2.3. Deterioration of fruit stem

For fruits that are commercialised with the stems attached, the appearance and hence quality of the stem is as important as the fruit itself. The stem of tamarillo fruit needs to be intact, green, or green/yellow stem to satisfy export standards (Sale & Pringle, 1999). However, physical damage and fast loss of moisture after harvest cause stem blackening of tamarillo (Sale & Pringle, 1999). A postharvest hot water dip, which successfully reduced fungal rots during storage, could not prevent the stem blackening (Yearsley *et al.*, 1987). Thus, the development of stem blackening is a crucial factor in determining whether a hot

water dip is useful and there is a need to develop an alternative method to maintain all tamarillo quality attributes during storage.

For cherries, the development of stem browning is related to moisture loss, chlorophyll degradation, and enzymatic browning (Siegelman, 1952). Methods to preserve the stem quality of cherry fruit are based on dipping fruit and stem in various kinds of wax coatings. Dipping sweet cherries in 100% *Aloe vera* gel for five minutes reduced respiration and microbial growth, and delayed weight loss, changes of fruit colour, softening, as well as changes of stem colour (Martinez-Romero *et al.*, 2006). Wax coatings, such as Vapor Guard, SF 215, and Fresh Cote, reduced fruit weight loss and stem discoloration for 'Van' sweet cherries (Lidster, 1981). However, for 'Bing' sweet cherries, fruit stored in a box with polyliner had higher stem moisture, more green stems, and less brown stem than fruit with wax coating (Drake *et al.*, 1988). These results agree with findings in tamarillo where the fruit in trays with polyliner had less stem discoloration compared to the application of wax which did not delay the changes of stem colour (Schotsmans *et al.*, 2005).

Controlled atmosphere storage (0.5-2.5% O_2 and 0.01-0.03% CO_2) (Chen *et al.*, 1981) and modified atmosphere packaging (MAP) (0.8% O_2 and 4.5% CO_2) (Meheriuk *et al.*, 1995) maintained cherry fruit quality and green stem colour for five and six weeks, respectively, compared to the control in a box with polyliner where the fruit and stem quality was maintained for three weeks (Drake *et al.*, 1988). Drake *et al.* (1988) noted that the loss of green stem of cherry was related to the loss of stem moisture. This possibly indicates that modified atmosphere storage intensively reduces the loss of moisture content in the stem, and finally stem discoloration. The addition of pure essential oils, especially eugenol, in a separated permeable package inside packaging enhanced the beneficial effect of MAP in terms of maintenance of cherry fruit quality for longer storage periods and delaying stem browning more intensively compared to the packaging without the added essential oils (Serrano *et al.*, 2005). The addition of essential oils reduces dehydration, chlorophyll degradation, and the activity of enzymatic browning although the exact mechanisms are unknown.

Since for cherries, the development of stem browning is related to moisture loss, chlorophyll degradation, and enzymatic browning (Siegelman, 1952), these are likely to be the factors involved in stem discoloration of tamarillo fruit.

2.3.1. Moisture content

The fruit stem contains a high amount of moisture and the loss of moisture coincides with the development of browning/blackening of cherry (Drake *et al.*, 1988) and tamarillo stems (Sale & Pringle, 1999), litchi peel (Huang *et al.*, 2005), and strawberry fruit (Nunes *et al.*, 2005). The loss of moisture reduces membrane integrity followed by decompartmentation, leading to the interaction between polyphenol oxidase in the chloroplasts and phenolic substrates in the vacuoles (Vamos-Vigyazo, 1981) causing tissue browning (Schick & Toivonen, 2002).

Temperature and relative humidity are the main factors causing moisture loss from the stem (Siegelman, 1952) with higher temperature and lower relative humidity promoting moisture loss.

MAP successfully maintains the quality of cherry fruit and stem for six weeks (Meheriuk *et al.*, 1995). In general, the low permeability to water vapour results in higher relative humidity inside the packaging (Kader *et al.*, 1989; Hertog, 2003). This reduces the driving force for water vapour transfer out of the stem to the surrounding environment, resulting in a decrease in water loss and stem quality is maintained. The addition of essential oils, especially eugenol, intensified the effects of MAP on maintaining qualities of cherry (Serrano *et al.*, 2005) and grape (Valverde *et al.*, 2005) and delaying stem browning during storage period. Essential oils are thought to reduce transpiration even though the exact mechanism is unknown (Serrano *et al.*, 2005; Valverde *et al.*, 2005).

2.3.2. Chlorophyll content

Chlorophyll is a green pigment that is important in photosynthesis, absorbing light to produce energy for plant growth and development (Scheer, 1991). The chlorophyll is located in the chloroplasts in the form of a chlorophyll-protein complex and chlorophyll *a*

and *b* are the most important pigments in green vegetable tissues. The chlorophyll biosynthesis can be classified into three steps (Eckhardt *et al.*, 2004). Glutamate is converted into 5-aminolevulinic acid (ALA) by glutamyl tRNA reductase. ALA molecules then react with each other to form protoporphyrin IX. In the final step, magnesium from the membrane is transferred into protoporphyrin IX forming Mg-protoporphyrin IX (MgProto), which is converted into chlorophyllide *a* by methyltransferase and MgProto monomethylester cyclase. Chlorophyllide *a* is catalysed into chlorophyll *a*, which can inter-converse into chlorophyll *b*.

Although the mechanism of chlorophyll degradation is not certain for all horticultural produce, the two critical factors in causing chlorophyll degradation are the presence of light and/or oxygen. Chlorophyll degradation can be through modification of the chlorophyll structure (reaction I) like the loss of Mg and phytol, or the cleavage of the macrocyclic ring system and subsequent degradation to smaller molecular weight (reaction II) (Brown *et al.*, 1991). Both reactions I and II involve several enzymes. In reaction I, the phytolester bond is catalysed by the enzyme chlorophyllase followed by the release of Mg due to the enzyme Mg-dechelatase leading to chlorophyll destruction (Eckhardt *et al.*, 2004). In litchi, a decrease in chlorophyll content coincided with an increase in the activity of chlorophyllase resulting in degreening and the occurrence of red colour (anthocyanins) on the fruit skin (Wang *et al.*, 2005). In reaction II, the chlorophyll is degraded due to photochemical and non-photochemical reaction which involves light and oxygen. The chlorophyll is bleached when exposed to light with oxygen availability called photooxidation (Brown *et al.*, 1991). Hendry *et al.* (1987) noted that in healthy tissues, the light did not influence the chlorophyll degradation, but when the tissues started to senesce, the light became a promoter of chlorophyll breakdown. The non-photochemical degradation is related to activated oxygen like peroxide and superoxide, which form non-photochemically and degrade chlorophyll in the presence of chlorophyll peroxidase (Brown *et al.*, 1991).

An increase in respiration rate and ethylene production accelerates the chlorophyll degradation as found in rocket leaf and coriander leaf (Jiang *et al.*, 2002; Koukounaras *et al.*, 2006; 2007). A higher respiration rate increases O_2 consumption leading to an increase

in photooxidative bleaching or chlorophyll peroxidase involved in the chlorophyll degradation (Brown *et al.*, 1991) and a higher ethylene concentration reduces chlorophyll and protein levels (Jiang *et al.*, 2002). Ethylene also increases the activities of chlorophyllase and peroxidase as the inhibition of ethylene activity by 1-MCP in lime delayed the chlorophyll degradation with lower activities of both enzymes compared to untreated fruit (Win *et al.*, 2006). Hendry *et al.* (1987) noted that the activity of enzymatic chlorophyll breakdown was low when chlorophyll was in the form of the chlorophyll-protein complex. Hence, the effect of ethylene may be the chlorophyll destruction and subsequently an increase in the activity of chlorophyll degrading enzymes resulting in an increase in the chlorophyll degradation.

Modified atmosphere storage effectively delays the loss of chlorophyll content in vegetable tissues as found in galega kale (1-2% O_2 and 15-20% CO_2) (Fonseca *et al.*, 2005), Bok Choy (5% O_2 and 2% CO_2) (Lu, 2007), and broccoli (5% O_2 and 6% CO_2) (Serrano *et al.*, 2006). Additionally, an enriched CO_2 atmosphere reduces the activity of chlorophyllase resulting in a decrease in the chlorophyll degradation (Guevara *et al.*, 2003).

The addition of essential oils, especially eugenol, to MAP successfully maintained the green colour of cherry stem (Serrano *et al.*, 2005) and grape rachis (Valverde *et al.*, 2005) during storage. Although the reaction between the essential oils and chlorophyll is unknown, eugenol has been found to prevent peroxidation (Lederer *et al.*, 2004), in which singlet oxygen like peroxide degrades chlorophyll (Brown *et al.*, 1991), leading to the inhibition of chlorophyll degradation.

2.3.3. Polyphenol oxidase activity

Polyphenol oxidase (PPO) is an enzyme and the interaction between PPO and phenolic substrates causes browning in fruit and vegetable tissues (Vamos-Vigyazo, 1981). Both PPO and phenolic substrates are higher in the outer layer of horticultural tissue as found in the skins of grape, peach, cherry, and plum. PPO is located in the chloroplasts, while phenolic substrates are found in the vacuoles. The PPO concentration decreases during ripening. The phenolic compounds vary among horticultural products and the most important substrates are catechins, cinnamic acid esters, 3,4-dihydroxy phenylalanine, and

tyrosine (Vamos-Vigyazo, 1981). Higher levels of PPO and phenolic compounds correlated to a greater incidence of brown spots on the mango peel (John *et al.*, 2002). However, an increase in degree of browning of peach related to only an increase in the PPO activity as phenolic compounds did not change when browning occurred (Brandelli & Lopes, 2005).

PPO activity correlates to the development of tissue browning in many fruits as found in strawberry skin (Nunes *et al.*, 2005), loquat flesh (Cai *et al.*, 2006), and mango peel (John *et al.*, 2002). The mechanism of browning can be classified into two reactions, which both require oxygen (Vamos-Vigyazo, 1981). PPO catalyses the hydroxylation of monophenols, leading to the formation of o-dihydroxy compounds. Then, the o-dihydroxy compounds are oxidised to o-quinones. The quinones react with each other to form higher molecular weight polymers that are brown. The requirement of oxygen in the browning reactions is revealed in the high susceptibility to browning of cut surfaces which is reflected in a dramatic increase in the PPO activity in sliced apple (Martinez & Whitaker, 1995).

The PPO activity is highest between pH 4 and pH 7, and increases with an increase in temperature from 3°C to 37°C (Vamos-Vigyazo, 1981). Temperatures below 0°C (Vamos-Vigyazo, 1981) or above 50°C (Martinez & Whitaker, 1995) affect the PPO activity as the low temperature causes cell membrane injury resulting in the interaction between PPO and phenolic compounds. At temperature above 50°C, PPO is destroyed.

Controlled and modified atmosphere in cold storage control the PPO activity as O_2 is required for the oxidation of substrates to produce brown polymers (Vamos-Vigyazo, 1981) and CO_2 is a competitive inhibitor of PPO (Deng *et al.*, 2006). Also, a decrease in temperature will slow down the PPO activity. The depleted O_2 and enriched CO_2 atmosphere reduced the PPO activity with a decrease in browning of banana peel (12% O_2 and 4% CO_2) (Nguyen *et al.*, 2004) and cherry flesh (5% O_2 and 10% CO_2) (Tian *et al.*, 2004). However, MAP (2% O_2 and 5% CO_2) did not inhibit the PPO activity in bamboo shoot, but it did prevent browning by preventing the accumulation of malondialdehyde and reducing the activity of peroxidase and phenylalanin ammonialyase, which are also involved in the development of tissue browning (Shen *et al.*, 2006).

The combination of essential oils, especially eugenol, with MAP successfully maintained the green colour of cherry stem (Serrano *et al.*, 2005) and grape rachis (Valverde *et al.*, 2005). Even though the relationship between the essential oils and browning is unknown, the benzene derivatives of the essential oils are inhibitors of PPO (Martinez & Whitaker, 1995) and can effectively prevent oxidation (Ruberto & Baratta, 2000), resulting in the inhibition of brown polymer formation.

2.4. Postharvest technologies

2.4.1. Waxing

Wax coating is relatively hydrophobic (Kester & Fennema, 1986), hence adding it to the fruit surface increases resistance to water movement from the fruit to the surrounding air, resulting in a decrease in water loss and an increase in firmness as found in cherry (Yaman & Bayondrl, 2002) and avocado (Jeong *et al.*, 2003). The application of wax coating also aims to improve fruit appearance and extend shelf life (Maguire *et al.*, 2001). Waxing increased lightness of cherry (Yaman & Bayondrl, 2002) and mango (Baldwin *et al.*, 1999) and greenness of avocado (Jeong *et al.*, 2003). Waxing can also modify the internal atmosphere by reducing O_2 and increasing CO_2 levels in mango and delaying fruit ripening (Baldwin *et al.*, 1999). However, the proportion of wax coating applied on the fruit skin must be considered as it can change the O_2 and CO_2 permeability of the fruit skin too much leading to anaerobic respiration and physiological disorders (Maguire *et al.*, 2001). The ethylene production rate decreased in waxed avocado, but wax coating did not delay the time to reach the ethylene climacteric peak (Jeong *et al.*, 2003). However, the application of wax coating to mamey sapote fruit neither affected respiration rate nor ethylene production (Ergun *et al.*, 2005).

The application of paraffin wax coating to purple passionfruit reduced weight loss and enhanced fruit appearance for five weeks (Pruthi, 1963). This is different from findings in purple passionfruit (Dagama *et al.*, 1991) where adding autocitrol wax on the fruit surface did not improve storage life. Hence, more studies are needed to investigate the effect of waxing on purple passionfruit.

2.4.2. Modifying the atmosphere

Modified atmosphere storage has been used successfully to maintain fruit quality during long term storage (Wills *et al.*, 1998). Controlled atmosphere (CA) storage generally refers to decreased O_2 and increased CO_2 by applying, monitoring and maintaining a precise amount of these gases. Modified atmosphere packaging (MAP) offers a cheaper alternative to modify the atmosphere surrounding fruit by relying on the permeability characteristics of the packaging material and the fruit metabolism to attain and maintain a certain atmosphere. Additionally, MAP results in high relative humidity inside the packaging and has been used successfully to prolong the storage life of fruits and vegetables (Kader *et al.*, 1989).

2.4.2.1. Passive modified atmosphere packaging

Passive MAP is established mainly through fruit respiration and the characteristics of the packaging film such as film structure, gas permeability, and thickness (Kader *et al.*, 1989). In a closed package, the fruit consumes O_2, and produces CO_2, water vapour, and ethylene. At the same time, these gases are allowed to move in and out of the packaging depending on its permeability. The permeability of the packaging material refers to the ability of O_2, CO_2, or water vapour to penetrate through the packaging film through passive diffusion by which the difference in gas concentrations on two sides of the packaging material will cause the movement of gas from high to low concentrations. MAP film generally has a low permeability to O_2, CO_2, and water vapour, hence high relative humidity, high CO_2, and low O_2 levels will develop inside the packaging (Kader *et al.*, 1989; Hertog, 2003). The low O_2 and high CO_2 levels in MAP will slow down the metabolism of the packaged fruit and micro-organism growth (Hertog, 2003), resulting in delay of fruit ripening, senescence and decay.

The high relative humidity inside the packaging also decreases the driving force for water vapour transfer from the fruit to the surrounding environment, resulting in a decrease in water loss, and subsequently weight and firmness loss as found in persimmon (Cia *et al.*, 2006), carambola fruit (Ali *et al.*, 2004), and avocado (Yahia & Gonzalez-Aguilar, 1998). Moreover, MAP delayed the changes of colour as found in persimmon (Cia *et al.*, 2006),

carambola fruit (Ali *et al.*, 2004), broccoli (Serrano *et al.*, 2006), and Bok Choy (Lu, 2007).

However, MAP can also induce unfavourable conditions to the packaged fruit as the O_2 and CO_2 levels developed in the packaging may exceed the tolerance limits of the fruit, causing off-flavours and physiological disorders (Kader *et al.*, 1989). Hence, successful MAP must maintain optimum O_2 and CO_2 levels to attain the beneficial effects of modified atmosphere without exceeding the tolerance limits of the fruit. The high relative humidity developed inside MAP may also cause water condensation on the surface of packaged fruit resulting in microbial growth and accelerated decay.

The time to reach the steady state with optimum gas composition in MAP should be short to retain the quality of packaged fruit (Hertog, 2003). A number of techniques have been introduced to create the steady-state gas conditions in a short time like reducing the volume inside the packaging and packing warm fruit (Hertog, 2003). However, the reduction of the volume inside the packaging reduces the buffering capacity of the modification. Consequently, the atmosphere developed in the packaging is more sensitive to storage temperature fluctuations as O_2 will decrease and CO_2 increase much faster with an increase in storage temperature in packaging with a lower gas volume. Packing warm fruit may also induce condensation, and subsequently fungal growth (Hertog, 2003).

Maintaining the steady-state gas conditions depends on the temperature as a change in temperature influences the respiratory metabolism of the packaged fruit and the permeability of the packaging film and hence the rate of gases penetrating through the packaging (Hertog, 2003). Ideally, the gas composition in the packaging could be maintained during long term storage if the activation energy of the respiratory enzymes of the packaged fruit was equal to the activation energy of the film permeability to gas exchange (Chen *et al.*, 2000). However, the respiratory mechanism of fruit is more sensitive to the change in temperature than the permeability of packaging film (Kader *et al.*, 1989).

2.4.2.2. Active modification

Active MAP is related to the active modification of the atmosphere inside the packaging and not relying on the film permeability and the fruit metabolism. This can involve removing gases from the packaging and then applying a precise amount of O_2 and CO_2, or adding an ethylene absorber (Kader *et al.*, 1989), or antimicrobial volatiles (Utto *et al.*, 2005) to the packaging.

Ethylene scavenging generally uses potassium permanganate ($KMnO_4$) to remove ethylene from packaging (Zagory, 1995). $KMnO_4$ is a stable purple solid and oxidises ethylene to CO_2 and water (Wills & Warton, 2004). In general, $KMnO_4$ is prepared by coating an inert substrate like perlite, alumina, silica gel, vermiculite, activated carbon, or celite, and packing it in a sachet to avoid contact with the packaged fruit as it is toxic (Zagory, 1995). Wills & Warton (2004) noted that the efficiency of $KMnO_4$ decreased with an increase in relative humidity as it took a longer time to remove ethylene under higher relative humidity compared to lower relative humidity. Adding an ethylene scavenger to MAP reduced ethylene and CO_2 concentrations, delayed ripening, and inhibited disorders in Japanese pear (Szczerbanik *et al.*, 2005) and kiwifruit (Ben-Arie & Sonego, 1985).

The release of natural compounds has been recently tested to prevent microbial growth in food products with a added benefit of reduction of the use of chemical additives (Burt, 2004). Eugenol (2-methoxy-4-(2-propenyl) phenol) is a major component (85%) of clove oil and effectively prevents the bacterial growth of *E. coli*, *S. typhimurium*, and *L. monocytogenes* (Burt, 2004). Eugenol disrupts the cell membranes as the hydroxyl groups on eugenol interact with the proteins in the membrane. As a result, cell membranes lose their structure, and the hydrophobic compound of eugenol further disrupts the membrane lipids, resulting in more leakage and the death of bacterial cells (Burt, 2004).

The addition of eugenol in a separated package inside MAP intensified the effects of the packaging on maintaining a green colour of cherry stem (Serrano *et al.*, 2005) and grape rachis (Valverde *et al.*, 2005) and the fruit quality. Eugenol can prevent peroxidation (Lederer *et al.*, 2004), meaning singlet oxygen like peroxide can no longer degrade chlorophyll (Brown *et al.*, 1991). The benzene derivatives of eugenol can prevent

oxidation (Ruberto & Baratta, 2000), resulting in the inhibition of brown polymers formation. Addition of eugenol to cherry (Serrano *et al.*, 2005) and grape (Valverde *et al.*, 2005; Valero *et al.*, 2006) in MAP decreased weight loss due to a reduction in transpiration, and delayed fruit ripening due to a decrease in ethylene concentration, the activities of PG and PME, soluble solids content, and acidity loss. Moreover, the addition of eugenol did not affect O_2 and CO_2 accumulation in the packaging.

CHAPTER 3

MATERIALS AND METHODS

3.1. Introduction

This chapter covers the materials and methods used in the experiments with passionfruit and tamarillo including the experimental design and the data analysis. As this work includes experiments on two fruit species, the experimental design will be discussed per species, but since the quality measurements for the two fruit species are similar, these will be discussed together.

3.2. Fruit source

3.2.1. Passionfruit

Passionfruit (*Passiflora edulis* cv. 'Black Beauty') were supplied by Mark Bloxwich from PassioNZ Limited (Northland, New Zealand) and harvested by gathering from the ground on 9 May 2006 for the first trial and 29 June 2006 for the second trial. The fruit were packed in commercial cardboard boxes with each fruit sitting in an individual cup within tray counts of 25, 28, 30, 33, 35, and 42. They arrived at the postharvest laboratory at Massey University (Palmerston North, New Zealand) on 10 May 2006 in the morning for the first trial and 30 June 2006 in the morning for the second trial.

3.2.2. Tamarillo fruit

The tamarillo fruit (*Cyphomandra betacea* cv. 'Mulligan Red') were harvested on 25 August 2006 at the Craig and Robyn Watson Redwood orchard (Maungatapere, New Zealand), and packed in commercial cardboard boxes with plastic tray and polyliner. The fruit arrived at Massey University on 26 August 2006 in the evening and was stored overnight at 4 ± 0.3°C.

3.3. Experimental design

3.3.1. Passionfruit

3.3.1.1. The first trial (maximum storage duration 42 days)

There were six treatments in a factorial design as outlined in Table 3-1. The modified atmosphere plastic bags were supplied by CONVEX Plastics (Hamilton, New Zealand). Fruit were randomised to ensure different sizes of fruit spread over all treatments. Bags were cut to an average size of 22.5 × 15.5 cm for Bag 1 and 32 × 11 cm for Bags 2-4. A 1 cm^2 area of the bags was covered with silicone to enable repetitive sampling of the atmosphere in the bags. All fruit were stored at the current commercial temperature of 8 ± 0.5°C.

Table 3-1. Application of control, waxing, and packaging treatments in passionfruit project.

Treatment	Code	Description
1	Control	Fruit in standard packaging (cardboard box with plastic tray)
2	Wax	Waxed fruit in standard packaging The wax used was Citrus gleam
3	Bag 1 (SC WO 23338)	Thickness = 50 μm OTR = 854 ml m^{-2} day^{-1} Oriented PE Laminate (two films joined together)
4	Bag 2 (MK WO 23339)	Thickness = 40 μm OTR = 3089 ml m^{-2} day^{-1} material = based on PE WVP = 1.56×10^{-12} mol m s^{-1} m^{-2} Pa^{-1}
5	Bag 3 (BL WO 23339)	Thickness = 50 μm OTR = 1437 ml m^{-2} day^{-1} material = based on PE WVP = 5.63×10^{-12} mol m s^{-1} m^{-2} Pa^{-1}
6	Bag 4 (BH WO 23339)	Thickness = 50 μm OTR = 2347 ml m^{-2} day^{-1} material = based on PE

OTR = oxygen transmission rate measured at 5°C using the Convex test method
PE = polyethylene
WVP = water vapour permeability measured at 20°C using gravimetric method as presented in Appendix F

At arrival, 40 fruit were selected to measure initial quality (week 0) and 120 fruit for the wax treatment. Wax was applied (Citrus Gleam®, Castle Chemicals, Australia) by dipping paper tissue in the wax and wiping it on the fruit surface. For the measurements during storage and shelf life, 40 fruit (or ten bags with four fruit each) of each treatment were removed from cold storage after 20, 28, and 42 days. Twenty fruit (or five bags) of each treatment were measured immediately and the rest of the bags was stored at room temperature (20°C) for seven days to assess their behaviour during shelf life.

3.3.1.2. The second trial (maximum storage duration 70 days)

There were three treatments as outlined in Table 3-2. The randomisation of fruit into the bags, the size of the bags, the application of silicone, and the storage condition were the same as for the first trial.

Table 3-2. Application of packaging treatments in the second trial of passionfruit

Treatment	Code	Description
1	Bag 2 (MK WO 23339)	As described in Table 3-1
2	Bag 3 (BL WO 23339)	As described in Table 3-1
3	Bag 4 (BH WO 23339)	As described in Table 3-1 with the addition of an ethylene scavenger

At arrival, 20 fruit were randomly selected to measure initial quality. After 42 days of storage, 20 fruit (or five bags with four fruit each) of each treatment were removed from cold storage and measured immediately. From this point on, fruit in cold storage was visually inspected on a regular basis to enable us to remove the fruit immediately should it start to deteriorate fast. After 70 days of storage, 40 fruit (or ten bags) of each treatment were removed from cold storage. Twenty fruit (or five bags) of each treatment were measured immediately and the rest was held at room temperature (20°C) for seven days of shelf life. The fruit was visually inspected during and measured after the shelf life period.

3.3.2. Tamarillo fruit

There were five treatments in a factorial design as outlined in Table 3-3. Bags were cut to an average size of 15 × 21 cm. A 1 cm^2 area of the bags was covered with silicone to

enable repetitive sampling of the atmosphere in the bags. All fruit were stored at 4±0.3°C with relative humidity (RH) of 85±10%.

Table 3-3. Treatments applied in tamarillo project

Treatment	Code	Description
1	Control	Fruit in standard packaging (cardboard box with plastic tray and polyliner)
2	Bag 2 (MK WO 23339)	As described in Table 3-1
3	Bag 2+oil (MK WO 23339)	As described in Table 3-1 2 with the addition of a clove oil sachet
4	Bag 3 (BL WO 23339)	As described in Table 3-1
5	Bag 3+oil (BL WO 23339)	As described in Table 3-1 with the addition of a clove oil sachet

At arrival, 16 fruit were randomly selected to measure initial fruit and stem qualities (week 0). For later measurements, 32 fruit (or eight bags with four fruit each) of each treatment were removed from cold storage after 14, 28, 42, and 56 days. Sixteen fruit (or four bags) of each treatment were measured immediately and the remaining bags were stored at room temperature (20°C) for three days of shelf life. Fifteen stems were selected to measure moisture content, polyphenol oxidase (PPO) content, and chlorophyll content with five stems of each measurement. PPO and chlorophyll content measurements were done the day after removal from cold storage and the stems were kept in a plastic bag and stored overnight at 4°C.

3.4. Active packaging

3.4.1. Ethylene scavenging

The ethylene scavenger used for the second trial with passionfruit was Purafil containing 4% of $KMnO_4$. 1 g of Purafil was added to a 4×5 cm sachet (paper-like) (Tyvek® brand spunbonded olefin, DuPont, Wilmington, DE) and then the sachet was sealed. Tyvek® is made of high density polyethylene and has a high moisture vapour transmission rate. It is resistant to salt solutions, oxidising and reducing agents, inorganic chemicals and organic solvents such as ethyl acetate and phenol.

3.4.2. Clove oil

Clove oil (*Eugenia caryophyllata*, AromaSence, Whangaparaoa, NZ) was used in the form of silica gel saturated with clove oil in a 4×5 cm sachet like the one containing the ethylene scavenger. The preparation of the saturated silica gel with clove oil is presented in Appendix A. The sachet was placed outside the tray to avoid contact with the fruit. After adding the sachet in the bags, they were sealed immediately using heat-sealing equipment. Each sachet contained clove oil at around 0.81 g (0.76 ml) and silica gel absorbed clove oil at around 0.64 g (0.60 ml) clove oil per g dried silica gel. The absorption of silica gel was calculated from Eq 1 and Eq 2.

$$\text{clove oil (g)} = \text{saturated silica gel with clove oil (g)} - \text{dried silica gel (g)} \quad \text{Eq 1}$$

$$\text{The absorbtion of silica gel} = \frac{\text{clove oil (g)}}{\text{dried silica gel (g)}} \quad \text{Eq 2}$$

3.5. Measurements

All measurements and laboratory analyses were conducted at the Postharvest Laboratory of the Institute of Food, Nutrition and Human Health (IFNHH), and the Food Chemical Laboratory of the Institute of Natural Resources (INR), Massey University.

3.5.1. Water vapour permeance

Fruit were placed in an airstream overnight (Figure 3-1). Wet and dry bulb temperatures were recorded during this time as well as the fruit temperature directly under the skin. All temperatures were recorded every five min using a data logger (Grant Squirrel 1200 series logger, Grant Instrument (Cambridge) Ltd, England). Water loss was determined during this period as weight loss corrected for respiration by weighing each fruit before and after air exposure. The measurement was done at room temperature (20°C). Water vapour permeance (mol s^{-1} m^{-2} Pa^{-1}) was calculated as shown in Appendix B.

Figure 3-1. Measurement of water vapour permeance with wet/dry bulb probe, data logger, and fruit skin temperature measurement

3.5.2. Weight loss

Fruit weight was obtained using a balance (0.001g Mettler-Toledo PG 503s, Medic Corporation Limited, US). The weight loss of fruit was expressed as percentage loss of original weight according to Eq 3.

$$\left(\frac{\text{initial weight} - \text{final weight}}{\text{initial weight}}\right)(100) \qquad \text{Eq 3}$$

3.5.3. Gas measurements

3.5.3.1. Respiration

Twenty fruit of the control and wax treatments for the passionfruit in the first trial, and eight fruit of the control and bag treatments for the tamarillo experiment were placed in individual closed white plastic containers (534 cm^3).

A 1 ml sample was taken from the headspace of the container directly after closing the containers and a second one 45 minutes later for the passionfruit trial and 60 minutes for the tamarillo experiment. The samples were injected in an O_2/CO_2 analyser to determine the O_2 and CO_2 concentration. The O_2/CO_2 analyser was equipped with an O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK), with O_2-freeN_2 as a carrier gas (flow rate 35 ml min^{-1}). From the difference in concentration, O_2 consumption and CO_2 production were calculated as presented in Appendix C and expressed in µmol kg^{-1} s^{-1}.

3.5.3.2. O_2/CO_2 concentration

To quantify the composition of the atmosphere in the MA bags, a 1 ml sample was taken from the atmosphere in the bag using a syringe and injected into the O_2/CO_2 analyser to determine O_2 and CO_2 concentration. The results were expressed as a volume percentage.

3.5.3.3. Ethylene production

Ethylene production was measured by flame-ionisation gas chromatography using a Varian® model 3400 gas chromatograph equipped with a 1/8 in. alumina packed column (AllTech Associates, NZ) (set at 120°C with N_2 as the carrier gas at 35 ml min^{-1}) and a flame ionisation detector (set at 150°C with H_2 and air flow rates of 20 and 300 ml min^{-1}, respectively).

Using the respiration setup, 1 ml of the headspace was sampled using a syringe after 1.4 hours for the control and waxed passionfruit of the first trial and after 1.3 hours for tamarillo fruit experiment. Additionally, ethylene in the bags was measured by taking a 1 ml sample from the atmosphere in the bags. The results for ethylene concentration in the bags were expressed as parts per million (ppm). Ethylene production was calculated as presented in Appendix C and expressed in nmol $kg^{-1}s^{-1}$.

3.5.3.4. Eugenol concentration

Eugenol concentration was measured by flame-ionisation gas chromatography using a GC 6000 VEGA Series 2 (Carlo Erba Instruments) equipped with a 30m×0.25mm×0.52μm capillary column using ZB-5 (Phenomenex® NZ, Ltd, NZ). The following operational conditions were chosen: detector temperature 260°C; injector temperature, 250°C, the optimal split flow rate was 200:1, and N_2 was used as the carrier gas at a flow rate of 2 ml min^{-1}. The column temperature was kept at 60°C for 50 sec, then linearly increased to 150°C (at 40°C per min) where it was kept for 30 sec followed by a final increase to 260°C (at 2°C per min). The optimal split flow rate was determined to be 200:1. The retention time of eugenol was 7.63 ± 0.02 min. A 1 ml sample was taken from the atmosphere in the bags using a 1 ml gas-tight syringe. Peak areas and retention time were recorded on an integrator (C-R6A Chromatopack Shimadzu, Japan) and compared with the response of injected samples of standard eugenol dissolved in ethyl acetate (99.5% BDH Laboratory Supplies, England). Eugenol concentration was calculated as presented in Appendix D. The results were expressed in mol m^{-3}.

3.5.4. Physicochemical characteristics

3.5.4.1. Colour

Skin colour of the fruit was determined using a calibrated (using the supplied standard white calibration plate) spectrophotometer (CM-2600D, Konica Minolta, Albany, New Zealand) at three positions on the fruit approximately evenly around the equator. The average value of lightness (L^*), redness/greenness (a^*), yellowness/blueness (b^*), colour intensity (C^*), and hue angle (h^o) was calculated using the supplied Spectramagic NX software (CM-S100w, Konica Minolta, Albany, New Zealand).

3.5.4.2. Stiffness

Stiffness of the fruit was measured using the Acoustic Firmness Sensor (AFS, AWETA, Nootdorp, The Netherlands) at three positions on the fruit (as above). Fruit were placed horizontally on the sensor, which was covered with a foam support and linked to a balance.

Within this balance, an upward directed microphone is mounted at a few millimetres from the fruit surface. Fruit are taped gently by impacting using a light plastic rod that is mounted beside the microphone. The first resonance frequency of the resulting frequency spectrum is selected and used to calculate stiffness. The results were averaged per fruit and expressed as $10^6 Hz^2 g^{2/3}$.

3.5.4.3. Compression firmness

The compression firmness of the fruit was determined using a texture analyser (TA-XT2i, Stable Micro System, Godalming England), equipped with a load cell of 25 N. A 60 mm diameter-disc compressed each individual fruit perpendicular to its axis at 2 mm s^{-1} to a threshold depth of 2 mm. The firmness of fruit was measured at room temperature and 2 measurements per fruit were taken on the equator on opposite sides of the fruit. The average value was expressed as the peak force in N.

3.5.4.4. Pulp yield for passionfruit

Passionfruit were cut and the pulp was removed and weighed. Percentage of pulp yield was expressed as (pulp weight divided by fruit weight) × 100. Subsequently, the pulp was sieved and the juice was collected for soluble solids content and titratable acidity determination.

3.5.4.5. Soluble solids content

Total soluble solids content (SSC) of the juice was determined for each fruit using a calibrated digital refractometer (PAL-1, Pocket Series, ATAGO Ltd., Japan). The results were expressed as °Brix.

3.5.4.6. pH and titratable acidity

The pH of the juice was recorded and titratable acidity (TA) was determined by titration with 0.1 M NaOH up to pH 8.2 using a titrator (DL21, Mettler Instruments AG, Zurich, Switzerland). The sample was prepared by diluting 1 ml of juice in 50 ml distilled H_2O. Since citric acid is the main organic acid in passionfruit (Shiomi *et al.*, 1996b) and

tamarillo (Romero-Rodriguez *et al.*, 1994), the results were expressed as g of citric acid equivalent per 100 ml juice.

3.5.5. Stem measurements

3.5.5.1. Moisture content

Five stems were selected to measure the moisture content by drying the excised stem to constant weight in a plastic weighing plate in an oven at 70°C. Drying took three days to reach a constant weight. The moisture content was calculated using Eq 4 and the results were expressed as percentage.

$$\left(\frac{\text{Fresh stem weight} - \text{Dry stem weight}}{\text{Fresh stem weight}}\right)(100) \qquad \text{Eq 4}$$

3.5.5.2. Chlorophyll content

Five stems were collected on the day the other quality characteristics were measured and kept overnight in the dark at 4°C. This course of action was chosen because storing the stems in a freezer at -18°C resulted in freezer burn. Each stem was ground with a mortar and pestle and 0.1 g of each ground stem was put into a 1.7 ml microcentrifuge tube and 1 ml of 80% acetone was added. The suspension was mixed using a shaker for ten min and then centrifuged at 10,000g for five min at 20°C. The supernatant was removed into a glass tube and kept in the dark. To the remaining tissue in the microcentrifuge tube, 1 ml of 80% acetone was added. The extraction was repeated until the tissue had a pale colour. All the supernatants were combined and their absorbance at 647 and 663 nm was measured using a spectrophotometer (UV-160A, Shumadzy Corporation, Japan). The amount of chlorophyll was calculated using Eq 5 as proposed by Wellburn (1994).

$$\text{Chlorophyll } a = 12.25A_{663.2} - 2.79A_{646.8} \quad (\mu\text{g ml}^{-1}) \qquad \text{Eq 5}$$

$$\text{Chlorophyll } b = 21.5A_{646.8} - 5.1A_{663.2} \quad (\mu\text{g ml}^{-1})$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b \quad (\mu\text{g ml}^{-1})$$

$$\mu g \text{ of chlorophyll} / g \text{ fresh weight} = \frac{CV}{W}$$

where

V = the volume of 80% acetone (ml)

C = chlorophyll a or b in Eq 5

W = the weight of sample (g)

3.5.5.3. Polyphenol oxidase (PPO) content

The method was adjusted from Cai *et al.* (2006). Five stems were collected on the day the other quality characteristics were measured and stored overnight in the dark at 4°C. PPO content was measured the following day. Each stem was powdered with a mortar and pestle and 0.2 g of each stem was added to the extraction solution (10 ml of 0.1 M sodium phosphate buffer, pH 6.5 (adjusted with 0.1 NaOH or 0.1 HNO_3), containing 0.1g of polyvinylpolypyrrolidone (PVPP)) and then mixed with a magnetic stirrer for four min at 5°C. The suspension was filtered through No. 1 filter paper and the total amount of the filtrate was measured. The filtrate was transferred into a 1.7 ml microcentrifuge tube and kept in ice to slow down PPO activity. The filtrates were centrifuged at 13,200g for 15 min at 5°C. The supernatant was kept in ice and used for the PPO assay. The supernatant (0.5ml), the substrate (1 ml of 0.1 M catechol) and a buffer (1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5)) were mixed in a glass tube using a vortex and then pipetted into a 4 ml cuvette. The increase in the absorbance at 420 nm at 30°C was measured using a temperature controlled spectrophotometer (UV-160A and TCC-240A, Shimadzy Corporation, Japan) and the enzyme activity was calculated from the linear portion of the curve in the first minute. The enzyme unit was expressed as the change in absorbance of 0.001 per minute. The calculation of PPO activity was shown as Eq 6.

$$\text{Unit enzyme/ml enzyme} = \frac{\Delta A_{420nm} / \min}{(0.001)(0.5)} \qquad \text{Eq 6}$$

0.001 = the change in A_{420nm}/min per unit of PPO in a 3 ml reaction mixture at pH 6.5 at 30°C

0.5 = volume (ml) of enzyme used

$$\text{Unit enzyme/g fresh weight} = \frac{\text{units/ml enzyme}}{\text{g fresh weight/ml enzyme}} \qquad \text{Eq 7}$$

$$\text{g fresh weight/ml enzyme} = \left(\frac{\text{Powdered stems (g)}}{\text{Total extraction (ml)}} \right) \quad \text{Eq 8}$$

3.5.6. Sensory test of passionfruit

Sensory analysis was carried out by the researcher. The juice was used to evaluate sweetness and sourness. Sweetness and sourness were separately rated as follows.

1 = very little sweet/sour

2 = little sweet/sour

3 = sweet/sour

4 = very sweet/sour

3.5.7. Appearance of tamarillo

3.5.7.1. Stem yellowing

Stem yellowing was graded according to the HortResearch scale shown in Figure A- 6 in Appendix E.

0 = no yellowing

1 = slight, less than 25% of yellowing

2 = moderate, 25-50% of yellowing

3 = severe, more than 90% of yellowing

If a rating is higher than 1.5, the fruit is considered commercially unacceptable.

3.5.7.2. Stem blackening

The incidence of stem blackening was graded according to the HortResearch scale shown in Figure A- 7 in Appendix E.

0 = no blackening

1 = slight, less than 25% of blackening

2 = moderate, 25-50% of blackening

3 = severe, more than 90% of blackening

If a rating is higher than 1.5, the fruit is considered commercially unacceptable.

3.5.7.3. Calyx blackening

Calyx blackening was graded according to the HortResearch scale shown in Figure A- 3 in Appendix E.

0 = calyx is healthy and green.

1 = slight, less than 25% of blackening.

2 = moderate, 25-50% of blackening.

3 = severe, more than 90% of blackening.

If a rating is higher than 1.5, the fruit is considered commercially unacceptable.

3.5.7.4. Calyx lifting

Calyx lifting was defined as the separation of the lobes of the calyx from the skin of the fruit and graded according to the HortResearch scale shown in Figure A- 2 in Appendix E.

0 = no lifting, calyx attaches to the skin surface.

1 = slight, 1-2 lobes slightly separate from the skin surface.

2 = moderate, 2-4 lobes moderately separate from the skin surface.

3 = severe, more than 3 lobes fully separate from the calyx.

If a rating is higher than 1.5, the fruit is considered commercially unacceptable.

3.5.7.5. Stem end rots

Stem end rot was defined as a soft brown rot occurring around the base of the stem and under the calyx and was graded according to the HortResearch scale shown in Figure A- 5. in Appendix E.

0 = no rot

1 = slight, less than 25% of the disorder

2 = moderate, 50% of the disorder

3 = severe, more than 90% of the disorder

If a rating is higher than 1, the fruit is considered commercially unacceptable.

3.5.7.6. Body disorders and discoloration

Body disorders and discoloration was graded in four categories according to the HortResearch scale shown in Figure A- 4 in Appendix E.

0 = no disorders and discoloration

1 = slight, 10% of spotting on the fruit surface

2 = moderate, 25-50% of spotting on the fruit surface

3 = severe, more than 50% of spotting on the fruit surface

A rating of more than 1 is considered commercially unacceptable.

3.6. Statistical methods

Data were averaged and mean comparisons were performed using a least significant difference at P=0.05. Statistical procedures were performed using the statistical analysis system (SAS) software version 9.1 (SAS Institute, Cary, NC, US).

CHAPTER 4

PHYSIOLOGICAL AND CHEMICAL CHARACTERISTICS OF PURPLE PASSIONFRUIT

4.1. Introduction

Purple passionfruit has been grown in New Zealand for many years and is currently exported to several markets; however, little information is available about their quality and storage behaviour. At full ripeness, the fruit are often significantly shrivelled but this is not necessarily a sign of decreased quality. Nevertheless, a literature review revealed only a few reports on quality assessment of these fruit. Quantitative evidence is also needed to assist in educating consumers regarding optimal eating quality of the fruit.

Artificial wax has been used to improve the appearance of fruit, decrease water loss, and attain a modified internal atmosphere (Maguire *et al.*, 2001). Also, modified atmosphere packaging (MAP) has been used successfully to maintain fruit quality during long term storage and extend shelf life (Hertog, 2003). However, little information is available on applying wax coating and MAP to purple passionfruit. Passionfruit is a climacteric fruit and will therefore respond to exogenous ethylene. Reducing external ethylene concentrations improves storage life of plum (Valero *et al.*, 2003), tomato (Guillen *et al.*, 2007), and avocado (Hershkovitz *et al.*, 2005) and can possibly extend the postharvest life of passionfruit.

In this chapter, the first objective is to establish objective quality parameters for passionfruit and the second is to test waxing, packaging, and ethylene scavenging to improve storage life.

4.2. Results of the first trial

4.2.1. Observations of disorders and resulting changes in the experiment

Fruit from Bag 1 developed red spots and bleeding after 20 days of storage and the fruit was unacceptable for consumption or sale (Figure 4-1). Hence, there were no further measurements of Bag 1 after 20 days. The concentrations of O_2, CO_2, and ethylene inside Bag 1 were 0.34±0.18%, 27.82±0.49%, and 276.08±44.86 ppm, respectively after 20 days of storage at 8°C. During shelf life after 28 days of cold storage, some fruit in Bag 3 had red spots and white fungal growth, on the fruit surface. During shelf life after 42 days of storage the red spots and fungal growth in Bag 3 was more pronounced, a few fruit in Bag 2 also had red spots and some fruit in Bag 4 had an off-flavour and red spots.

A

B

Figure 4-1. Fruit in Bag 1 (A) development of red spots and bleeding after 20 days of storage at 8°C, (B) development of red spots and white fungus after 20 days of storage with 7 days of shelf life at room temperature (20°C)

4.2.2. Water related measures

4.2.2.1. Water vapour permeance

There were no combination effects of waxing, storage duration, and shelf life on water vapour permeance (WVP) (Table 4-1).

Table 4-1. Influence of waxing, cold storage at 8°C (days), and shelf life at 20°C (days) on water vapour permeance (WVP)

Treatment		WVP (μmol s^{-1} m^{-2} Pa^{-1})
Factor (F)	control	0.10*
	wax	0.09
Storage duration (Sd)	0	1.69a
	20	0.11b
	28	0.09c
	42	0.10c
Shelf life (Sl)	0	0.10NS
	7	0.10
Interactions	F × Sd, Sd × Sl	*
	F × Sl, F × Sd × Sl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

The control had a slightly higher WVP than waxed fruit. WVP significantly decreased in storage in the first 28 days and remained constant thereafter. Shelf life did not cause a change in WVP of the control and waxed fruit after cold storage.

4.2.2.2. Weight loss

Weight loss was significantly affected by waxing, packaging, storage duration, and shelf life (Figure 4-2). The control and waxed fruit lost more weight than the fruit in packaging. The rate of weight loss of the control and waxed fruit was similar and remained high throughout storage duration. The rate of weight loss of packaged fruit was similar and remained low and constant over the storage period. For the control and waxed fruit, weight loss dramatically increased during shelf life; for the fruit in packaging it did not.

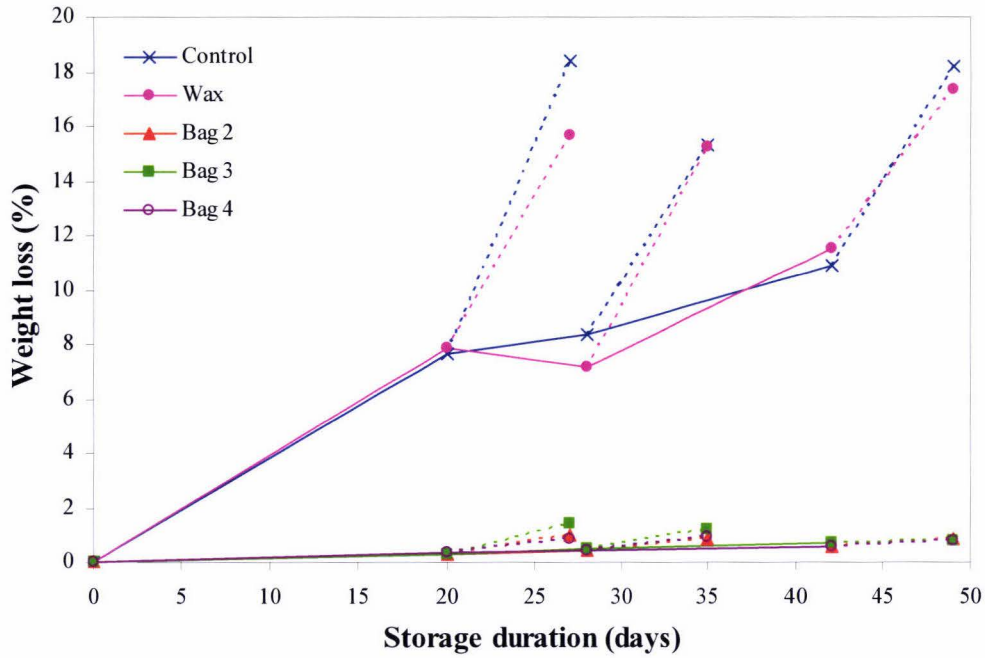

Figure 4-2. Weight loss of the control, waxed fruit, fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 1.1, n = 20 fruit)

Shrivelling occurred in the control and waxed fruit, but not in the packaged fruit. However, packaged fruit did have more disorders and rots than the control and waxed fruit. Shrivelling of the control and waxed fruit first appeared after 20 days of storage at 8°C and gradually increased thereafter. Shelf life storage increased the shrivelling of the control and waxed fruit, but for the packaged fruit it did not.

4.2.3. Gas related measures

4.2.3.1. Respiration

The CO_2 production rate of the control and waxed fruit remained constant during storage (Figure 4-3). Although the CO_2 production rate of the control slightly increased after 20 days of storage, it was not significantly different from the initial CO_2 production rate. The CO_2 production rate of the control and waxed fruit did increase during shelf life. After 28 days of storage, the CO_2 production rate of the control no longer significantly increased during shelf life, whereas it still increased significantly for waxed fruit.

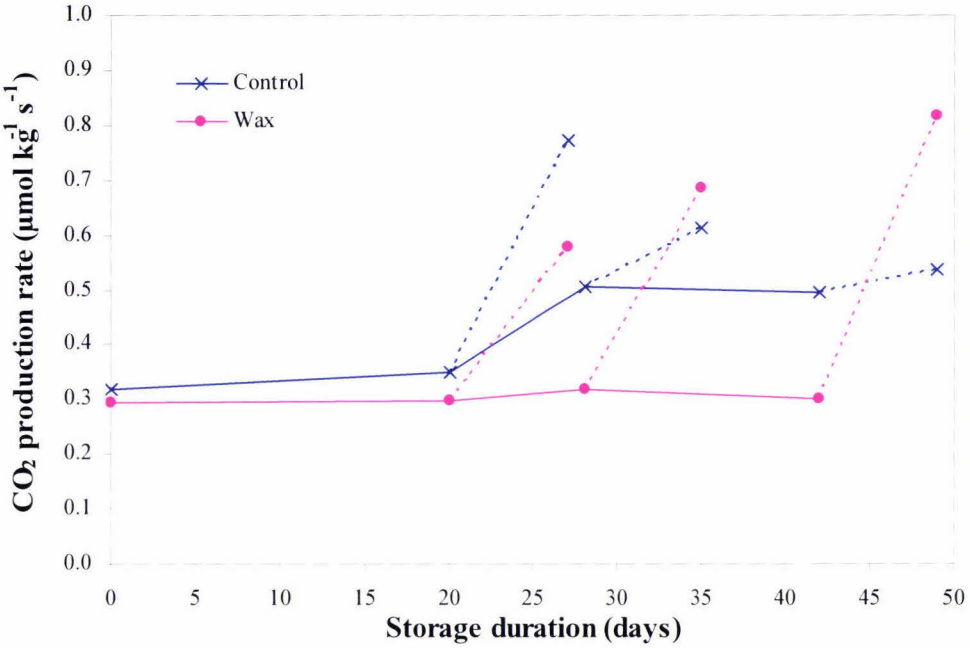

Figure 4-3. CO_2 production rate of the control and waxed fruit during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.05, n = 20 fruit)

The O_2 consumption rate of control and waxed fruit was similar and constant during the first 28 days of storage and increased after 42 days of storage (Table 4-2). Shelf life did cause a significant increase in the O_2 consumption rate.

Table 4-2. Influence of waxing, cold storage at 8°C (days), and shelf life at 20°C (days) on the O_2 consumption rate (rO_2)

Treatment		rO_2 (µmol kg^{-1} s^{-1})
Factor (F)	Control	0.49^{NS}
	Wax	0.44
Storage duration (Sd)	20	0.43b
	28	0.42b
	42	0.55a
Shelf life (Sl)	0	0.40*
	7	0.53
Interactions	F × Sd, F × Sd × Sl	NS
	F × Sl, Sd × Sl	*

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

The respiratory quotient (RQ) of the control and waxed fruit was similar and remained constant during storage at around 1.0 (Figure 4-4).

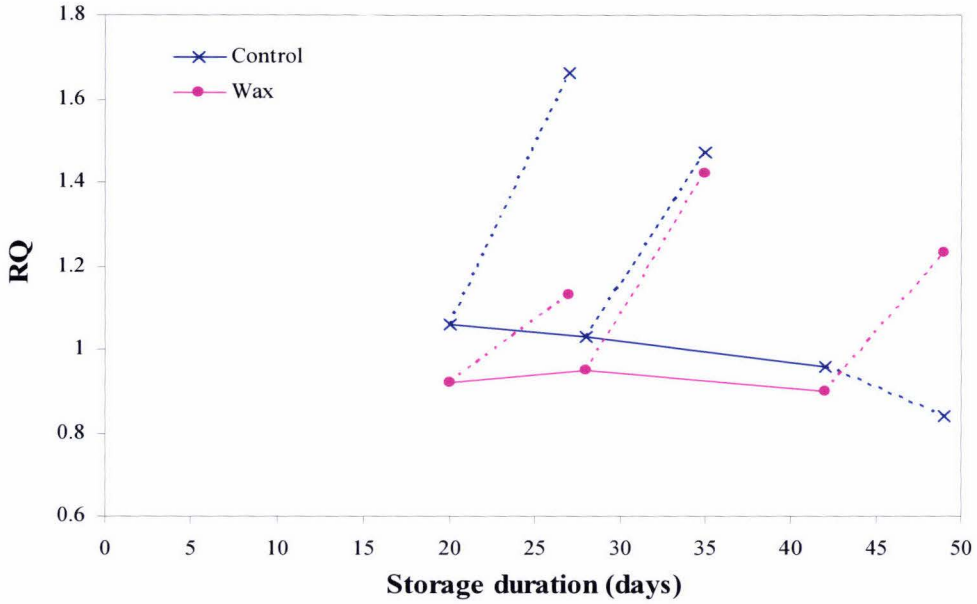

Figure 4-4. The respiratory quotient of the control and waxed fruit during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.23, n = 20 fruit)

After 20 days of storage with seven days of shelf life, the RQ of the control increased to 1.66 and the RQ of waxed fruit increased to 1.42 after 28 days of storage with seven days of shelf life. After 42 days of storage with shelf life, the RQ of the control was lower than that of waxed fruit although it was not significantly different.

4.2.3.2. Gas composition in the bags

The O_2 concentration in the bags depended on the combination of packaging, storage duration, and shelf life (Figure 4-5). The O_2 concentration reduced in all the bags during the first 20 days of storage at 8°C. Thereafter, the O_2 concentration in Bags 2 and 4 remained constant, but that in Bag 3 kept decreasing between 20 and 28 days of storage and remained constant thereafter. During shelf life, the O_2 concentration increased in Bags 2 and 3 after 20 and 28 days of storage, respectively. The O_2 concentration in Bag 4 was not affected by shelf life nor was the O_2 concentration in Bags 2 and 3 after 42 days of storage. The high O_2 concentration measured in Bag 2 after 28 days of storage with seven

days of shelf life was because of a puncture in all five bags due to bad adhesion of the silicone.

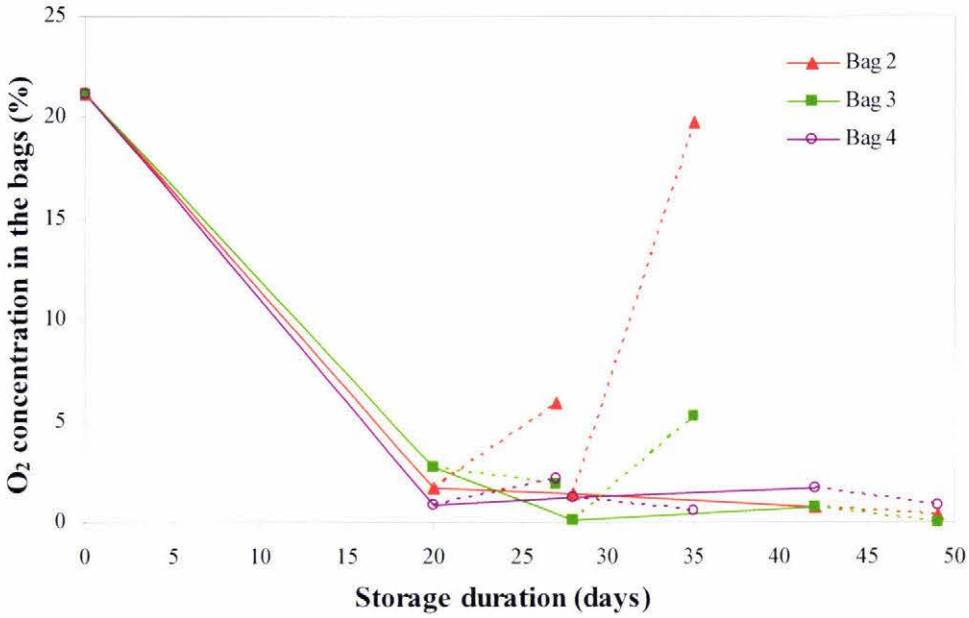

Figure 4-5. O_2 concentration in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.68, n = 2-5 bags)

The CO_2 concentration increased in all the bags during the first 20 days of storage and remained constant thereafter with the highest CO_2 concentration in Bag 3 (Figure 4-6).

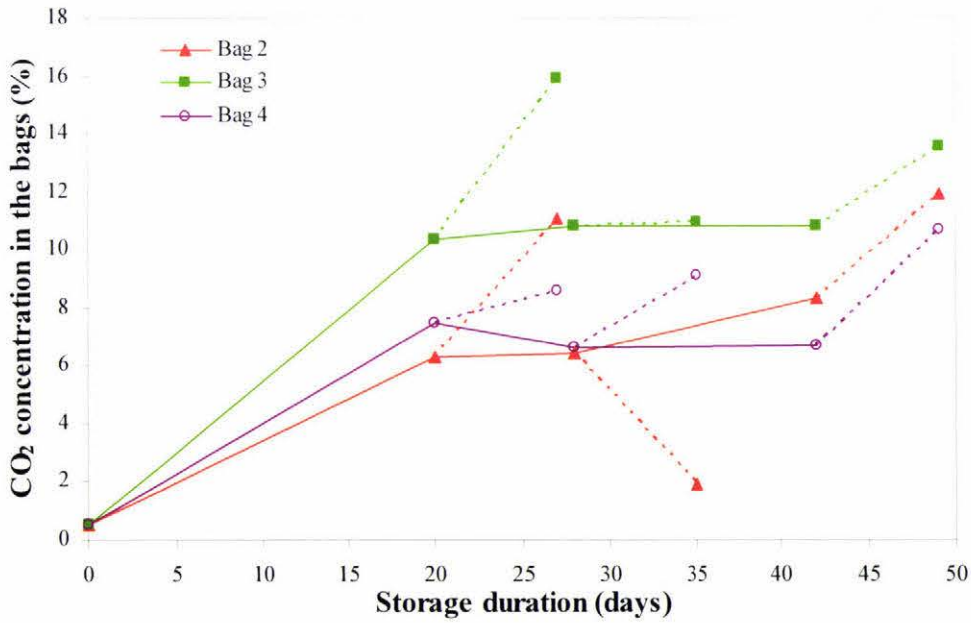

Figure 4-6. CO_2 concentration in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.79, n = 2-5 bags).

The CO_2 concentration in Bags 2 and 4 was similar during the cold storage period. After 42 days of storage, the CO_2 concentration in all the bags differed slightly within the range of 6-12%. The CO_2 concentration increased during shelf life in Bags 2 and 3 after 20 days of storage. After 42 days of storage, shelf life caused a similar increase in CO_2 concentration in all the bags. As mentioned before, Bag 2 had a leak after 28 days of storage with shelf life which explains the decreasing CO_2 concentration.

4.2.3.3. Ethylene production

The ethylene production rate of the control and waxed fruit significantly depended on the combination of storage duration and shelf life ($P<0.05$). The ethylene production rate slightly decreased during storage and increased during shelf life (Figure 4-7). The control and waxed fruit had a similar ethylene production rate. The ethylene production rate significantly increased during shelf life but to a lower level with progressing storage time. The increase in ethylene production rate was slightly faster for control fruit compared to waxed fruit after 28 and 42 days of storage.

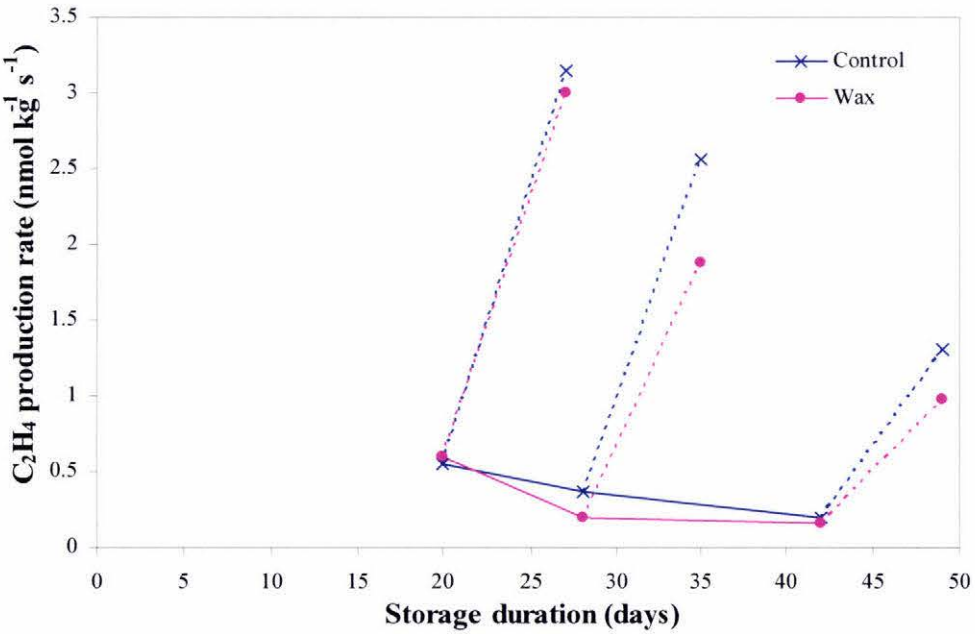

Figure 4-7. Ethylene production of the control and waxed fruit during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.17, n = 20 fruit)

Ethylene concentration in the bags also depended on the combination of storage duration and shelf life ($P<0.05$). Although the ethylene concentration was not significantly different in all packaging, Bag 4 had the highest ethylene accumulation during storage period with or without shelf life (Figure 4-8). The ethylene concentration increased during the first 20 days of storage, and significantly decreased thereafter up to 28 days, after which the decrease was minor.

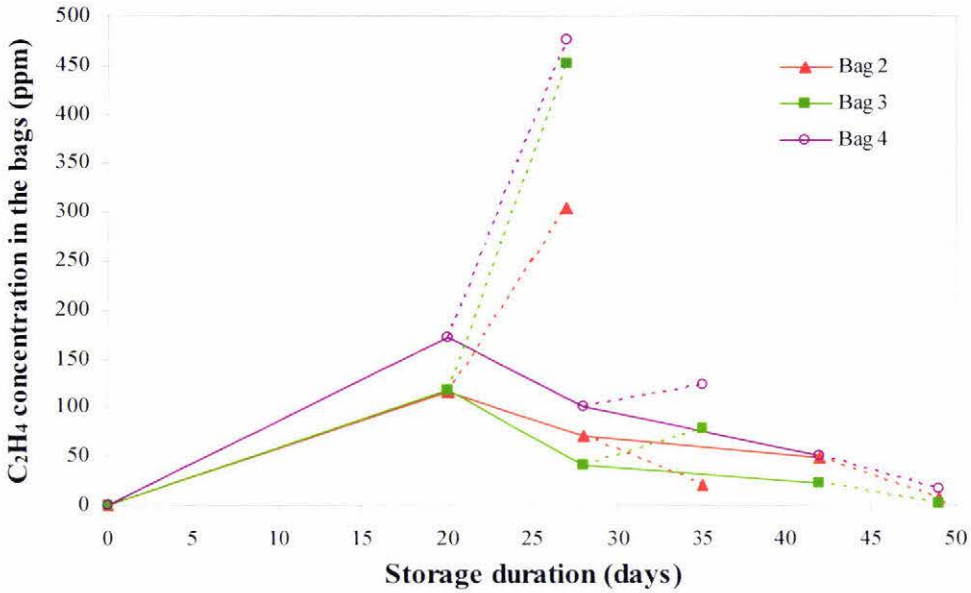

Figure 4-8. Ethylene concentration in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 43.13, n = 2-5 bags)

Shelf life caused a significant increase in the ethylene concentration only after 20 days of storage with a similar increase in the ethylene production for Bags 3 and 4, whereas a smaller increase was seen for Bag 2 (Figure 4-8). After 28 and 42 days of storage at 8°C, the ethylene concentration did not increase during shelf life.

4.2.4. Non destructive measures

4.2.4.1. Colour

All colour parameters depended on waxing, packaging, storage duration, and shelf life (Table 4-3). The control and waxed fruit had similar L^* and a^* values with lower b^*, C^* and h° for the waxed fruit compared to the control. Packaged fruit had higher values for L^*, b^*,

and h° compared to waxed and control fruit. Bags 2 and 4 had lower values for a^* compared to Bag 3 where a^* was similar to the control and waxed fruit. C^* values for Bags 2 and 4 were also lower compared to Bag 3, but similar to that of the control, whereas fruit in Bag 3 had the highest C^* value. All colour parameters of fruit in Bags 2 and 4 were similar except for L^*, which was higher in Bag 2.

Table 4-3. Influence of waxing, packaging (Bags 2, 3, and 4), cold storage at 8°C (days), and shelf life at 20°C (days) on lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*), and hue angle (h°)

Treatment		L^*	a^*	b^*	C^*	h°
Factor (F)	Control	31.97b	2.99a	4.11b	5.22b	53.07c
	Wax	32.05b	3.14a	3.62c	4.88c	48.48d
	Bag 2	33.29a	2.46b	4.60a	5.32b	60.92a
	Bag 3	32.57c	2.91a	4.63a	5.68a	57.25b
	Bag 4	32.72c	2.48b	4.65a	5.39b	61.44a
Storage duration (Sd)	0	32.78^{NS}	1.85c	4.38a	4.87^{NS}	65.79a
	20	32.55	2.69b	4.53a	5.40	58.12b
	28	32.54	2.88ab	4.34a	5.36	55.60c
	42	32.39	2.93a	4.07b	5.17	53.70c
Shelf life (Sl)	0	32.59^{NS}	2.63*	4.32^{NS}	5.17*	57.68*
	7	32.41	2.99	4.31	5.43	54.47
Interactions	F × Sd, F × Sl	*	*	*	NS	*
	Sd × Sl	NS	NS	*	NS	NS
	F × Sd × Sl	NS	NS	NS	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

The positive a^* value of all treatments refers to fruit being more red than green. Waxed fruit appeared to be more red although the a^* value was not significantly higher than the control and fruit in Bag 3 (Table 4-3). Fruit in Bags 2 and 4 were least red. During storage, a^* increased gradually for all fruit, and during shelf life, a^* also increased significantly.

The positive b^* value refers to fruit being more yellow than blue. Fruit in all packaging had the same higher b^*, whereas waxed fruit had the lowest b^* with the b^* value for the control

fruit in the middle (Table 4-3). b^* did not change during the first 28 days of storage, but was slightly lower after 42 days of storage.

C^* or chroma refers to the saturation of colour (pigment of colour). Only main effects of waxing or packaging and shelf life caused differences in the value for C^* (Table 4-3). The highest C^* value was found in Bag 3, whereas that of waxed fruit was the lowest. The control and fruit in Bags 2 and 4 had similar values for C^*. During storage at 8°C, the C^* value remained constant, but C^* increased slightly during shelf life.

L^* was not affected by waxing, packaging, storage duration, or shelf life with values in the range of 32-33 during cold storage and 30.5-34 during shelf life (Figure 4-9). L^* of fruit in all treatments remained constant over the entire storage period, except for fruit in Bag 3, where an increase was seen in the first 20 days and a decrease thereafter bringing L^* at the end of storage (42 days) lower than all other treatments. With an additional seven days of shelf life following 42 days of cold storage, L^* was still higher compared to the control and waxed fruit since L^* of fruit in Bag 3 did not change during shelf life, whereas L^* of waxed and control fruit decreased markedly.

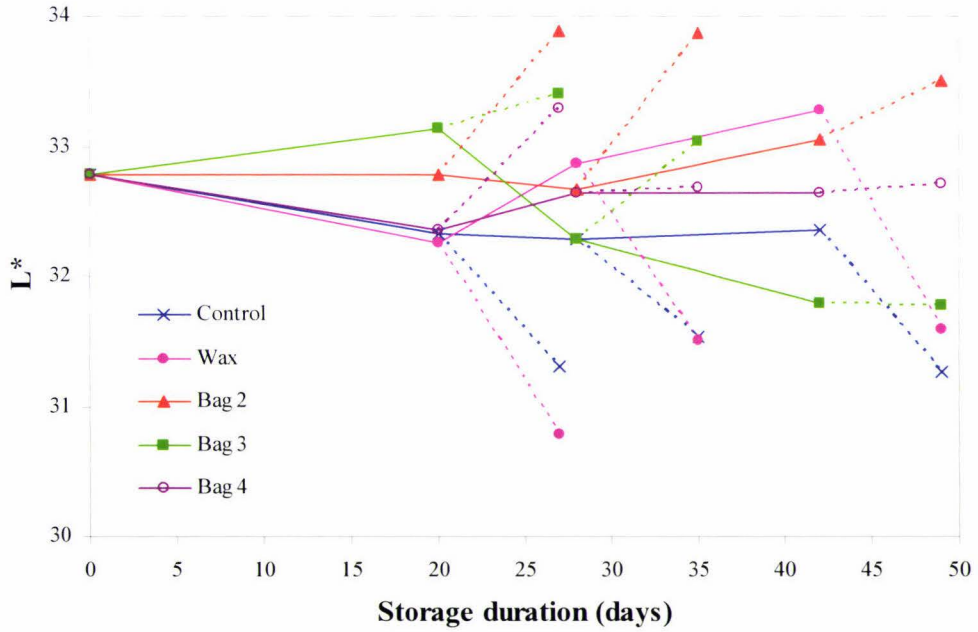

Figure 4-9. Lightness of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.88, n = 20 fruit)

During shelf life L^* seemed to increase for packaged fruit, especially fruit in Bag 2, and decrease for the control and waxed fruit.

h° or hue angle refers to the basic colour of fruit. The h° value of all treatments was between 50° and 70° which puts it in the colour wheel between red (0°) and yellow (90°) (Table 4-3). After 20 and 28 days of cold storage, the h° value of the different treatments were not significantly different except for the two extremes, Bag 3 had a significantly higher h° value compared to waxed fruit (Figure 4-10). The h° values of all treatments were similar after 42 days of storage.

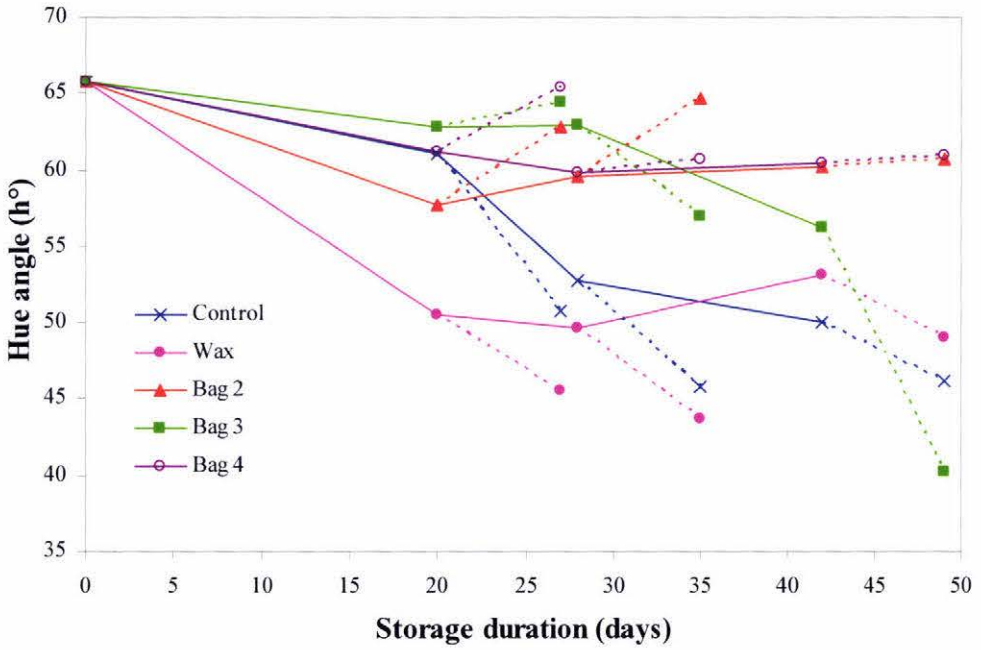

Figure 4-10. The hue angle of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 2.70, n = 20 fruit)

The h° value decreased during storage and increased during shelf life for fruit in Bags 2 and 4, but decreased during shelf life for control and waxed fruit after each storage period (Figure 4-10). The h° value of waxed fruit decreased from 65° to 50° during the first 20 days, whereas the h° value of all the other fruit remained between 60° and 65° (Figure 4-10). After 28 days of storage, the h° values of the control and waxed fruit were similar and considerably (10°) lower compared to the packaged fruit. This difference of about 10° remained up to 42 days of storage. The h° value of fruit in Bag 3 decreased after 28 days of

storage with or with shelf life resulting in an h° value similar to that of the control and waxed fruit after 42 days of storage with or without shelf life.

4.2.4.2. Stiffness

Stiffness of packaged fruit significantly increased during the first 20 days of storage (Figure 4-11), with the biggest increase seen for fruit in Bag 4 followed by Bags 3 and 2, resulting in different stiffness after 20 days of storage. After 20 days, stiffness of packaged fruit decreased at different rates negating the previously found difference. After 28 days of storage, all packaged fruit had similar stiffness, but thereafter stiffness of fruit in Bags 2 and 4 decreased at a higher rate than for fruit in Bag 3 to the same level as the control and waxed fruit but remaining slightly higher compared to the initial stiffness. Loss of stiffness of fruit in Bag 3 was slower from 28 days onwards resulting in significantly higher stiffness after 42 days of storage.

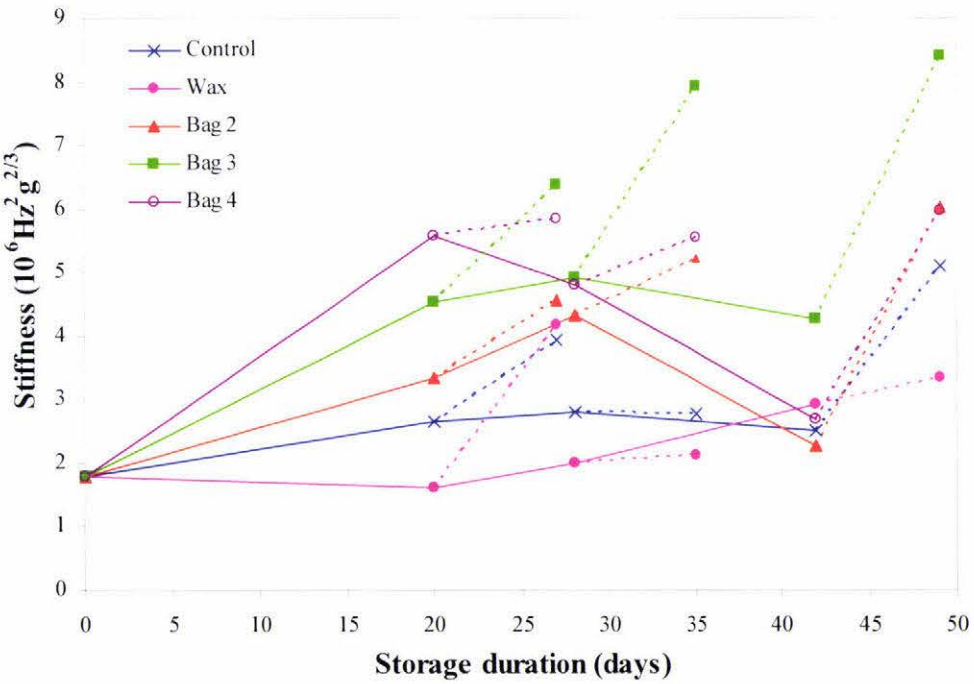

Figure 4-11. Stiffness of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.94, n = 20 fruit)

The control and waxed fruit had lower stiffness than packaged fruit during 28 days of storage (Figure 4-11). Stiffness of the control significantly increased during the first 20

days of storage, while it did not change for waxed fruit. After 20 days of storage, stiffness of waxed fruit gradually increased, whereas stiffness of the control only slightly changed. Stiffness of all treatments significantly increased during shelf life storage at room temperature.

4.2.4.3. Compression firmness

Compression firmness did not significantly change during cold storage for any of the treatments (Figure 4-12). During shelf life, the compression firmness of packaged fruit did not change significantly, but that of control and waxed fruit dramatically decreased, especially after 20 and 28 days of storage.

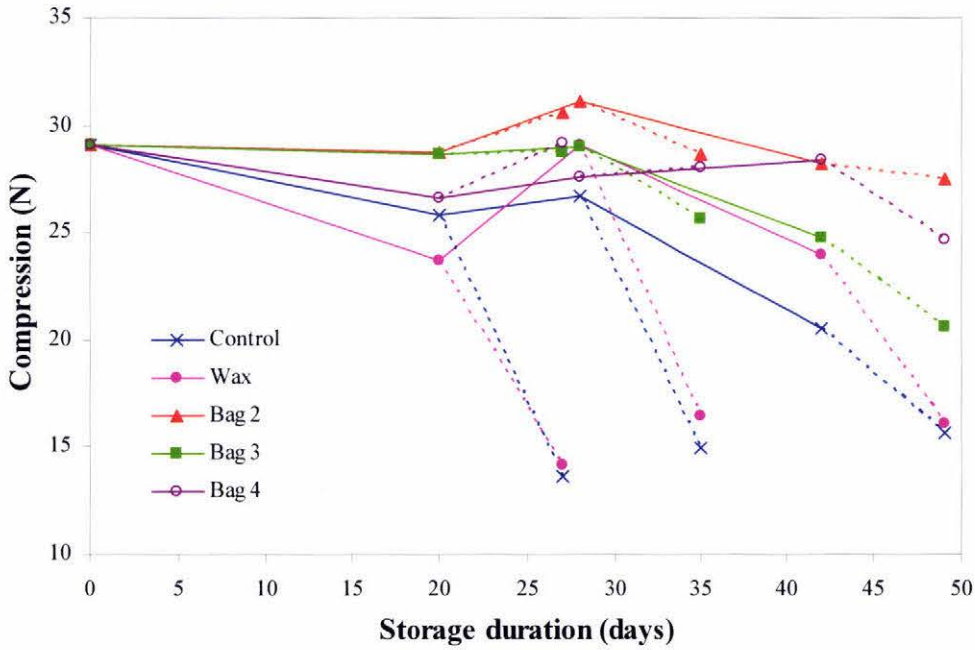

Figure 4-12. Compression firmness of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 1.45, n = 20 fruit)

4.2.5. Pulp and juice characteristics

4.2.5.1. Pulp yield

Pulp yield was measured by comparing the weight of the pulp scooped out with a spoon with the total fruit weight. Pulp yield of the control and waxed fruit was higher than that of

packaged fruit (Table 4-4). Fruit in Bag 4 had similar pulp yield as fruit in Bag 2 and higher pulp yield than fruit in Bag 3. During cold storage, pulp yield gradually decreased, but increased during the seven days of shelf life at room temperature. To determine whether the changes in % pulp yield were due to changes in the pulp or the husk, the absolute pulp weight was also analysed i.e. the actual weight of the scooped out pulp.

Table 4-4. Influence of waxing, packaging (Bags 2, 3, and 4), cold storage at 8°C (days), and shelf life at 20°C (days) on pulp yield and absolute pulp weight

Treatment		Pulp yield (%weight)	Absolute pulp weight (g)
Factor (F)	Control	54.02a	34.08ab
	Wax	54.20a	32.91bc
	Bag 2	49.75bc	34.27a
	Bag 3	48.53c	32.02c
	Bag 4	50.30b	33.97ab
Storage duration (Sd)	0	54.47a	33.70ab
	20	54.06a	35.51a
	28	50.04b	32.33b
	42	49.93b	32.54b
Shelf life (Sl)	0	50.53*	33.66^{NS}
	7	52.21	33.27
Interactions	F × Sd	*	NS
	F × Sl	NS	NS
	Sd × Sl	NS	NS
	F × Sd × Sl	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

The pulp weight of all treatments slightly increased during the first 20 days of storage and decreased thereafter (Table 4-4). After 28 days of storage, the pulp weight remained constant and ended up at a similar value compared to the initial fruit. The control seemed to have a higher pulp weight than waxed fruit although it was not significantly different. The pulp weight of fruit in Bags 2 and 4 was similar and significantly higher than that of fruit in Bag 3. During shelf life, the pulp weight of all treatments did not change.

4.2.5.2. Soluble solids content

Soluble solids content (SSC) was slightly higher in the control and waxed fruit compared to fruit in the bags (Table 4-5). SSC of fruit in Bags 2 and 4 was similar and higher than that of fruit in Bag 3. It decreased during the first 20 days of storage and the decrease slowed down thereafter. SSC slightly decreased during shelf life.

Table 4-5. Influence of waxing, packaging (Bags 2, 3, and 4), cold storage at 8°C (days), and shelf life at 20°C (days) on soluble solids content (SSC)

Treatment		SSC (°brix)
Factor (F)	Control	12.82a
	Wax	12.69ab
	Bag 2	12.34c
	Bag 3	11.94d
	Bag 4	12.41bc
Storage duration (Sd)	0	13.27a
	20	12.67b
	28	12.35c
	42	12.26c
Shelf life (Sl)	0	12.87*
	7	12.00
Interactions	F × Sd	*
	F × Sl, Sd × Sl	NS
	F × Sd × Sl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

4.2.5.3. pH and titratable acidity of the juice

The change in pH of the juice was small at 3.3±0.2 during the cold storage period (Figure 4-13). The pH of the control and waxed fruit did not change during the first 20 days of storage, thereafter it decreased but after 28 days started to increase again, ending up at a higher value compared to the start of the storage period. The pH of packaged fruit decreased during the first 20 days of storage, and thereafter it remained constant for fruit in Bags 2 and 4 but declined for fruit in Bag 3. After 28 days of storage, the pH of fruit in

Bag 3 started to increase ending up at a slightly lower level compared to that of the initial fruit. After 42 days of storage, the pH of the control and waxed fruit was significantly higher than that of packaged fruit. The pH of fruit in Bag 3 was lowest compared to fruit in the other treatments.

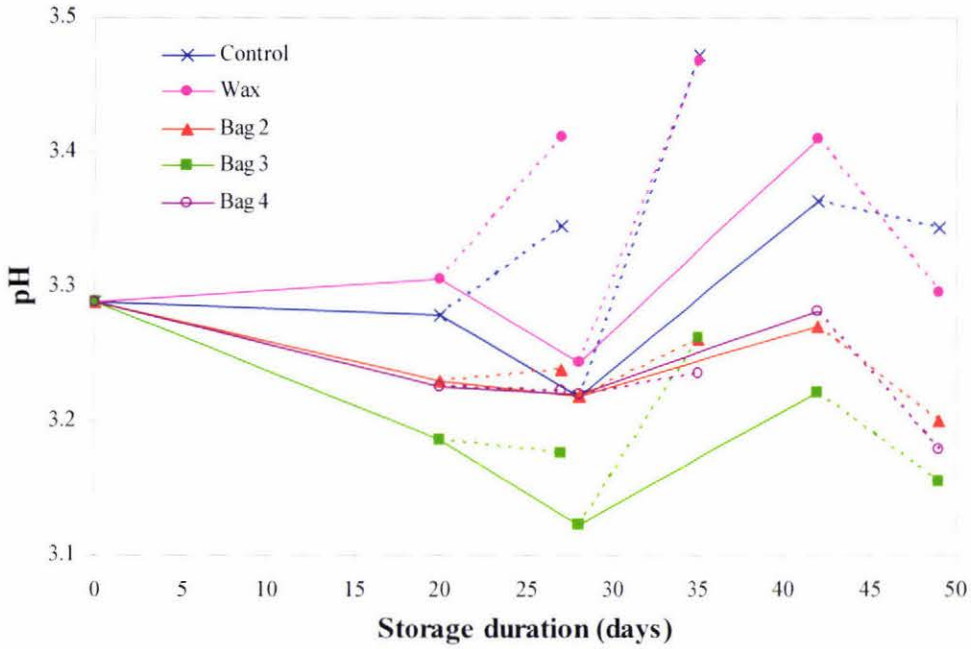

Figure 4-13. pH of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.002, n = 20 fruit)

Whereas the pH of the packaged fruit was not affected by shelf life, the pH significantly increased during shelf life for the control and waxed fruit after 20 and 28 days of cold storage, but after 42 days of storage, the apparent pH decrease during shelf life was not significant (Figure 4-13).

Titratable acidity (TA) was affected by the combination of waxing, packaging, storage duration, and shelf life (Figure 4-14). During the first 20 days of storage, TA of fruit in Bag 3 did not change, but significantly decreased for the other treatments. Thereafter, TA of all treatments decreased although not significantly. After 28 days of storage, TA of packaged fruit slightly increased again but not to the initial level, whereas it decreased for the control and waxed fruit resulting in a significantly higher TA of packaged fruit compared to non-packaged fruit after 42 days of storage.

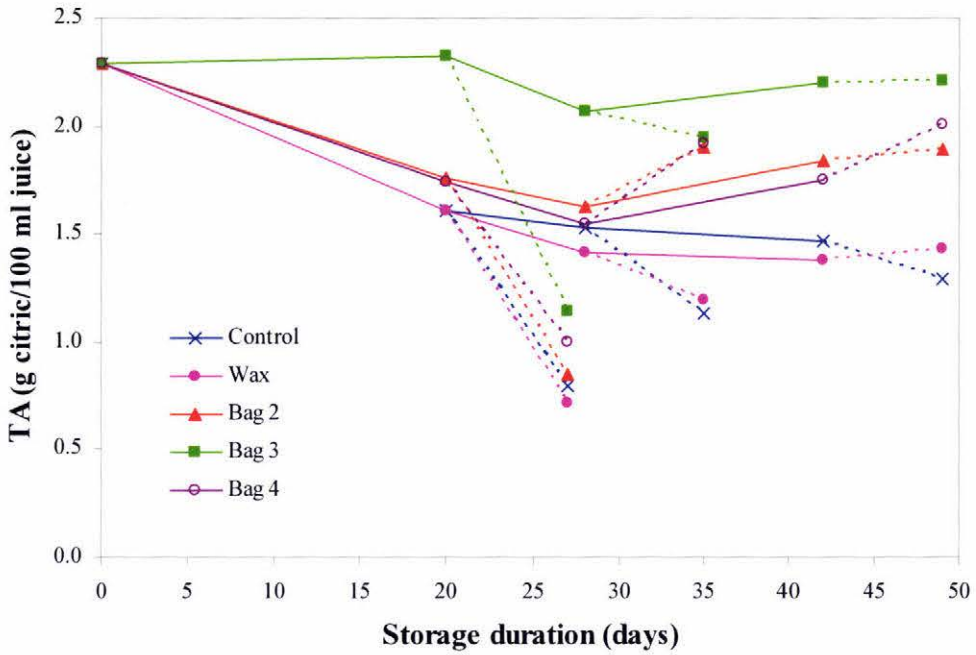

Figure 4-14. Titratable acidity of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.04, n = 20 fruit)

TA of fruit in Bag 3 seemed to remain highest during cold storage but after 42 days of storage it was not significantly different from that of fruit in Bags 2 and 4 (Figure 4-14). During shelf life following 20 days of cold storage, TA noticeably decreased for fruit in all treatments and final TA was at similar for all treatments, but for longer storage periods no significant change in TA during shelf life was noted. During shelf life after 28 and 42 days of storage, there was a slight but insignificant increase in TA for Bags 2 and 4, whereas TA for control and waxed fruit decreased resulting in a significant difference between packaged and non-packaged fruit after shelf life at that point.

4.2.5.4. The ratio of SSC to TA

The ratio of SSC to TA or sweet/acid ratio sometimes gives a better impression of the changes in taste. The SSC/TA ratio was affected by the combination of waxing, packaging, storage duration, and shelf life (Figure 4-15). The SSC/TA ratio of fruit in Bag 3 was lowest over the entire cold storage period. The control, waxed fruit, and fruit in Bags 2 and 4 had a similar SSC/TA ratio during cold storage. After 20 days of cold storage, the SSC/TA ratios of all treatments dramatically increased during shelf life.

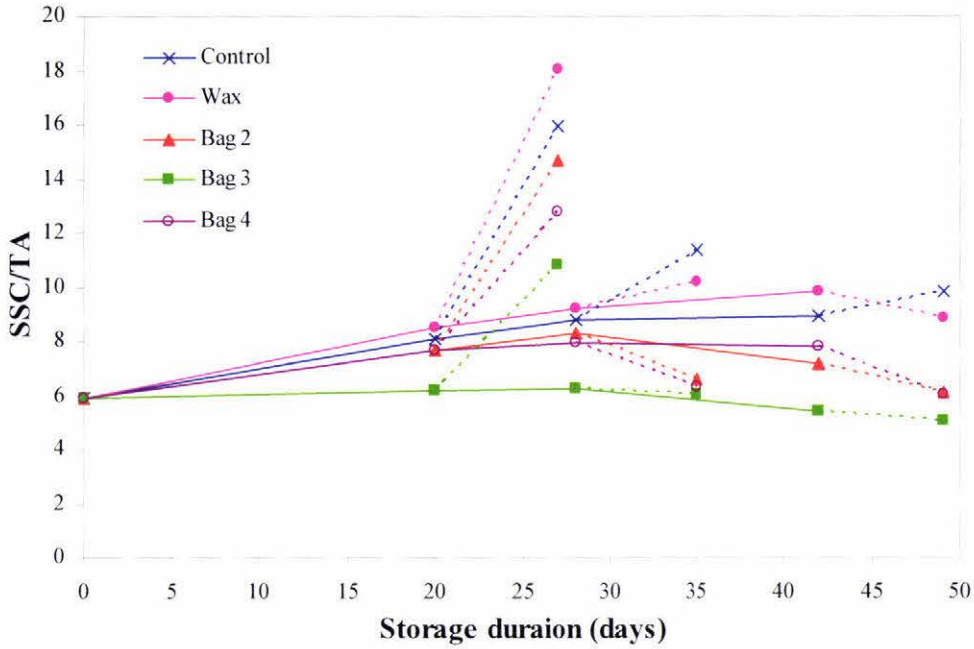

Figure 4-15. The SSC/TA ratio of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 3.0, n = 20 fruit)

After 28 days of storage, the SSC/TA ratios of the control and waxed fruit slightly increased during shelf life, while those of fruit in Bags 2 and 4 decreased during shelf life and that of Bag 3 remained constant, resulting in a clear difference in SSC/TA ratios between packaged fruit and non-packaged fruit (Figure 4-15). After 42 days of storage, the SSC/TA ratio remained constant during shelf life for all treatment, but the SSC/TA ratio of the control and waxed fruit was still higher than those of packaged fruit.

4.2.6. Sensory test

Fruit pulp and juice was also tasted by the researcher and scored for sweetness and sourness. Sensory sweetness of the control and waxed fruit increased faster than that of packaged fruit during the first 28 days of storage and changed at the same rate thereafter (Figure 4-16). Sweetness of fruit in Bag 2 was similar to that of fruit in Bag 4 after 20 days of storage, and gradually increased to the same level as the control and waxed fruit by 42 days of storage, while sweetness of fruit in Bag 4 still remained lower. Sweetness of fruit in Bag 3 appeared lowest over the entire storage period but was not significantly lower than that of fruit in the other bags. During shelf life, sweetness of the packaged fruit did

not significantly change except for Bag 3 after 20 days of storage, whereas sweetness of the control and waxed fruit dramatically increased after 28 days of storage with the control fruit having the highest score.

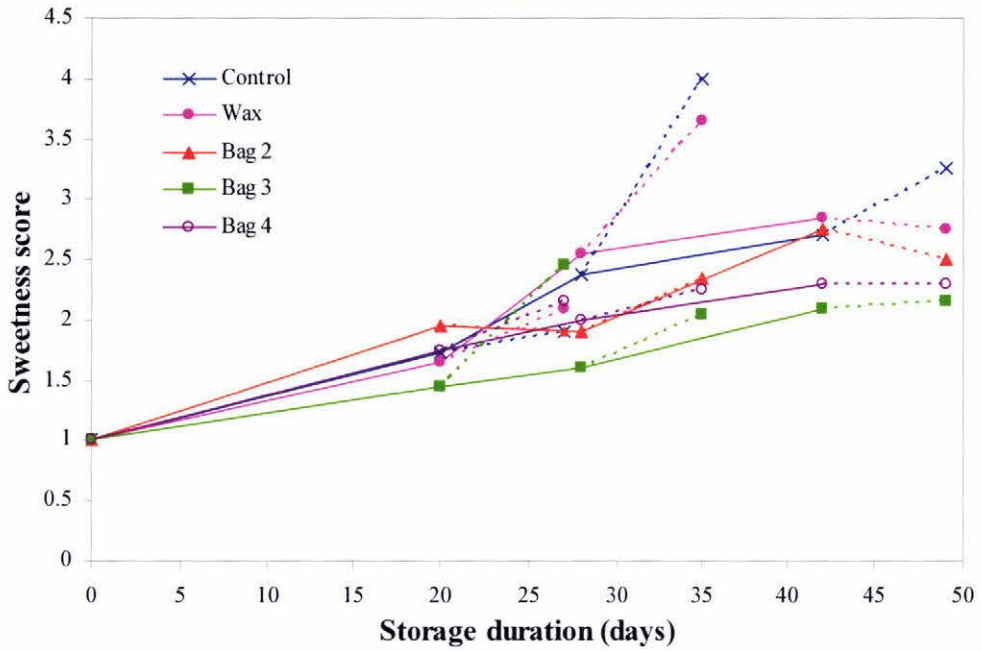

Figure 4-16. Sweetness score of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.11, n = 20 fruit)

Sourness decreased at the same rate for all treatments during the first 20 days of cold storage, after 28 days of storage the rate of decrease increased except for fruit in Bag 3 where sourness remained constant (Figure 4-17). After 28 and 42 of cold storage, the sourness of the different treatment was not significantly different except for the two extremes, Bag 3 had a significantly higher sourness compared to waxed fruit. Shelf life storage caused an additional decrease in sourness score for the control, but did not significantly affect sourness of the other treatments after 28 and 42 days of cold storage.

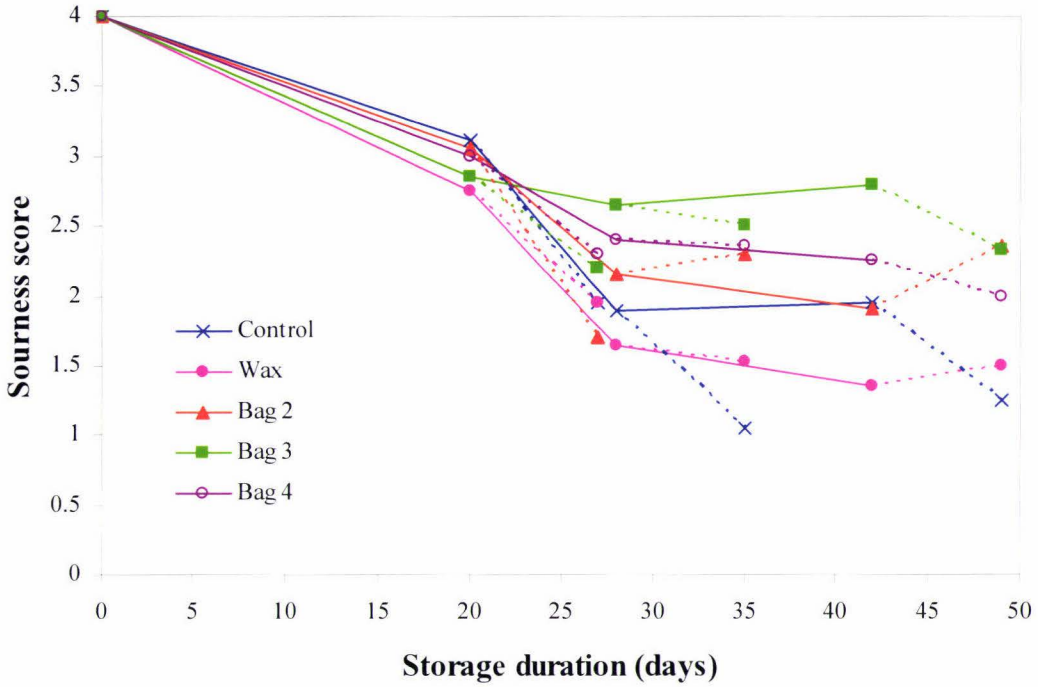

Figure 4-17. Sourness score of the control, waxed fruit, and fruit in Bags 2, 3, and 4 during storage at 8°C without shelf life (—), and with 7 days of shelf life at 20°C (---). (s.e. = 0.15, n = 20 fruit)

4.3. Results of the second trial

Since the fruit of the first trial with the exception of Bag 1 was still in good condition at the end of the trial, we wanted to see if the storage duration could be extended. Therefore, a second trial was carried out. Additionally, the quality of fruit in Bags 2 and 4 was similar in the first trial but after 42 days of storage with seven days of shelf life, the juice from fruit in Bag 4 had an off-flavour, possibly due to more ethylene accumulation in Bag 4. To assess this, an ethylene scavenger was added into Bag 4 in the second trial.

L^*, b^*, C^*, h°, compression firmness, and stiffness were similar between the initial fruit of the first trial and the second trial (Table 4-6). The a^* value, SSC, pH, TA, and sourness score of the initial fruit of the second trial were lower than those of the first trial, whereas respiration rate and sweetness score were higher. The pulp yield of the initial fruit of the second trial was lower than that of the first trial although it was not significantly different.

Table 4-6. Quality of the passionfruit at harvest for the first and second trials

	Initial fruit	
Quality parameters	First trial	Second trial
Lightness (L*)	32.78^{NS}	32.73
Redness (a*)	1.85^{*}	1.43
Yellowness (b*)	4.38^{NS}	4.02
Chroma (C*)	4.87^{NS}	4.32
Hue angle (h°)	65.79^{NS}	68.65
Respiration rate (rCO_2, μmol kg^{-1} s^{-1})	0.31^{*}	0.41
Stiffness ($10^6 Hz^2 g^{2/3}$)	1.79^{NS}	2.37
Compression firmness (N)	29.07^{NS}	29.73
Pulp yield (%weight)	54.47^{NS}	48.16
SSC (°brix)	13.27^{*}	12.02
pH	3.29^{*}	3.09
TA (g citric/100 ml juice)	2.29^{*}	1.99
Sweetness score	1.00^{*}	1.5
Sourness score	4.00^{*}	2.65

NS, Non significant or *, significant *T* test at $P<0.05$ respectively

4.3.1. Observation of disorders and resulting changes in the experiment

In Figure 4-18 we show fruit in Bag 3 after 49 days of storage.

Figure 4-18. Fruit in Bag 3 after 49 days of cold storage at 8°C

The fruit developed unacceptable red juicy bleeding and extensive white fungus growth. Therefore, Bag 3 was removed from the trial and will thus not be discussed any further. Fruit in Bag 2 also developed large indentations in the fruit surface and a white powder-like (not resembling fungal growth) substance appeared on the surface after 70 days of storage with four days of shelf life (Figure 4-19). Thus, there were no quality measurements of Bag 2 at the end of the seven days of shelf life period following 70 days of cold storage.

Figure 4-19. Fruit in Bag 2 after 70 days of storage at 8°C with 4 days of shelf life at 20°C

After 70 days of cold storage with seven days of shelf life, fruit in two of the five bags of Bag 4 had bleeding, large indentations, and the same white powder-like substance and were unacceptable (Figure 4-20). However, fruit in three bags of Bag 4 still looked healthy.

Figure 4-20. Fruit in Bag 4 with $KMnO_4$ after 70 days of cold storage at 8°C with 7 days of shelf life at 20°C (left) fruit with bleeding, large indentations, and white powder-like substance in 2 bags and (right) healthy fruit in 3 bags

4.3.2. Weight loss

Weight loss was similar for Bags 2 and 4 and increased significantly between 42 and 70 days of storage at 8°C (Table 4-7). During shelf life, the weight loss in Bag 4 significantly increased.

Table 4-7. The weight loss of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	Weight loss (%)	
(days)	Bag 2	Bag 4+$KMnO_4$
42	0.64*	0.56
70	1.01	1.01
70 + 7 days of shelf life	-	1.38*

* significant F test at $P<0.05$

4.3.3. Gas composition in the bags

The O_2 and C_2H_4 content was not significantly different for Bag 2 and Bag 4 and remained constant during cold storage at 8°C (Table 4-8). However, C_2H_4 accumulation in the bags of the second trial was much lower compared to the first trial. Additional shelf life in Bag 4 after 70 days of storage did not change the O_2 and C_2H_4 concentrations.

Table 4-8. O_2, CO_2, and C_2H_4 content in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	O_2 (%)		CO_2 (%)		C_2H_4 (ppm)	
(days)	Bag 2	Bag 4 + $KMnO_4$	Bag 2	Bag 4 + $KMnO_4$	Bag 2	Bag 4 + $KMnO_4$
42	3.84^{NS}	1.18	7.00*	8.50	0.10^{NS}	1.89
70	3.38	4.21	9.39	9.12	1.38	0.70
70+7 days of shelf life	-	3.56^{NS}	-	8.63^{NS}	-	1.38^{NS}

NS, Non significant or *, significant F test at $P<0.05$ respectively

After 42 days of storage, the CO_2 concentration in Bag 2 was lower than that in Bag 4 and significantly increased after from 42 to 70 days of storage (Table 4-8). No significant increase was observed in Bag 4. Because of this the CO_2 concentration was similar for Bag 2 and Bag 4 after 70 days of storage. Shelf life caused a slight but not significant decrease in CO_2 concentration in Bag 4 after 70 days of storage.

4.3.4. Non destructive measures

4.3.4.1. Colour

L^*, a^*, and b^* were similar for fruit in Bag 2 and Bag 4 and remained constant during storage (Table 4-9). During shelf life, only a^* of fruit in Bag 4 decreased, while L^* and b^* did not significantly change.

Table 4-9. The values of lightness (L^*), redness (a^*), and yellowness (b^*) of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	L^*		a^*		b^*	
(days)	Bag 2	Bag 4+ $KMnO_4$	Bag 2	Bag 4+ $KMnO_4$	Bag 2	Bag 4+ $KMnO_4$
42	32.31^{NS}	32.78	1.76^{NS}	1.71	4.99^{NS}	4.49
70	30.73	31.61	2.20	2.28	4.25	4.21
70+7 days of shelf life	-	33.72^{NS}	-	1.10^{*}	-	5.06^{NS}

NS, Non significant or *, significant F test at $P<0.05$ respectively

C^* and h° were similar in Bag 2 and Bag 4 and also remained constant during storage (Table 4-10). During shelf life, the h° value of fruit in Bag 4 significantly increased leading to a higher final value compared to the initial fruit, but C^* did not.

Table 4-10. The values of chroma (C^*) and hue angle (h°) of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	C^*		h°	
(days)	Bag 2	Bag 4+$KMnO_4$	Bag 2	Bag 4+$KMnO_4$
42	5.36^{NS}	4.85	69.06^{NS}	68.29
70	4.97	5.00	60.51	59.76
70+7 days of shelf life	-	5.22^{NS}	-	77.26^{*}

NS, Non significant or *, significant F test at $P<0.05$ respectively

4.3.4.2. Stiffness

Stiffness of fruit in Bag 2 was higher than that of fruit in Bag 4 although the difference was not significant (Table 4-11). Stiffness of fruit in Bag 4 slightly decreased from 42 to 70 days of storage and increased, but not significantly, during subsequent shelf life.

Table 4-11. Stiffness of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	Stiffness ($10^6 Hz^2 g^{2/3}$)	
(days)	Bag 2	Bag 4+$KMnO_4$
42	6.62^{NS}	4.59
70	6.32	3.30
70+7 days of shelf life	-	4.81^{NS}

NS, Non significant *F* test at $P<0.05$

4.3.4.3. Compression firmness

Compression firmness of fruit in Bag 2 and Bag 4 was similar and decreased from 42 to 70 days of storage although the decrease was not significant for Bag 2 and Bag 4 (Table 4-12). During shelf life after 70 days of cold storage, compression firmness of fruit in Bag 4 increased, although not significantly.

Table 4-12. Compression firmness of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	Compression (N)	
(days)	Bag 2	Bag 4+$KMnO_4$
42	31.17^{NS}	30.66
70	28.53	25.88
70+7 days of shelf life	-	30.59^{NS}

NS, Non significant *F* test at $P<0.05$

4.3.5. Pulp and juice characteristic

4.3.5.1. Pulp yield

Pulp yield of fruit in Bag 2 and Bag 4 was similar after 42 and 70 days of cold storage and did not change during shelf life at 20°C after 70 days of cold storage (Table 4-13).

Table 4-13. Pulp yield of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	Pulp yield (%weight)	
(days)	Bag 2	Bag 4+$KMnO_4$
42	48.24^{NS}	48.71
70	48.32	45.75
70+7 days of shelf life	-	45.27^{NS}

NS, Non significant F test at $P<0.05$

4.3.5.2. Soluble solids content

Soluble solids content of fruit in Bag 2 and Bag 4 was similar and slightly decreased between 42 and 70 days of storage although the change was not significant for Bag 2 and Bag 4 (Table 4-14). During shelf life after 70 days of storage, SSC of fruit in Bag 4 remained constant.

Table 4-14. Soluble solids content (SSC) of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	SSC (°brix)	
(days)	Bag 2	Bag 4+$KMnO_4$
42	10.44^{NS}	10.54
70	9.73	9.50
70+7 days of shelf life	-	9.48^{NS}

NS, Non significant F test at $P<0.05$

4.3.5.3. pH and titratable acidity of the juice

The pH of fruit in Bag 2 and Bag 4 was similar and constant from 42 to 70 days of storage (Table 4-15). Titratable acidity (TA) of fruit in Bag 2 and Bag 4 was also similar, but decreased between 42 and 70 days of storage. During shelf life after 70 days of cold storage, the pH of fruit in Bag 4 did not change, whereas TA significantly increased bringing a higher value compared to the initial fruit.

Table 4-15. pH and titratable acidity (TA) of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	pH		TA (g citric/100 ml juice)	
(days)	Bag 2	Bag 4+$KMnO_4$	Bag 2	Bag 4+$KMnO_4$
42	3.24^{NS}	3.27	1.74*	1.76
70	3.34	3.27	1.22	1.28
70+7 days of shelf life	-	3.21^{NS}	-	2.40*

NS, Non significant or *, significant F test at $P<0.05$ respectively

4.3.5.4. The SSC to TA ratio

The SSC/TA ratio of fruit in Bag 2 and Bag 4 was similar and increased between 42 and 70 days of storage, but only the increase in Bag 2 was significant (Table 4-16). During shelf life after 70 days of cold storage, the SSC/TA ratio of fruit in Bag 4 markedly decreased.

Table 4-16. The SSC/TA ratio of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	SSC/TA	
(days)	Bag 2	Bag 4+$KMnO_4$
42	6.18*	6.35
70	8.73	7.84
70+7 days of shelf life	-	4.12*

* significant F test at $P<0.05$

4.3.6. Sensory

Sweetness and sourness scores of fruit in Bag 2 and Bag 4 were similar (Table 4-17). However, sweetness significantly decreased after 42 days of storage, whereas sourness slightly increased although not significantly. During shelf life after 70 days of storage, sweetness and sourness remained the same.

Table 4-17. The scores of sweetness and sourness of fruit in Bag 2 and Bag 4 with $KMnO_4$ during storage at 8°C for 42 days and for 70 days with or without 7 days of shelf life at 20°C

Storage duration	Sweetness score		Sourness score	
(days)	Bag 2	Bag 4+$KMnO_4$	Bag 2	Bag 4+$KMnO_4$
42	2.50*	2.25	1.90^{NS}	1.50
70	1.17	1.52	2.17	2.10
70+7 days of shelf life	-	1.63^{NS}	-	2.04^{NS}

NS, Non significant or *, significant F test at $P<0.05$ respectively

4.4. Discussion

One of the big problems during storage and export of purple passionfruit is excessive shrivelling due to water loss. Although this shrivelling does not change the pulp quality, it does affect the appearance of the fruit which is very important in consumer purchase decisions. A high respiration rate and ethylene production of passionfruit (Pruthi, 1963) also lead to a shorter shelf life for storage and transportation to both local and export markets. Hence, wax coating, modified atmosphere packaging (MAP), and an ethylene scavenger were applied in this study to reduce shrivelling and improve storage life. The influence of these treatments on changes in fruit characteristics and physiology were assessed through observation and sensory tests, as well as the measurement of respiration, ethylene production, colour, texture, soluble solids content (SSC), pH, titratable acidity (TA), and the SSC/TA ratio.

Passionfruit in the second trial was more mature than fruit in the first trial as fruit had lower SSC, pH, TA, and sourness with higher respiration rate and sweetness. In general, passionfruit at different stages of maturity does not show marked difference in SSC, the SSC/TA ratio, and acidity (Pruthi, 1963). Shiomi *et al.* (1996b) showed that less mature fruit (70 days after flowering) has a lower respiration than more mature fruit (90 days after flowering) after harvest. Hence, the different maturity of passionfruit may have had an effect on the quality parameters in the second trial.

4.4.1. Water related measures

As with other fruits, weight loss of passionfruit mainly depends on transpiration and respiration (Pruthi, 1963). For unpackaged passionfruit, weight loss in this study was lower at 8°C (7%) and higher at 20°C (18%), and increased with an increase in storage time similar to the linear increase in weight loss with storage time of passionfruit stored at 25°C (10-25%) found by Shiomi *et al.*(1996b). Weight loss of packaged fruit was much lower than that of unpackaged fruit but there was no difference between the different packaging materials. In the second trial, the addition of an ethylene absorber ($KMnO_4$) to Bag 4 did not affect weight loss. Excessive loss of water can cause a shrivelled appearance (Maguire *et al.*, 2001). In this study, the control and waxed fruit shrivelled increasingly with cold storage duration and even more obviously during shelf life at room temperature. However, fruit packed in Bags 2, 3, and 4 did not shrivel even during shelf life at room temperature.

Water vapour permeance is a characteristic of the fruit describing the ease with which water vapour can move through the skin and is related to the cuticle structure of the fruit. The cuticle is the outermost layer of the fruit covering the epidermis and consists of a cutin and wax layer (Maguire *et al.*, 2001). Adding an extra layer of artificial wax can often be used to improve the appearance of fruit, decrease the water vapour permeance and hence the water loss from the fruit as well as attain a modified internal atmosphere (Wills *et al.*, 1998; Maguire *et al.*, 2001). Although the water vapour permeance of the waxed fruit in this study was slightly lower than that of the control fruit, the similar weight loss and the development of shrivelling of the control and waxed fruit during storage and shelf life may indicate that the wax applied by wiping it on instead of traditional dipping did not effectively reduce the permeance of the skin of the passionfruit to water vapour. Hence, the application of waxing in this study did not prevent weight loss of passionfruit.

The decrease in water loss and shrivelling of the packaged fruit in this study is similar to findings in yellow passionfruit (Arjona *et al.*, 1994), where film-wrapped fruit shrivelled less than non-wrapped fruit. Thus, packaging reduces the weight loss and maintains the appearance and weight of the fruit during long term cold storage at 8°C, and extends shelf life at room temperature (20°C). The faster weight loss during shelf life is due to an

increase in temperature which increases transpiration and respiration (Maguire *et al.*, 2001) as well as the permeability of the packaging material (Hertog, 2003).

In the second trial, all fruit in Bag 2 developed large indentations in the fruit surface and a white powder-like substance, possibly due to *Fusarium oxysporum* (Pruthi, 1963), appeared on the surface (Figure 4-19) after only four days at room temperature following 70 days of storage, whereas only 40% of fruit in Bag 4 with the ethylene absorber was affected after seven days of shelf life. Possibly, the addition of an ethylene absorber enhances the effect of the packaging in extending shelf life. No difference in weight loss was observed between Bag 2 and Bag 4 with the ethylene absorber, this is similar to finding in strawberries (Picon *et al.*, 1993), where fruit packaged in MAP with or without an ethylene absorber had a similar weight loss.

4.4.2. Gas related measures

4.4.2.1. Respiration

Passionfruit is a climacteric fruit and its respiration rate increases with an increase in temperature (Pruthi, 1963; Shiomi *et al.*, 1996b). Pruthi (1963) noted that passionfruit reaches the climacteric peak at around 0.64-1.72 μmol CO_2 kg^{-1} s^{-1} at the end of four to five weeks of storage at 6.5°C. The climacteric peak refers to the onset of the mechanism of fruit ripening, when respiration increases with an increase in ethylene production (Wills *et al.*, 1998). In this study, the respiration rates of the control and waxed fruit were low at 0.45 and 0.31 μmol CO_2 kg^{-1} s^{-1}, respectively during 42 days of storage at 8°C.

The respiratory quotient (RQ) is used to characterise the balance between aerobic and anaerobic respiration and remained constant and around 1 during storage for the control and waxed fruit. This indicates aerobic respiration as the rate of O_2 uptake was equal to the rate of CO_2 production. After 20 days of cold storage, the respiration rate of the control fruit increased to 0.77 μmol CO_2 kg^{-1} s^{-1} during shelf life at room temperature; that of the waxed fruit remained below 0.6 μmol CO_2 kg^{-1} s^{-1}, but was not significantly different from the control. As a result, the RQ of the control increased earlier than that of the waxed fruit when stored at room temperature indicating an accelerated respiratory metabolism leading

to a lower O_2 availability inside the fruit (Lippert & Blanke, 2004). Hence, the control fruit possibly started ripening earlier than the waxed fruit when stored at room temperature.

4.4.2.2. Gas composition in the bags

Bag 1 had the lowest O_2, and highest CO_2 and C_2H_4 concentrations compared to the other bags after 20 days of cold storage which is due to the lower permeability values compared to the other bags. As fruit respires, the O_2 in the package is used up and more CO_2 is produced; however, the lower permeability of Bag 1 caused the slowest replacement of O_2 and slowest release of CO_2. Thus, the atmosphere inside Bag 1 would quickly have a very low concentration of O_2 and high concentration of CO_2. The levels of O_2 and CO_2 inside Bag 1 probably exceeded the tolerance limits of passionfruit within 20 days of cold storage at 8°C, causing tissue injury and resulting in the development of disorders such as red spots and bleeding on the fruit surface and making the fruit susceptible to fungal attack. The development of disorders on the fruit surface in this study is also similar to circular, sunken, and brown spots on the passionfruit surface caused by *Alternaria passiflorae* (Rodriguez-Amaya, 2003). Moreover, the high ethylene concentration in Bag 1 possibly accelerated and/or intensified fruit senescence. In avocado fruit, the higher ethylene accumulation in a PE bag corresponded to higher decay development (Pesis *et al.*, 2002).

During cold storage at 8°C, Bags 2, 3, and 4 had an atmosphere with low O_2 (1-3%) and high CO_2 (6-12%) concentrations. The depletion of O_2 in the different packaging was similar, but the increase in CO_2 of Bag 3 was much higher than that of Bags 2 and 4. This is related to the characteristics of the packaging as Bags 2 and 4 had the higher permeabilities, and Bag 3 had the lowest permeability of the bags. The depleted O_2 and enriched CO_2 in the packaging mainly results from the gas exchange characteristic of the packaged fruit coupled with the packaging material applied (Chen *et al.*, 2000). During shelf life at room temperature, the concentrations of O_2 (2-5%) and CO_2 (9-16%) increased. In general, a change in temperature influences the respiratory metabolism of the fruit and also the rate of gases penetrating through the packaging material (Hertog, 2003), but the respiratory mechanism of fruit is more sensitive to a change in temperature than the permeability of packaging film (Kader *et al.*, 1989). Hence, the different atmosphere

developing inside the packaging of passionfruit during storage at 8°C and 20°C may indicate a higher activation energy for passionfruit respiration compared to that for film permeability to gas exchange. Concentrations of O_2 and CO_2 in packaging should decrease and increase, respectively at higher temperature as an increased temperature accelerates O_2 consumption and CO_2 production (Hertog *et al.*, 1998). The increase in O_2 and decrease in CO_2 concentration in the package during shelf life after 28 days of cold storage, especially in Bag 2, was presumed to be due to a leak in the bags.

The development of low O_2 and high CO_2 concentrations in MAP is expected to inhibit the metabolism of the produce and micro-organism growth (Hertog, 2003). Although the rates of respiration and ethylene production of the packaged fruit were not directly measured, the fact that the rates of O_2 decrease and CO_2 increase in the packaging levelled out after 20-28 days indicates that respiration had slowed down. As for micro-organism growth inhibition, during shelf life after 28 days of cold storage, some fruit in Bag 3 had red spots and white fungal growth, *Fusarium oxysporum* (Pruthi, 1963), on the fruit surface. These incidences became serious during shelf life after 42 days of cold storage. Also, during shelf life a few fruit in Bag 2 were observed to have red spots after 42 days of storage. Some fruit in Bag 4 had an off-flavour and red spots after 42 days of storage with seven days of shelf life. Possibly, critically low O_2 and high CO_2 levels for fruit were reached in Bag 3 due to the low permeability, and the fruit was under more stress and as a result, more susceptible to attacks by micro organisms. Tissue of fruit stored under low O_2 and high CO_2 levels for extensive periods of time has been found to initiate anaerobic respiration or fermentation, which causes off-flavours (Kader *et al.*, 1989; Wills *et al.*, 1998). The development of off-flavours in fruit in Bag 4 during shelf life following 42 days of cold storage may indicate that the extremely low O_2 (0.85%) and high CO_2 (10.67%) levels at room temperature could have induced anaerobic respiration. Also, the development of physiological disorders during shelf life following 42 days of cold storage may indicate that abiotic stress caused by low O_2 and high CO_2 inside the package made the fruit in Bag 3 more prone to physiological disorders than those in Bags 2 and 4.

In the second trial, the levels of O_2 (1-4%) and CO_2 (7-9%) in Bag 2 and Bag 4 were similar to those in the first trial. In apple, the addition of an ethylene absorber reduced

respiration resulting in a lower CO_2 and higher O_2 concentrations in the packaging (Shorter *et al.*, 1992). Passionfruit is climacteric meaning that in general respiration of passionfruit increases concurrent with an increase in ethylene production during ripening (Pruthi, 1963). In other research on passionfruit, the application of ethylene after harvest did enhance fruit colour development (Arjona & Matta, 1991) and increase O_2 uptake (Pruthi, 1963) indicating that passionfruit is sensitive to external ethylene. However, in these experiments, after 42 days of cold storage, the concentrations of O_2 and CO_2 in Bag 4 (with $KMnO_4$) were similar to those in Bag 4 (without $KMnO_4$) in the first trial. This indicates that the addition of an ethylene absorber, and thus reduction of the ethylene concentration inside the packaging, did not lower respiration and did not result in lower CO_2 and higher O_2 concentrations inside the packaging. However, ethylene production was 100 times smaller in the second trial compared to the first trial and this could be the reason why there was no difference in respiration. Additionally, a study of kiwifruit (Ben-Arie & Sonego, 1985) demonstrated that the reduction of the CO_2 concentration inside the packaging with an ethylene absorber resulted from the different permeability of the packaging rather than a reduced respiration rate. Thus, further investigation of the effect of an ethylene absorber on O_2 and CO_2 consumption of passionfruit is warranted.

4.4.2.3. Ethylene production

The pattern of ethylene production of the control and waxed fruit in this study agreed with Pruthi (1963) where ethylene production peaked around 20 days after harvest and reduced thereafter. Ethylene production is decreased by low temperatures (Zamorano *et al.*, 1994) as seen during storage at 8°C. In contrast, the higher temperature (20°C) during shelf life resulted in an increase in the ethylene production rate. Therefore, ripening of passionfruit is delayed by cold storage and accelerated when fruit is warmed up again. Waxed fruit had a lower level of ethylene production compared to the control fruit in this study but the storage and shelf life effects were similar. This concurs with findings by Jeong *et al.* (2003) that the application of wax did not change the pattern of ethylene production and respiration of avocado, but reduced total ethylene production.

Low O_2 and high CO_2 concentrations also reduce ethylene production as O_2 is required to activate 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, which oxidises ACC to ethylene (Saltveit, 1999). High CO_2 is thought to interfere in ethylene activity as it may accumulate in the intercellular space and compete with ethylene for its binding site (Yang, 1985) or high CO_2 itself reduces the availability of ethylene, as it did in tomato (de Wild *et al.*, 2005). The reduction in the concentration of ethylene in the packaging is a result of a decrease in general metabolism by low O_2 and high CO_2 concentrations in the packaging during storage. Fruit in Bag 3 showed disorders and fungal growth earlier than fruit in Bags 2 and 4, although Bag 3 had a lower ethylene concentration compared to Bag 4. Possibly, the low O_2 and high CO_2 levels in Bag 3 had more influence on passionfruit quality than the low ethylene concentration. Ethylene concentration significantly increased in all packaging during shelf life due to the higher temperature (Kader *et al.*, 1989).

In the first trial, ethylene concentration in Bag 4 was insignificantly higher compared with the others, therefore an ethylene absorber was added in the second trial resulting in similar ethylene accumulation in Bag 2 and Bag 4. However, the accumulation of ethylene was much lower in all packaging in the second trial. Fruit in Bag 2 developed disorders within four days of shelf life following 70 days of cold storage, whereas 60% of fruit in Bag 4 with an ethylene absorber was still healthy. Since there was no difference in ethylene concentration between packaging types, the explanation for this difference in disorder development might be that the ethylene sachet absorbed some of the excess moisture (Ben-Arie & Sonego, 1985). This warrants further research into the optimal moisture level for passionfruit storage and the benefits of an ethylene absorber or relative humidity regulation system in the packaging.

4.4.3. Non destructive measures

4.4.3.1. Colour

Waxing increased redness of the fruit and gave it more shine, reflected in the higher lightness value compared with the control fruit. The fast decline of hue angle of waxed fruit during shelf life represented the rapid change from light to dark red peel colour due to the degradation of anthocyanin (Pruthi, 1963) as also found in the control fruit. Waxing

can create a modified internal atmosphere (Maguire *et al.*, 2001) which may slow down fruit ripening. In avocado fruit, waxing did not delay fruit ripening but it did maintain green peel colour, possibly due to the modified atmosphere inside the waxed fruit (Jeong *et al.*, 2003). However, waxing by wiping in this study did not delay the change of colour of passionfruit.

Colour development of fruit in the packaging, especially Bags 2 and 4, was slower compared to the control and waxed fruit as indicated by the higher hue angle and yellowness, and lower redness. The hue angle of fruit in Bags 2 and 4 decreased only slightly indicating a very subtle and slow change from light to dark red skin. Moreover, during shelf life the lightness and the hue angle of fruit in Bags 2 and 4 did not decrease as fast as for the control and waxed fruit. Therefore, we can conclude that Bags 2 and 4 effectively delay colour development of passionfruit during long term cold storage at 8°C and delayed the development of full colour during shelf life at room temperature (20°C).

The colour parameters of fruit in Bag 2 and Bag 4 were similar in both trials except for lightness in the first trial which was slightly lower for Bag 4. In the first trial, redness increased in all treatments during shelf life, whereas it decreased in Bag 4 with the ethylene absorber. In tomato, the change in redness was reduced in fruit with inhibited ethylene action (Guillen *et al.*, 2007). The hue angle of fruit in Bags 2 and 4 in the first trial did not change during shelf life, while it increased in fruit in Bag 4 with the ethylene absorber. This is similar to results for avocado fruit (Hershkovitz *et al.*, 2005) where fruit with inhibited ethylene action retained more green colour indicated by higher lightness, chroma, and hue angle. This indicates that the addition of an ethylene absorber to the packaging may also delay full colour development of passionfruit.

4.4.3.2. Firmness

Stiffness indicates fruit texture by measuring the acoustic impulse response of fruit after tapping it with a light plastic rod. Stiffness of packaged fruit increased during the first 28 days of storage at 8°C but then decreased again, whereas stiffness of non-packaged fruit only marginally changed during storage at 8°C. During shelf life, stiffness increased in all treatments. A decrease in stiffness correlates with a decrease in fruit firmness of tomato

(Schotte *et al.*, 1999), pear (Gomez *et al.*, 2005), mandarin (Wang *et al.*, 2006), and avocado (Galili *et al.*, 1998). Change in stiffness is influenced by temperature, water vapour pressure deficit, and relative humidity factors that also affect water loss (Hertog *et al.*, 2004a). Packaged fruit had a higher stiffness than non-packaged fruit, related to the lower water loss in packaged fruit. Additionally, stiffness increased during shelf life at room temperature concurrent with an increase in weight loss in contrast to other fruit where stiffness dramatically decreases at higher temperature and water vapour pressure deficit with low relative humidity as they raise water loss from the fruit (Hertog *et al.*, 2004a). Higher water loss causes lower turgor pressure in the cells and thus lower stiffness. At the end of the experimental period, stiffness of all treatments was higher than that of the initial fruit, while a decrease in stiffness is normally seen for instance in tomato (Schotte *et al.*, 1999), pear (Gomez *et al.*, 2005), mandarin (Wang *et al.*, 2006), and avocado (Galili *et al.*, 1998). Additionally, stiffness increased during shelf life; this is possibly related to an increase in water loss due to an increase in temperature. From the pulp yield measurements we can conclude that the water loss was mainly situated in the peel of the passionfruit, more specifically the whitish inner layer of the peel or the outermost layer of the rind making the peel tougher due to desiccated cells. Additionally, in watermelons, stiffness increases when internal voids appear (Diezma-Iglesias *et al.*, 2004). However, an internal void was observed in non-packaged fruit more than packaged fruit in this study. Concluding, all these factors make that the measurement of stiffness is not a good predictor for the quality of passionfruit. Waxing had no influence on stiffness which is expected since waxing in this study did not decrease water loss. Stiffness of fruit was not influenced by packaging material, neither did it change because of the addition of an ethylene absorber.

Contrary to stiffness, compression firmness of passionfruit reduced during storage and markedly decreased during shelf life, especially in the control and waxed fruit. Compression force has the same relation with firmness and water loss as stiffness and is also influenced by temperature, water vapour pressure deficit, and relative humidity factors that also affect water loss (Hertog *et al.*, 2004a). Thus, compression firmness appears a better predictor for the quality of passionfruit than acoustic firmness. Compression firmness of packaged fruit, especially in Bag 2, remained higher than that of the non-

packaged fruit during cold storage with or without shelf life due to the lower weight loss of packaged fruit. Bag 2 maintained the highest average compression firmness in the first trial, but in the second trial, the addition of an ethylene absorber to Bag 4 resulted in a similar compression firmness to fruit in Bag 2. Additionally, compression firmness of fruit in Bag 4 with the ethylene absorber did not change during shelf life following 70 days of storage. Similar positive effects of an ethylene absorber added to modified atmosphere packaging was found in kiwifruit (Ben-Arie & Sonego, 1985). This indicates that the addition of an ethylene absorber to packaging can enhance the effect of packaging on delaying softening of passionfruit and extending shelf life.

4.4.4. Pulp and juice characteristics

4.4.4.1. Pulp yield

The higher pulp yield of the non-packaged fruit compared to the packaged fruit in this study was similar to a previous study where film-wrapped fruit had a lower pulp yield than unwrapped fruit (Arjona *et al.*, 1994). Arjona *et al.* (1994) reasoned that the higher pulp yield of non-wrapped fruit resulted from a higher weight loss and in this study, the non-packaged fruit also had a higher weight loss than the packaged fruit. In yellow pitaya fruit, weight loss of the fruit was mainly related to an increase in water loss from the peel, while the pulp was still accumulating water (Nerd & Mizrahi, 1999). The non-packaged fruit had a significantly higher pulp yield compared to packaged fruit, but the absolute pulp weight was not different among treatments showing that the main difference between the treatments was located in the weight of the peel with the non-packaged fruit loosing more water from the peel than the packaged fruit. The gradual decrease in pulp yield during storage is a result of the general weight loss in this study. The increase in pulp yield during shelf life can be explained by the increase in water loss from the peel during shelf life due to the higher temperature (Hertog *et al.*, 2004a). The absolute pulp weight did not change during shelf life, whereas the pulp yield increased. This indicates that the water loss during shelf life was mainly situated in the peel, making the contribution of the peel to the total weight decrease.

4.4.4.2. Soluble solids content

Soluble solids content (SSC) was slightly higher for non-packaged fruit compared to packaged fruit contrary to similar SSC found in film wrapped and non-wrapped yellow passionfruit (Arjona *et al.*, 1994). A decrease in SSC during cold storage and shelf life is found in most fruit and is a result of the general use of these soluble solids as a substrate for the general metabolism of fruit that is separated from the tree like a conversion of sugar to energy and carbon dioxide in the respiratory system (Wills *et al.*, 1998). The greater reduction in SSC during shelf life is due to an increase in metabolic rate with the increase in temperature (Arjona *et al.*, 1992).

The addition of the ethylene absorber to Bag 4 did not change the reduction in SSC during storage; however, SSC of fruit in Bag 4 with the ethylene absorber remained constant during shelf life following 70 days of storage. Nevertheless, ethylene did not influence the total sugar contents in purple passionfruit (Arjona & Matta, 1991) or kiwifruit (Ben-Arie & Sonego, 1985) in previous studies.

4.4.4.3. pH and titratable acidity of the juice

An increase in pH and decrease in TA is found in most fruit and is a result of the use of acids as a substrate for the general metabolism of fruit that is separated from the tree like the tricarboxylic acid cycle, a reaction in the respiratory system (Wills *et al.*, 1998). The higher pH and lower TA of the non-packaged fruit compared to the packaged fruit resulted from a higher respiration rate. The lowest pH and the highest TA was found in Bag 3 and is associated with the lowest O_2 and highest CO_2 atmosphere inside Bag 3 which would inhibit metabolism more than Bags 2 and 4. The increase in pH and the reduction in TA of the non-packaged fruit during shelf life were relative to an increase in temperature raising the respiration rate. However, shelf life did affect the pH and TA of packaged fruit; the packaging, especially Bags 2 and 4, delayed the reduction of organic acids during long term storage with additional shelf life.

The addition of the ethylene absorber to Bag 4 did not affect pH or TA similar to previous results for kiwifruit (Ben-Arie & Sonego, 1985). In this study, TA of fruit in Bag 4 with an

ethylene absorber also reduced similarly to that of fruit in Bag 2 from 42 to 70 days of storage. However, TA of fruit in Bag 4 with the ethylene absorber doubled during shelf life following 70 days of storage similar to findings in tomato (Wills & Ku, 2002) where reduction of ethylene perception by 1-MCP treatment inhibited the loss of TA. Possibly, the addition of an ethylene absorber did not affect the reduction of TA of fruit in the packaging during cold storage, but did reduce the loss of TA during shelf life.

4.4.4.4. The ratio of SSC to TA

The SSC/TA ratio is one parameter to define maturity or ripening index (Wills *et al.*, 1998). The SSC/TA ratio was constant for all treatments during storage at 8°C due to a decrease in both SSC and TA. Shelf life caused an increase in the SSC/TA ratio of all treatments after 20 days of storage due to a greater decrease in TA compared to SSC. During shelf life after 28 days of storage, the SSC/TA ratio increased for non-packaged fruit due to a greater loss of TA compared to SSC; decreased for packaged fruit in Bags 2 and 4 due to a higher TA, but did not affect the SSC/TA ratio of fruit in Bag 3. The lowest SSC/TA ratio of fruit was found in Bag 3 which had the lowest SSC and highest TA. Relating the SSC/TA ratio to the sensory test in this study, the control and waxed fruit had the highest SSC/TA ratios (11.36 and 10.21, respectively) resulting in the highest sweetness and the lowest sourness score. The much lower SSC/TA ratio of packaged fruit, between 5 and 7 corresponds with lower sweetness and higher sourness scores. The SSC/TA ratio may be a good tool to predict the eating quality of passionfruit with the best flavour around 10-11. This is also the case for other fruits. An increase in the SSC/TA ratio correlates to an increase in consumer perception of sweetness of sweet cherries (Guyer *et al.*, 1993). Also, the SSC/TA ratio increases with ripening and is used to determine an optimum flavour quality of strawberry within the range of 10-12 (Kafkas *et al.*, 2007), tomato within the range of 9-10 (Guillen *et al.*, 2007), and table grape within the range of 55-60 (Valverde *et al.*, 2005).

In the first trial, fruit in Bags 2 and 4 had a similar SSC/TA ratio. The addition of the ethylene absorber to Bag 4 in trial 2 did not cause a difference. However, the increase in

the SSC/TA ratio of fruit in Bag 4 with the ethylene absorber was slightly slower than that of fruit in Bag 2 during cold storage at 8°C and was mainly due to a decrease in TA.

4.4.5. Sensory test

The similar sweetness and sourness between the control and waxed fruit indicated that waxing did not delay the change of taste of passionfruit in this study. The lower sweetness and higher sourness of the packaged fruit compared to the non-packaged fruit was related to lower SSC and higher TA, respectively. Fruit in Bag 3 had the lowest sweetness and the highest sourness as they had the lowest SSC and pH, and the highest TA. Sweetness and sourness of fruit in Bags 2 and 4 were similar with and without the addition of the ethylene absorber. However, fruit in Bag 4 had an off-flavour during shelf life following 42 days of storage, while fruit in Bag 2 did not. Based on this information, Bag 2 appears the best packaging to maintain sweetness and sourness of passionfruit in long term cold storage with additional shelf life. During shelf life after 70 days of storage, 60% of fruit from Bag 4 with an ethylene absorber was still healthy without an off-flavour, whereas this was not the case for fruit from Bag 2. Although the addition of an ethylene absorber did not affect the flavour of passionfruit, it may have eliminated the off-flavour development. The increase in sensory sweetness score during storage seems contrary to the SSC decrease. Generally, the measurement of SSC and TA refers to sweetness and sourness, respectively with a higher SSC corresponding with more sweetness and a higher TA with more sourness (Harker *et al.*, 2002). However, in sensory tests, sourness has been shown to overshadow the sweetness sensation, and as sourness decreased with a decrease in TA during storage, sweetness was unmasked similar to the study of Shiomi *et al.*(1996a) where eating quality of purple passion fruit improved mainly due to a decrease in acidity.

4.5. Conclusion

To assess quality changes of passionfruit during storage, compression firmness is a better tool than stiffness as compression firmness reduces during cold storage and shelf life, whereas stiffness does not. Titratable acidity is a better tool than soluble solids content to predict eating quality of passionfruit because titratable acidity reduces during storage

resulting in an increase in perceived sweetness and a decrease in sourness. The SSC/TA ratio may be a good tool to identify the optimal eating quality of passionfruit with the best flavour around 10-11.

The application of waxing and different types of packaging (Bag 1; SC-WO-23338, Bag 2; MK-WO-23339, Bag 3; BL-WO-23339, and Bag 4; BH-WO-23339) was investigated as a technique to prolong postharvest life of passionfruit and reduce shrivelling. Waxing did not improve the quality of passionfruit as water vapour permeance, water loss, redness, stiffness, compression firmness, pulp yield, SSC, pH, TA, and sweetness were similar to those of the control fruit. Packaging reduced weight loss, slowed down the changes in colour, pH, TA, sweetness, and sourness, maintained fruit firmness, and extended shelf life. Also, packaged fruit did not develop a shrivelled appearance. However, the very low permeability of Bag 1 caused the highest accumulation of CO_2 and ethylene, and the lowest O_2 with the development of bleeding, red spots, and fungal growth after only 20 days of storage. This rules out Bag 1 as an appropriate packaging to extend the postharvest life of passionfruit. Although O_2 and ethylene concentrations in Bags 2, 3, and 4 were similar, Bag 3 had a higher accumulation of CO_2 and when transferred to room temperature after 28 days of storage, fruit developed red spots and fungal growth earlier in Bag 3 than in Bags 2 and 4. Fruit in Bags 2 and 4 behaved similarly during storage. However, in Bag 4 more ethylene accumulated and fruit developed an off-flavour during shelf life following 42 days of storage, while few fruit in Bag 2 had red spots.

The addition of the ethylene absorber to Bag 4 did not cause a different atmosphere to develop inside the packaging nor did it change the behaviour of the fruit. However, fruit in Bag 2 could not be kept for more than four days at room temperature after 70 days of storage as fruit had large indentations and a white power-like substance on the fruit surface. In contrast, 60% of fruit in Bag 4 with the ethylene absorber was healthy with lower redness and SSC/TA ratio, higher hue angle and TA, and constant lightness, yellowness, chroma, firmness, pulp yield, SSC, pH, sweetness, and sourness during seven days of shelf life following to 70 days of storage. Thus, the addition of an ethylene absorber in the packaging may have a major effect on extending shelf life after storage up

to 70 days. Nevertheless, further study is necessary to elucidate the effect of an ethylene absorber or moisture absorber in the packaging on the quality of passionfruit.

CHAPTER 5

PHYSIOLOGICAL AND CHEMICAL CHARACTERISTICS OF 'MULLIGAN RED' TAMARILLO FRUIT AND STEM

5.1. Introduction

Tamarillo fruit is harvested and packed with the stems attached and the export standard requires stems to be intact and green (Sale & Pringle, 1999). As fruit ripens, its skin develops a full red colour, firmness decreases and the stem changes in colour from green to yellow and eventually detaches. Many postharvest treatments are aimed at reducing fungal infections of tamarillo while preserving their healthy appearance, but they do not successfully inhibit stem blackening, which affects the acceptance by the market.

MAP has been used successfully in other products and maintains fruit and vegetable quality during long term storage and extends shelf life (Hertog, 2003). However, no study has investigated the effect of MAP on the quality of tamarillo fruit and stem.

The addition of an essential oil like eugenol, which is a major component in clove oil (Burt, 2004), successfully improves the quality of fruit and stem of cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006). Hence, the addition of an essential oil may intensify an effect of MAP on preserving tamarillo fruit and stem quality.

The first objective of this chapter is to investigate the effect of MAP with or without the addition of clove oil on storage life of tamarillo fruit and stem. The second objective aims at determining the mechanism behind stem discoloration.

5.2. Observations of disorders and resulting changes in the experiment

Fruit in Bag 2 (Figure 5-1A) and Bag 3 (Figure 5-1B) with clove oil developed unacceptable discoloration on the fruit surface within 28 days of cold storage, especially on

the side of the fruit close to the clove oil sachet, and the discoloration became more serious during shelf life (Figure 5-1). Hence, there were no further measurements of respiration, ethylene production, fruit colour and firmness, and juice characteristics after 28 days of cold storage. However, stems were still healthy, so the concentrations of O_2, CO_2, ethylene, and eugenol released from the clove oil sachet inside the bags were still measured as they may have a positive effect on the stem characteristics.

Figure 5-1. Discoloration on the surface of fruit in Bag 2 (A) and Bag 3 (B) with the addition of clove oil after 28 days of cold storage at 4°C (left) and during 3 days of shelf life at 20°C (right)

Although fruit in Bag 3 without clove oil looked healthy after 28 days of cold storage, the locule structure of the fruit was disrupted, potentially caused by the enriched CO_2 and depleted O_2 atmosphere inside the bag (Figure 5-2).

Figure 5-2. Comparison of the locule of the control fruit (left) and fruit in Bag 3 without the addition of clove oil (right) after 28 days of cold storage

During shelf life following 28 days of cold storage, the skin of fruit in Bag 3 without clove oil also discoloured at the fruit base (Figure 5-3) and the damage to the locule of the fruit intensified. Thus, fruit in Bag 3 without clove oil were considered unacceptable for consumption and sale and there were no further measurements of fruit quality after 28 days of cold storage. However, the stem of fruit in Bag 3 without clove oil still looked healthy. Hence, the measurements of O_2, CO_2, and ethylene concentrations inside the bag were continued as they may affect stem quality.

Figure 5-3. Discoloration at fruit base (left) and disrupted locule (right) of fruit in Bag 3 without the addition of clove oil during 3 days of shelf life after 28 days of cold storage

5.3. Results

5.3.1. Weight loss

Weight loss depended on the combination of packaging, storage duration, and shelf life ($P<0.05$). Overall weight loss was low, with a maximum directly after storage of 0.7% and after shelf life of 1.0% (Figure 5-4). The weight loss in all treatments seemed to increase substantially during the first 14 days of cold storage. However, since this was followed by

an apparent decrease for the packaged fruit and a significant decrease for the control, the measurement at 14 days was likely compromised. It was probably a measurement error since the rise and subsequent decrease occurred for all treatments and since it was physically impossible for fruit to gain weight during storage and weight loss to decrease. After 28 days in storage, the highest weight loss was noted in Bag 3 with clove oil which was significantly higher compared to Bag 3 without clove oil and Bag 2 with clove oil. The lowest weight loss was noted in Bag 2 without clove oil and in the control, and these did not change substantially after this.

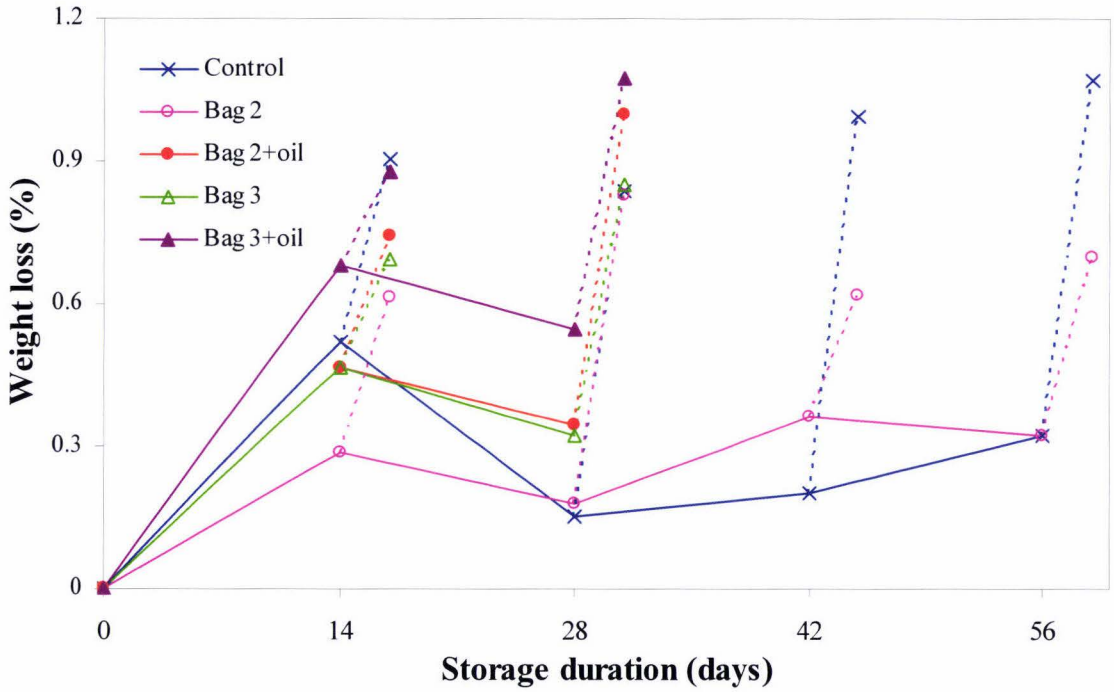

Figure 5-4. Weight loss of the control fruit and fruit in Bags 2 and 3 with or without the addition of clove oil during storage at 4°C (days) without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.007, n = 16 fruit)

During shelf life at room temperature (20°C), the weight loss in all treatments significantly increased with the highest weight loss after 14 days of storage for the control fruit and fruit in Bag 3 with clove oil, and after 28 days of storage for fruit in Bags 2 and 3 with clove oil (Figure 5-4). During shelf life after 42 and 56 days of storage, the weight loss of the control fruit was considerably higher compared with fruit in Bag 2 without clove oil.

5.3.2. Gas related measures

5.3.2.1. Respiration

The CO_2 production rate depended on the combination of packaging and storage duration ($P<0.05$). The addition of clove oil to the packaging did not affect the CO_2 production rate during the first 28 days of storage with or without shelf life (Figure 5-5).

The CO_2 production rate of packaged fruit, especially in Bag 3, increased during the first 14 days of cold storage and remained constant thereafter, while the CO_2 production rate of the control fruit remained constant during cold storage (Figure 5-5). The CO_2 production rate of fruit in Bag 3 was insignificantly higher than that of fruit in Bag 2, but it was significantly higher than the CO_2 production rate of the control fruit during the cold storage period. The CO_2 production rate of fruit in Bag 2 was significantly higher than that of the control only after 56 days of cold storage.

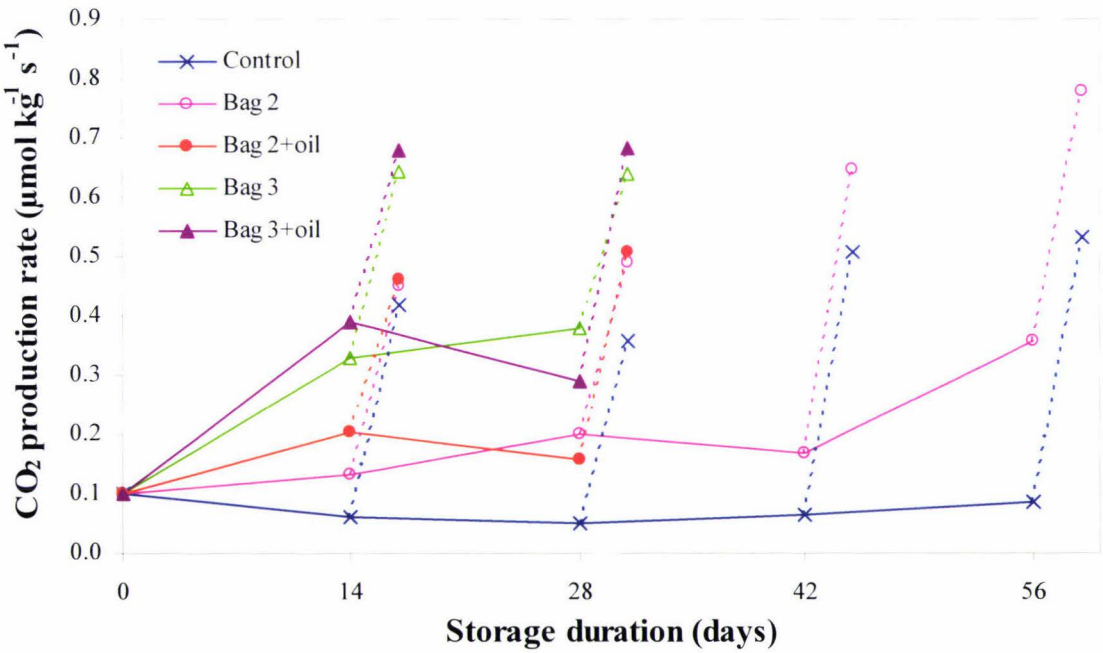

Figure 5-5. CO_2 production rate of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.004, n = 8 fruit)

Shelf life caused a significant increase in the CO_2 production rate of all treatments with the highest increase for fruit in Bag 3 and the lowest for the control fruit (Figure 5-5). During

shelf life, fruit in Bag 2 had a significantly higher CO_2 production rate compared to the control fruit only after 56 days of storage.

The O_2 consumption rate of fruit in Bag 3 with or without clove oil and fruit in Bag 2 with clove oil did not significantly change with levels ranging between 0.2-0.3 μmol O_2 $kg^{-1}s^{-1}$, during the first 14 days of cold storage, and thereafter it kept decreasing for fruit in Bags 2 and 3 with clove oil, but insignificantly increased for fruit in Bag 3 without clove oil (Figure 5-6). After 28 days of cold storage, the O_2 consumption rate of all treatments was not significantly different although fruit in Bag 3 had a higher O_2 consumption rate compared to the others. The control fruit and fruit in Bag 2 without clove oil had a similar O_2 consumption rate from 28 to 56 days of cold storage.

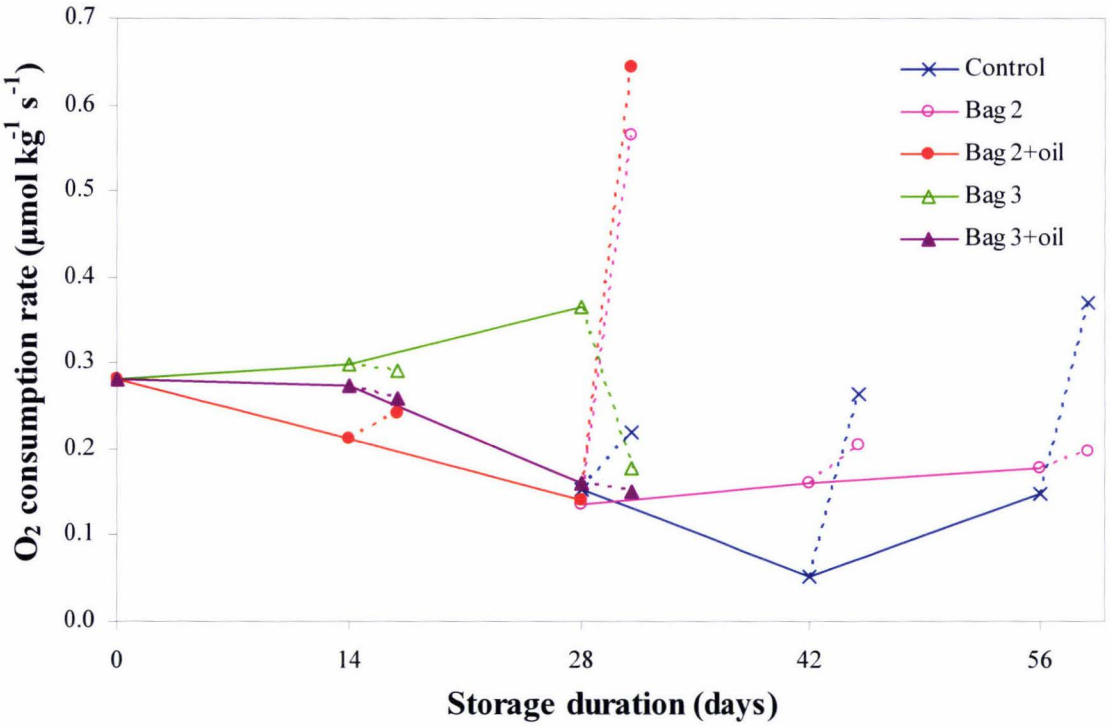

Figure 5-6. O_2 consumption rate of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.02, n = 8 fruit). Data for control and Bag 2 after 14 days are missing due to equipment failure.

Shelf life did not affect the O_2 consumption rate of fruit in Bag 3 with or without clove oil and Bag 2 with clove oil after 14 days of storage (Figure 5-6). After 28 days of cold storage, shelf life caused a dramatic increase in O_2 consumption rate of fruit in Bag 2 with

or without clove oil, while it caused a decrease for fruit in Bag 3 without clove oil resulting in a similar level to the control fruit and fruit in Bag 3 with clove oil, where the O_2 consumption rate was not affected by shelf life. During shelf life following 42 and 56 days of storage, the control fruit had an insignificantly higher O_2 consumption rate compared to fruit in Bag 2 without clove oil.

The respiratory quotient (RQ) of the initial fruit (0.39) is very low, this may be due to O_2 measurement uncertainty. The RQ of fruit in Bag 3 with or without clove oil and fruit in Bag 2 with clove oil increased equally during the first 14 days of cold storage and remained constant thereafter at around 1.3 (Table 5-1). The RQ of the control fruit and fruit in Bag 2 without clove oil did not significantly change from 28 to 56 days of cold storage with a higher value for fruit in Bag 2 without clove oil.

Table 5-1. The respiratory quotient of the control fruit and fruit in the bags with or without clove oil during cold storage at 4°C without and with 3 days of shelf life at 20°C. (s.e. = 0.34, n = 8 fruit)

Treatments	RQ				
	Storage duration (days)				
	0	14	28	42	56
Control	0.39	NA (2.39)	0.35 (2.01)	1.12 (2.23)	0.83 (1.51)
Bag 2	0.39	NA (2.57)	1.89 (0.99)	1.51 (3.57)	1.87 (3.77)
Bag 2+clove oil	0.39	1.22 (2.07)	1.24 (0.99)	-	-
Bag 3	0.39	1.34 (2.24)	1.27 (3.76)	-	-
Bag 3+clove oil	0.39	1.68 (2.53)	1.28 (3.89)	-	-

NA, the data are not available and the data in the brackets represent the results after 3 days of shelf life following cold storage at 4°C

Shelf life caused a rapid increase in RQ of all treatments, except Bag 2 with or without clove oil after 28 days of cold storage (Table 5-1). During shelf life, the RQ of fruit in Bag 3 with or without clove oil increased to more than 3 after 28 days of storage. The RQ of fruit in Bag 2 without clove oil doubled to 3.7 during shelf life following 42 and 56 days of cold storage compared to that of the control fruit which was around 1.9.

5.3.2.2. Gas composition in the bags

The O_2 and CO_2 concentrations in the bags depended on the combination of packaging, storage duration, and shelf life ($P<0.05$). Clove oil did not affect O_2 and CO_2 concentrations in the packaging.

Bag 2 had a higher O_2 concentration than Bag 3 during the entire storage period with or without shelf life (Figure 5-7). The O_2 concentration in Bag 3 remained constant within the range 1-2% during cold storage, while that in Bag 2 quickly decreased after 14 days of cold storage and remained constant within the range 3-4% after 28 days of cold storage. Shelf life did not affect O_2 concentration in Bag 3, but it caused a decrease in O_2 concentration in Bag 2 only after 14 days of cold storage, and thereafter the O_2 concentration in Bag 2 was not affected by shelf life.

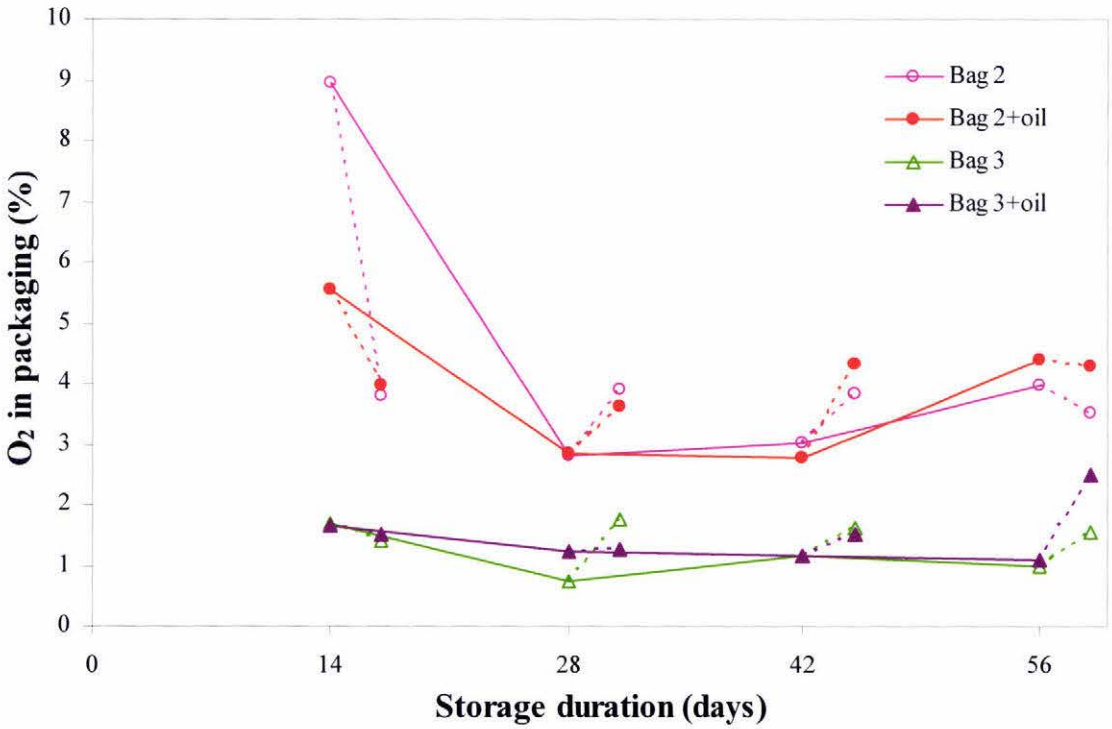

Figure 5-7. O_2 concentration in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.52, n = 4 bags)

The CO_2 concentration significantly increased in Bags 2 and 3 during the first 28 days of cold storage with a higher CO_2 concentration in Bag 3 (Figure 5-8). The CO_2 concentration

in Bag 2 remained constant after 28 days of cold storage, while that in Bag 3 gradually decreased. This resulted in the CO_2 concentration in Bag 3 being similar to that in Bag 2 at the end of the storage period. During shelf life after each storage period, the CO_2 concentration in both types of packaging significantly increased. Bag 3 had a higher CO_2 concentration than Bag 2 during shelf life following 14 and 28 days of storage, but after 42 and 56 days of storage, shelf life caused a similar increase in the CO_2 concentration in Bags 2 and 3.

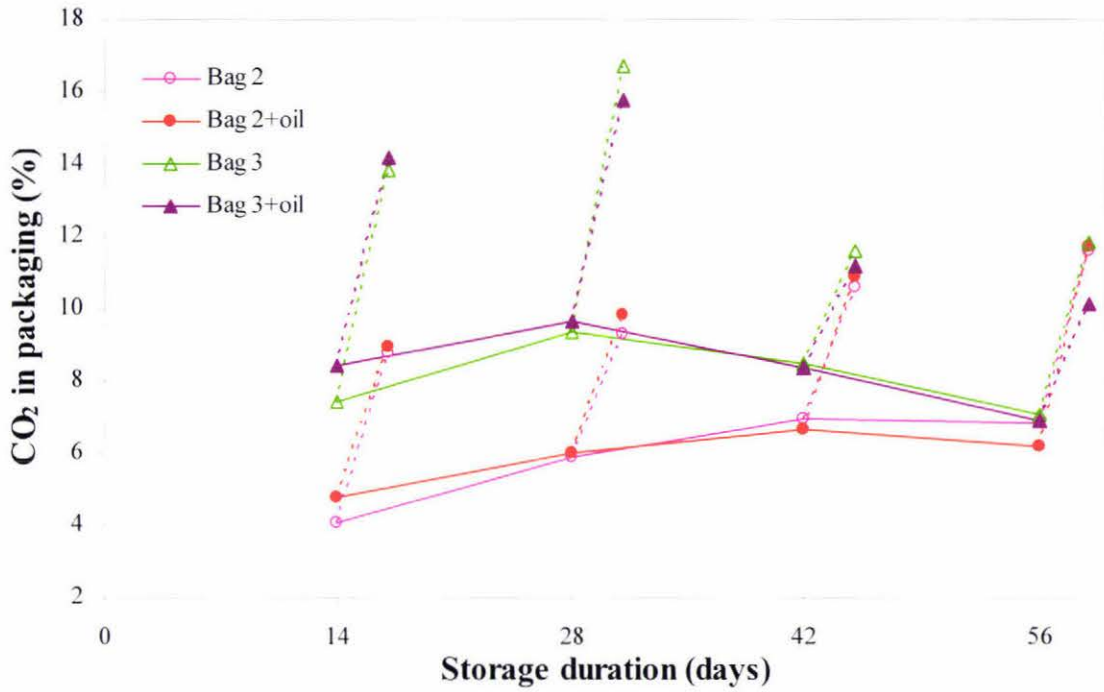

Figure 5-8. CO_2 concentrations in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.23, n = 4 bags)

5.3.2.3. Ethylene production

Ethylene production rate depended on the combination of packaging, storage duration, and shelf life ($P<0.05$). Clove oil had no effect on the ethylene production rate. The ethylene production rate of fruit in the bags was minimal up to 14 days of cold storage, whereas that of the control fruit was minimal up to 42 days of cold storage (Figure 5-9). The ethylene production rate slowly increased with storage duration. After 42 and 56 days of cold

storage, fruit in Bag 2 without clove oil had a higher ethylene production rate compared to the control fruit although it was not significantly different.

During shelf life the ethylene production rate significantly increased with progressing storage time and accelerated the ethylene production rate of the control fruit being detected after 14 days of storage (Figure 5-9). The ethylene production rate of fruit in Bag 2 without clove oil was much higher than the control fruit during shelf life. However, the ethylene production rate of fruit in Bag 3 with or without clove oil was not affected by shelf life following 14 and 28 days of storage.

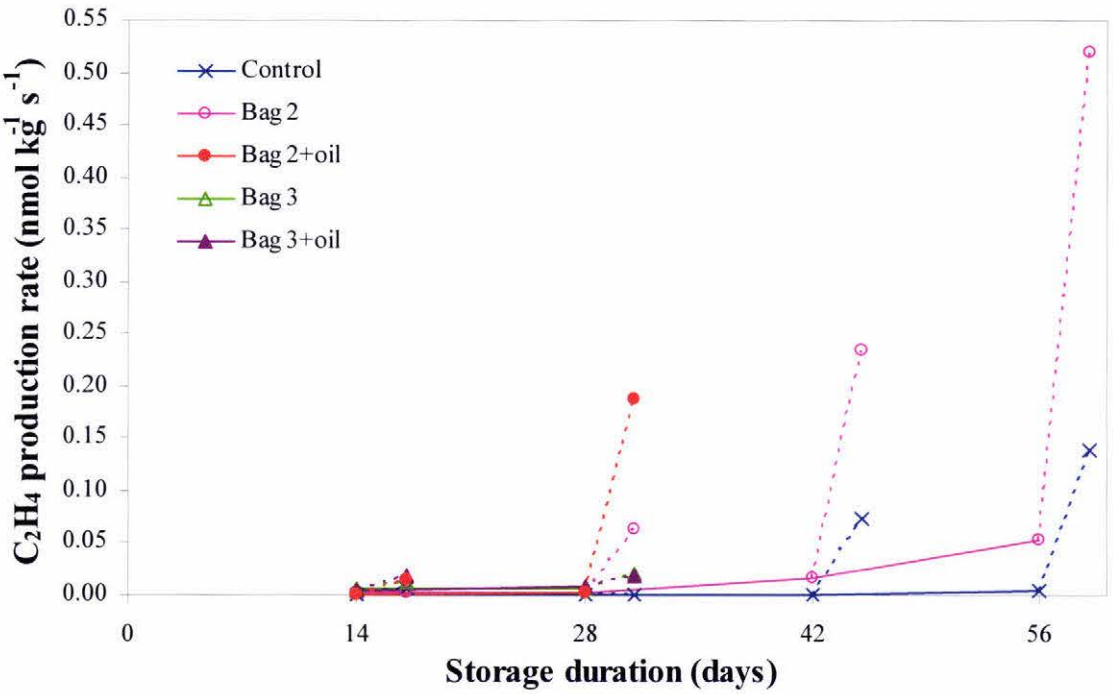

Figure 5-9. Ethylene production rate of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 7×10^{-4}, n = 8 fruit)

Ethylene concentration in the bags depended on the combination of packaging, storage duration, and shelf life ($P<0.05$). Clove oil did not affect the ethylene concentration in the packaging.

Ethylene concentration increased with storage duration for fruit in Bag 2, whereas it increased during the first 42 days of cold storage and slightly decreased thereafter for fruit in Bag 3 (Figure 5-10). Bag 2 appeared to have a higher ethylene concentration than Bag 3

after 42 and 56 days of cold storage but this was not significant. Shelf life caused a significant increase in the ethylene concentration in Bag 2 after 42 and 56 days of storage, but a smaller increase was seen for Bag 3.

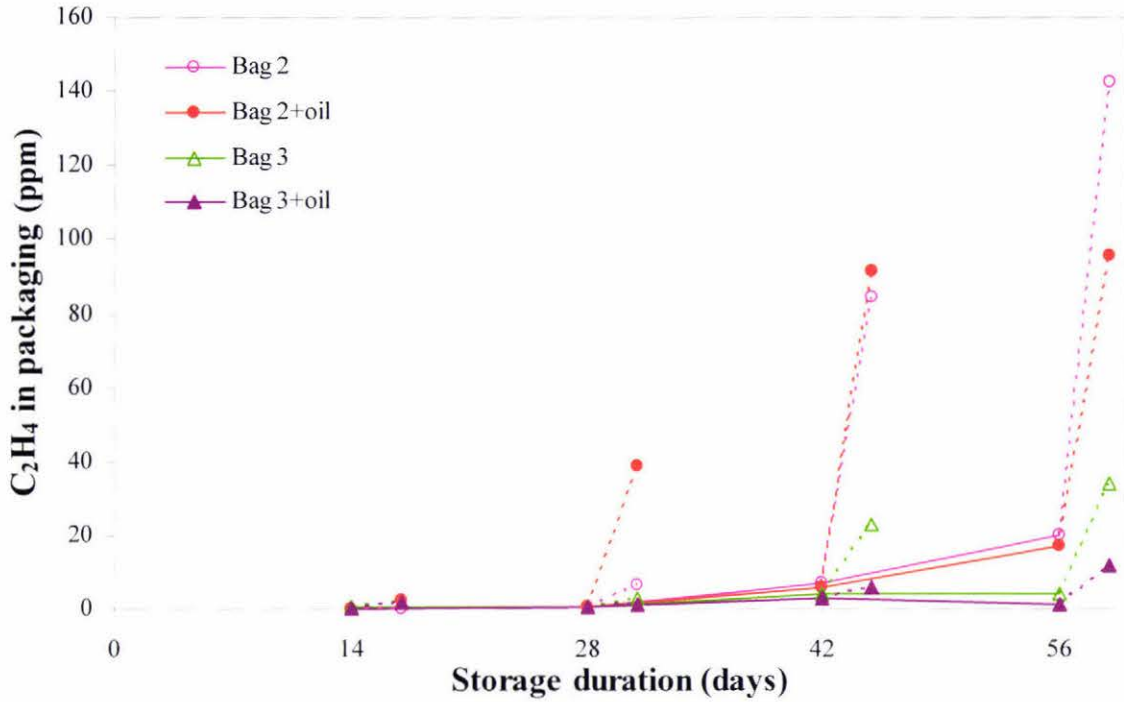

Figure 5-10. Ethylene concentration in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 5.79, n = 4 bags)

5.3.2.4. Eugenol released from a clove oil sachet

The effect of clove oil on tamarillo fruit and stem quality was mainly focused on eugenol released from a clove oil sachet. Hence, eugenol concentration in the headspace of sealed packaging was measured. The eugenol concentration depended on the combination of packaging and shelf life ($P<0.05$).

The eugenol concentration was similar between Bags 2 and 3 during storage period with or without shelf life (Figure 5-11). Eugenol could be detected in the packaging after 28 days of cold storage. The higher eugenol concentration in Bag 3 compared to Bag 2 after 28 days of cold storage and the large standard deviation indicate the presences of outliers. An average of four bags is represented, one of which had no detectable eugenol and the other

two extremely high eugenol concentrations of 0.22 and 0.30 mmol m^{-3}. After 42 days of cold storage, the eugenol concentration was the same in both types of packaging and equal to the concentration in Bag 2 after 28 days. Thereafter, the eugenol concentration in both packaging types decreased ending up at a similar level as after 14 days of cold storage. Shelf life caused a similar increase in eugenol concentration in Bags 2 and 3.

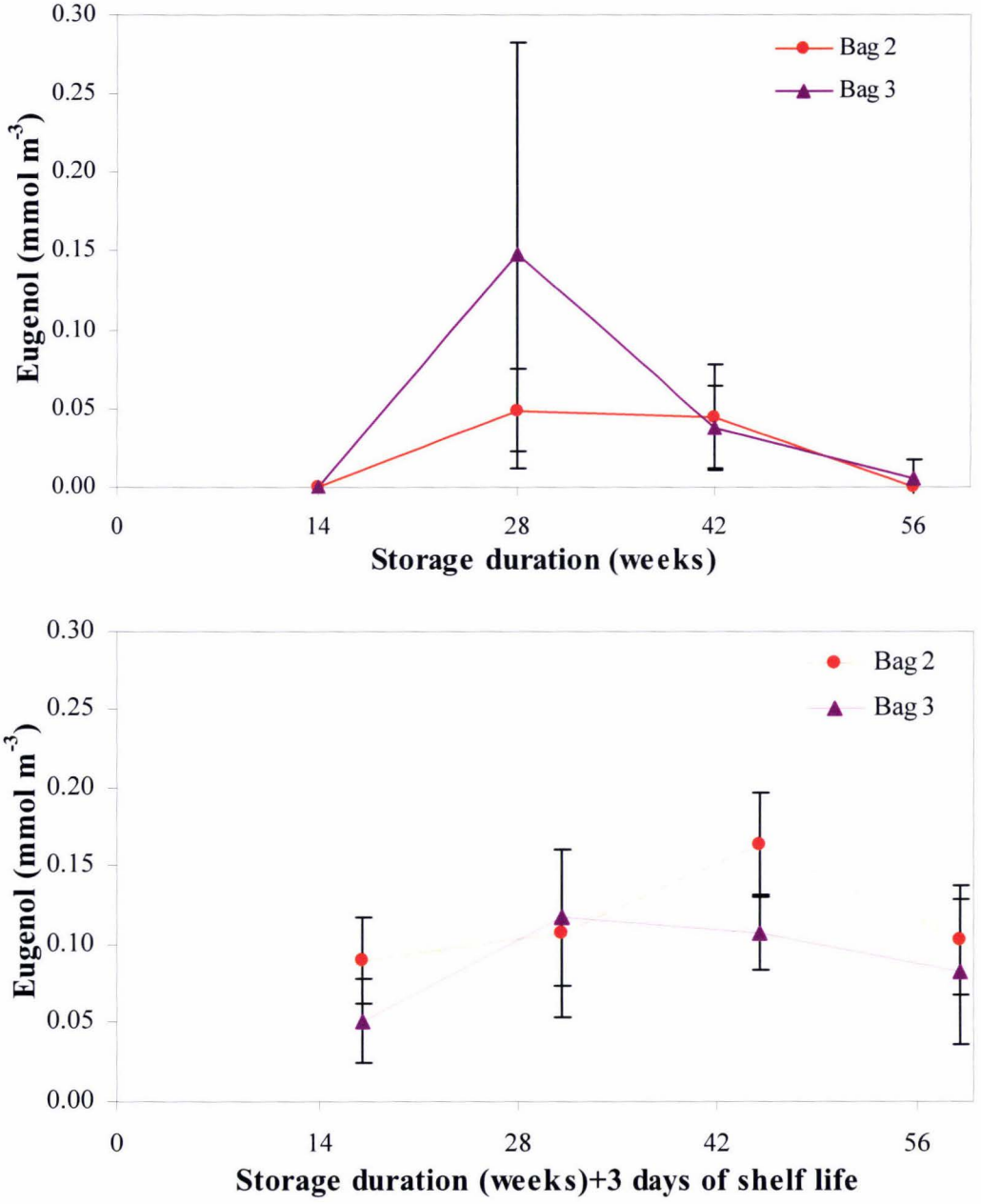

Figure 5-11. Eugenol concentration in Bags 2 and 3 during storage at 4°C (above) and with 3 days of shelf life at 20°C (below). Vertical bars indicate standard deviations surrounding the mean.

5.3.3. Non destructive measures

5.3.3.1. Colour

L^* of all treatments increased during the first 14 days of cold storage and decreased thereafter except for fruit in Bag 2 without clove oil, where L^* remained constant up to 42 days of cold storage and slightly increased at the end of storage period (Figure 5-12). After 28 days of cold storage, L^* of the control fruit gradually increased to a similar value as fruit in Bag 2 without clove oil. During shelf life, L^* of all treatments was similar and significantly increased only after 28 days of cold storage, except for fruit in Bag 2 without clove oil.

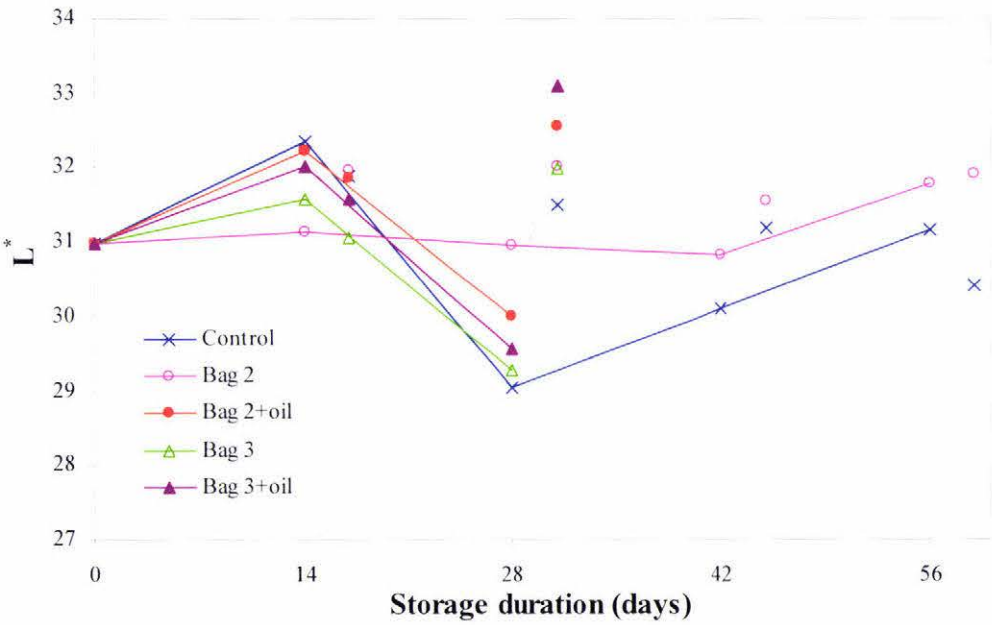

Figure 5-12. Lightness of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.48, n = 16 fruit)

a^*, b^*, and C^* of all treatments were similar during the cold storage period and decreased during the first 14 days of cold storage (Figure 5-13). From 14 to 28 days of cold storage, a^*, b^*, and C^* increased for all treatments with a significant increase for fruit in Bag 2 without clove oil. From 28 days of cold storage onwards, a^*, b^*, and C^* of the control fruit and fruit in Bag 2 without clove oil gradually decreased, ending up at a lower level compared to those of the initial fruit. a^*, b^*, and C^* of all treatments decreased similarly

during shelf life except b^* of fruit in Bag 3 with clove oil after 28 days of storage with three days of shelf life, when an insignificant increase was seen.

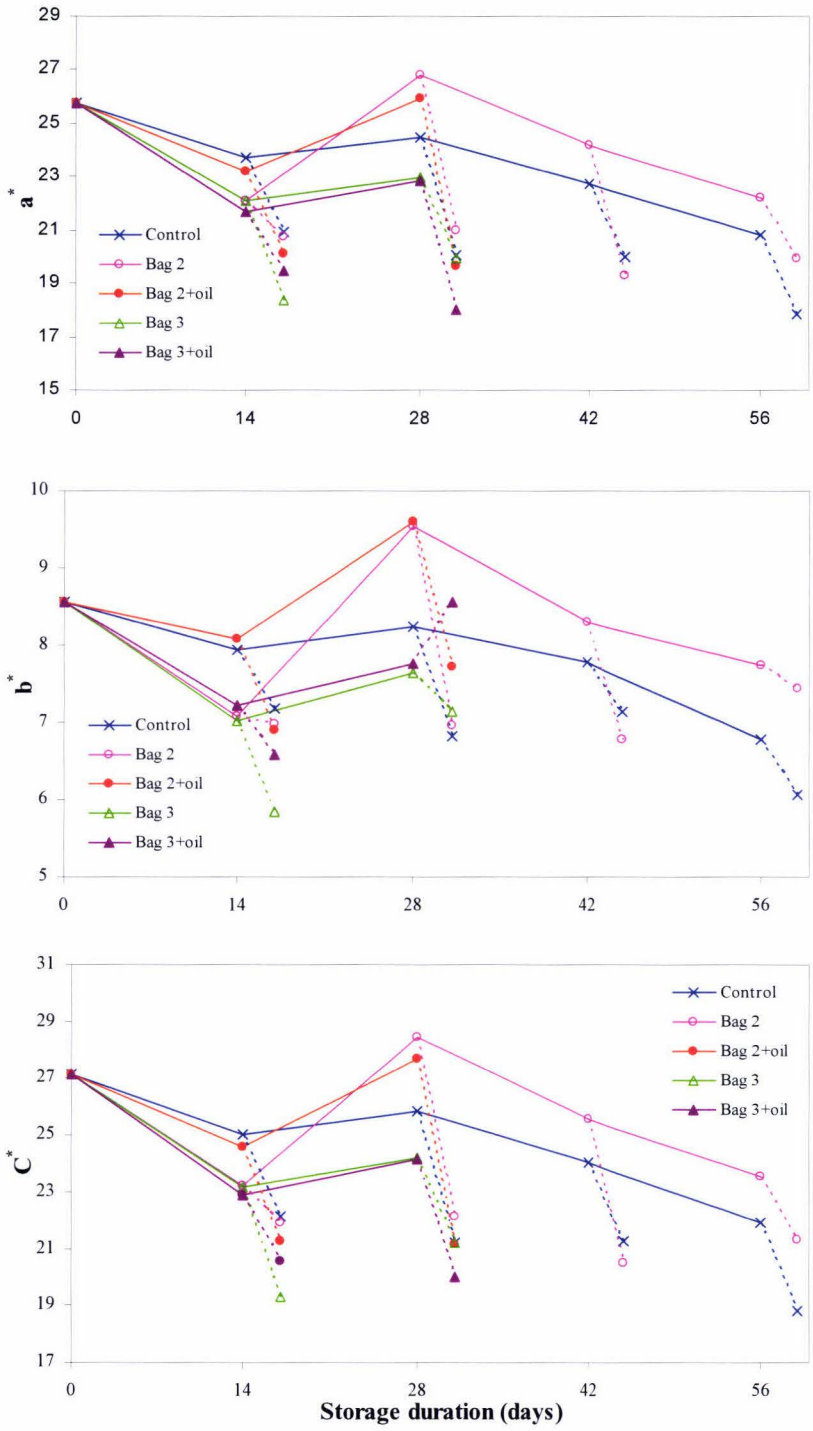

Figure 5-13. Redness (a*), yellowness (b*), and chroma (C*) of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. of a^*, b^*, and $C^* = 2.02$, 0.59, and 2.45, respectively, n = 16 fruit)

The h° value depended on the combination of storage duration, shelf life, and clove oil ($P<0.05$), while the packaging had no effect. The h° value of all treatments was similar and did not significantly change with the values ranging between 17 and 20 during cold storage (Figure 5-14).

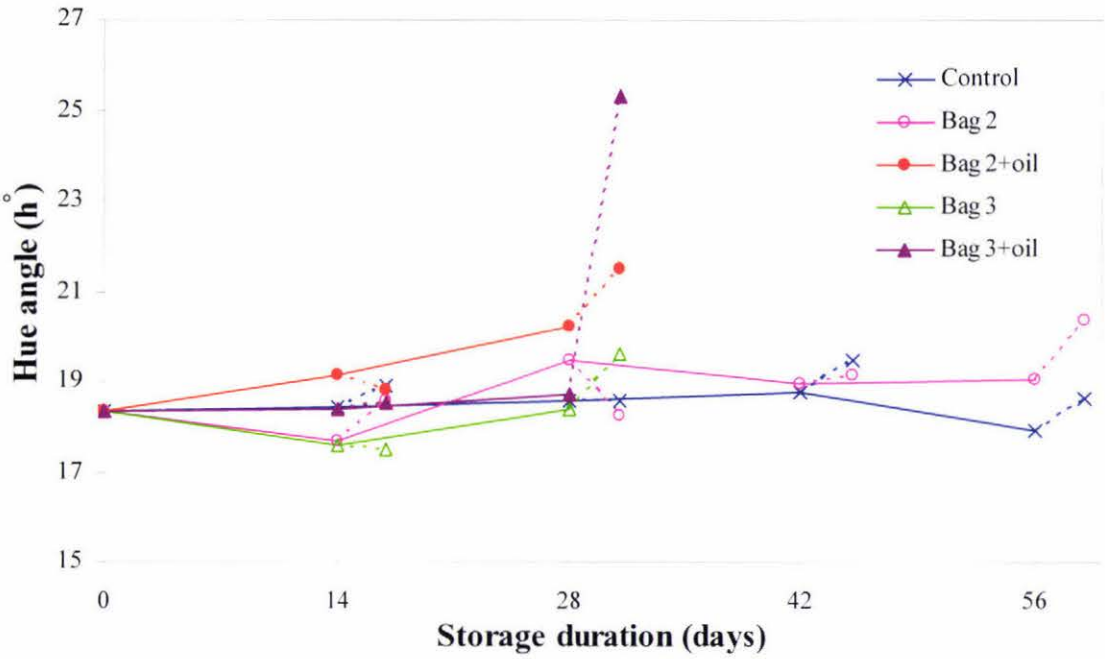

Figure 5-14. Hue angle of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 1.15, n = 16 fruit)

Shelf life did not cause a significant change in h° of all treatments, except for fruit in Bag 3 with clove oil after 28 days of storage when h° increased significantly having the highest value during shelf life.

5.3.3.2. Stiffness

Stiffness was affected by the combination of packaging, storage duration, and shelf life ($P<0.05$). Clove oil had no effect on stiffness during 28 days of cold storage with or without shelf life. Stiffness of all treatments was similar and increased during the first 14 days of cold storage (Figure 5-15). Thereafter, stiffness decreased except for fruit in Bag 2 without clove oil. Between 14 and 28 days of cold storage, the control fruit and fruit in Bag 2 with clove oil lost stiffness at a similar rate, which was lower than the rate of stiffness

loss of fruit in Bag 3 with or without clove oil; in that same period stiffness of fruit in Bag 2 without clove oil increased. After 28 days of cold storage, the rate of stiffness loss was higher for fruit in Bag 2 without clove oil compared with control fruit, resulting in similar values after 42 and 56 days of cold storage.

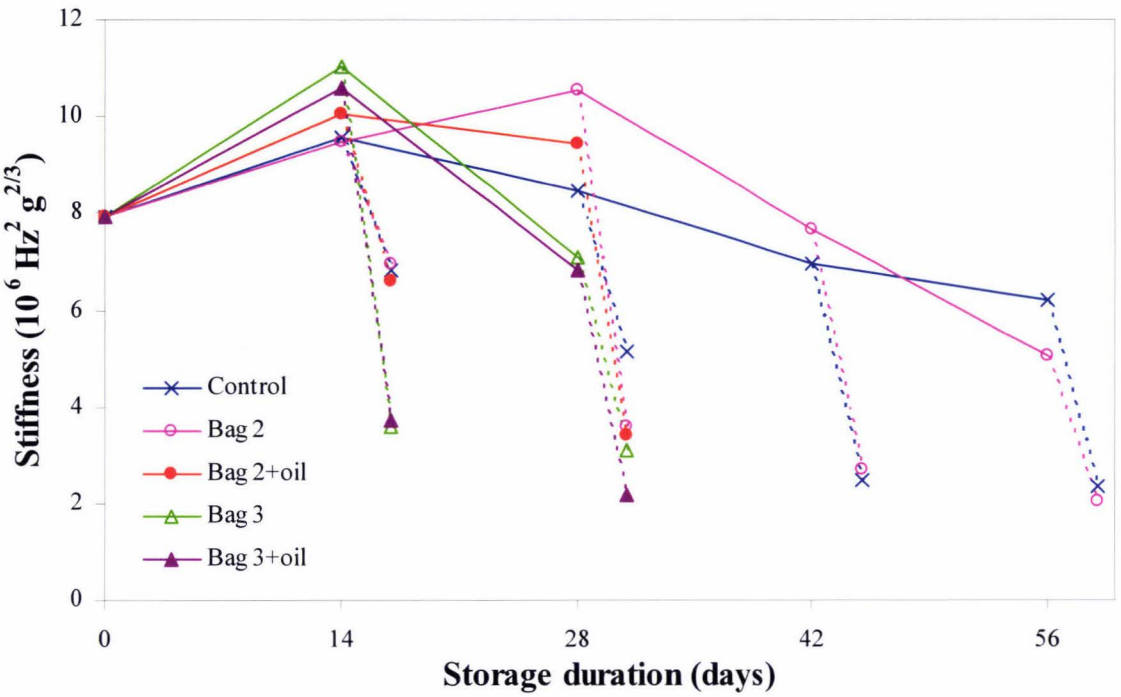

Figure 5-15. Stiffness of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.83, n = 16 fruit)

Shelf life caused a significant loss of stiffness for all treatments (Figure 5-15). Stiffness loss of fruit in Bag 3 with or without clove oil during shelf life following 14 days of storage was significantly higher compared with that of the other treatments. Stiffness loss during shelf life following longer storage was significant and similar for all treatments.

Although the addition of clove oil had no effect on stiffness during 28 days of storage, the surface of the packaged fruit close to a clove oil sachet was softer as measured by touch and compared with the surface of healthy fruit in the same package.

5.3.3.3. Compression firmness

Compression firmness was affected by the combination of packaging, storage duration, and shelf life ($P<0.05$). The addition of clove oil insignificantly decreased compression firmness of packaged fruit during 28 days of cold storage (Figure 5-16).

Compression firmness of packaged fruit increased during the first 14 days of cold storage with a bigger increase for fruit in Bag 3 (Figure 5-16). Between 14 and 28 days of storage, compression firmness decreased for fruit in Bag 3 and increased for fruit in Bag 2 resulting in higher compression firmness of fruit in Bag 2 than Bag 3 after 28 days of cold storage. Compression firmness of the control fruit did not significantly change with the values ranging between 15 and 16 N during the first 28 days of cold storage but slowly decreased thereafter ending up at a lower level compared to the initial values. The compression firmness of fruit in Bag 2 without clove oil decreased after 28 days of cold storage to a similar level as the control fruit by the end of the storage period.

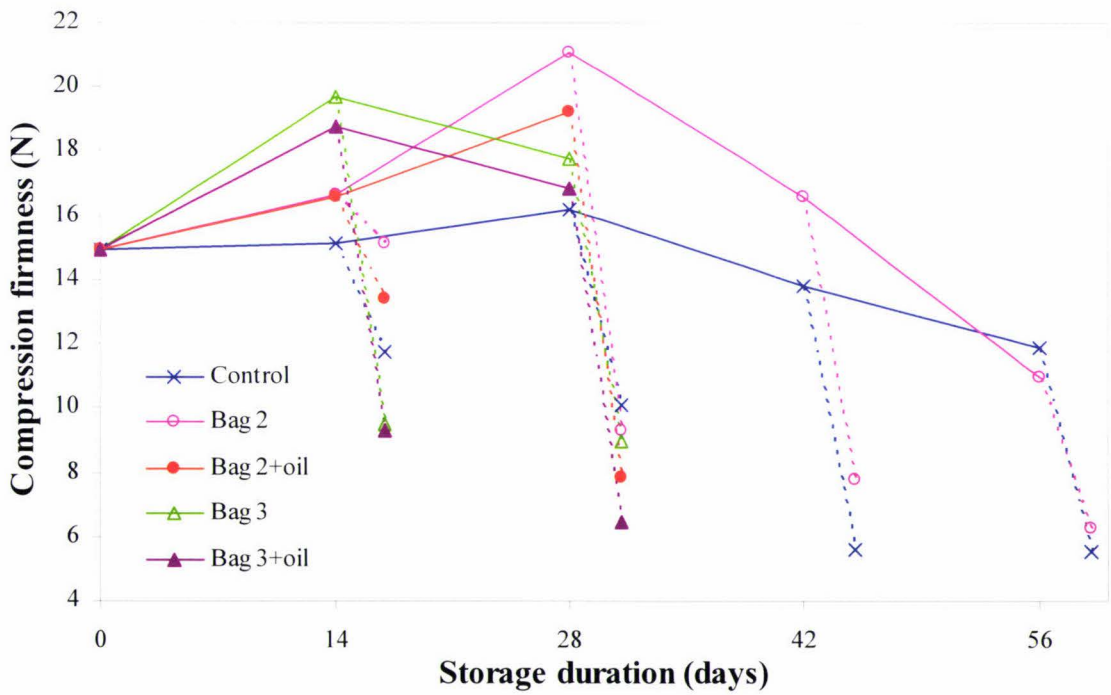

Figure 5-16. Compression firmness of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 2.64, n = 16 fruit)

Compression firmness decreased during shelf life for all treatments (Figure 5-16). Compression firmness of fruit in Bag 3 with or without clove oil decreased significantly more than for the other treatments during shelf life after 14 days of storage. After 28, 42 and 56 days of storage, the decrease of compression firmness during shelf life was similar for all treatments.

5.3.4. Juice characteristics

5.3.4.1. Soluble solids content

Soluble solids content depended on the main effects of storage duration and clove oil, but the packaging and shelf life had no effect on it (Table 5-2). There were also no interaction effects.

Table 5-2. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on soluble solids content (SSC)

Factors		SSC (°brix)
Packaging (P)	Control	11.08^{NS}
	Bag 2	10.93
	Bag 3	11.06
Storage duration (Sd)	0	10.89ab*
	14	11.06ab
	28	11.13a
	42	10.81b
	56	10.82b
Shelf life (Sl)	0	11.09^{NS}
	3	10.93
Clove oil (Cl)	No	11.21*
	Yes	10.89

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly ($P<0.05$)

SSC of the control and fruit in the packaging was similar and slightly increased during the first 28 days of cold storage (Table 5-2). Thereafter, it reduced to the similar level as the beginning of storage and remained constant till the end of cold storage period. SSC was

not affected by shelf life. The addition of clove oil to the packaging significantly reduced SSC during 28 days of storage.

5.3.4.2. pH and titratable acidity of the juice

The pH was affected by the combination of packaging, storage duration, shelf life, and clove oil ($P<0.05$). The change in pH of all treatments was relatively small at 3.3-3.5 during the cold storage period with or without shelf life (Figure 5-17). The pH of all treatments similarly increased during the first 14 days of cold storage, and thereafter it slightly decreased except for fruit in Bag 3 with clove oil, in which the pH kept increasing. After 28 days of cold storage, the pH of fruit in Bag 3 with or without clove oil was significantly higher than that of fruit in Bag 2 with or without clove oil and the control fruit. The pH of the control fruit and fruit in Bag 2 without clove oil was similar and did not change after 28 days of cold storage. The pH of all treatments insignificantly increased during shelf life.

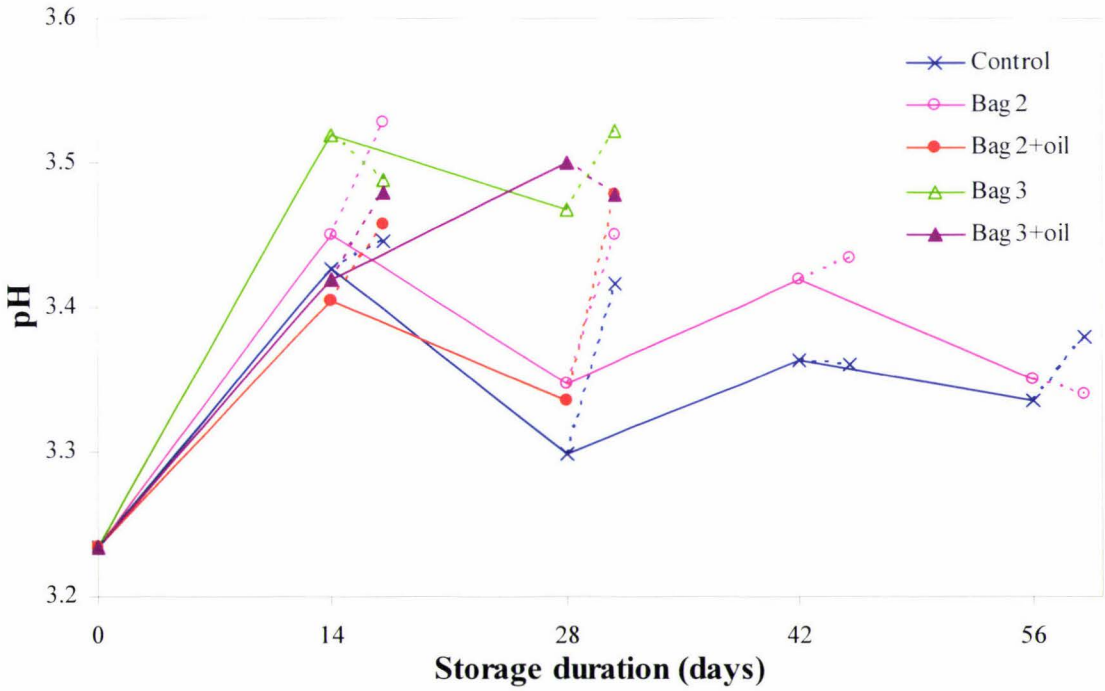

Figure 5-17. pH of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.003, n = 16 fruit)

Titratable acidity (TA) was affected by the combination of packaging, storage duration, shelf life, and clove oil ($P<0.05$) (Figure 5-18). TA of all treatments was similar during cold storage with or without shelf life and decreased during the first 14 days of cold storage with a fast decrease for the control fruit and fruit in Bag 3 without clove oil. Thereafter, TA of fruit in Bag 2 with or without clove oil and Bag 3 with clove oil kept decreasing, while those of the control fruit and fruit in Bag 3 without clove oil remained constant resulting in a similar TA level of all treatments after 28 days of cold storage. From this point on, TA of the control and fruit in Bag 2 without clove oil remained constant.

The overall decrease in TA during shelf life after 14 days of storage was insignificant. The apparent increase after 28 days of storage was likely due to measurement uncertainty (Figure 5-18). For longer (42-56 days) storage periods, TA of the control fruit decreased faster than that of fruit in Bag 2 without clove oil during shelf life although the final value was not significantly different.

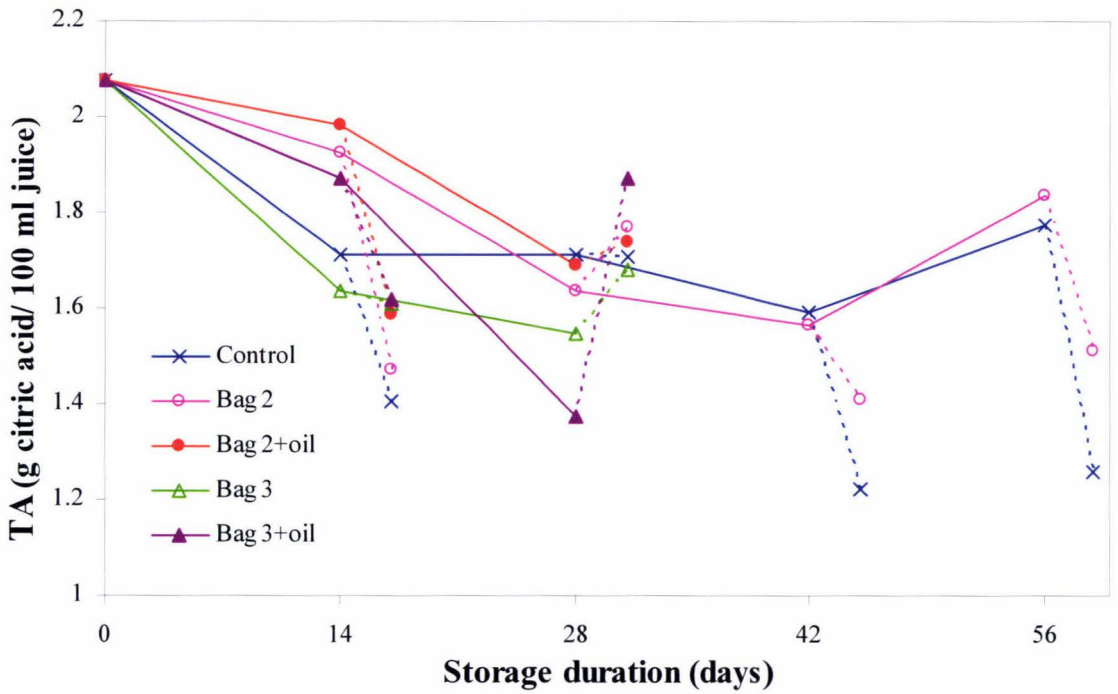

Figure 5-18. Titratable acidity of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.02, n = 16 fruit)

5.3.4.3. The ratio of SSC to TA

The SSC/TA ratio was affected by the combination of packaging, storage duration, shelf life, and clove oil ($P<0.05$) (Figure 5-19). The SSC/TA of all treatments was not significantly different during cold storage. The SSC/TA ratio of all treatments, except for fruit in Bag 2 with clove oil, increased during the first 14 days of cold storage. Thereafter, the SSC/TA ratio of the control fruit and fruit in Bag 3 without clove oil remained constant, but that of the others increased. After 28 days of cold storage onwards, the SSC/TA ratio of the control fruit and fruit in Bag 2 was constant.

The SSC/TA ratio of all treatments was similar during shelf life following 14 and 28 days of storage (Figure 5-19). Shelf life caused an insignificant increase in SSC/TA ratio of all treatments except Bag 3 without clove oil after 14 days of storage, while it caused a decrease with a significant decrease for fruit in Bag 3 with clove oil after 28 days of storage. The SSC/TA ratio of the control fruit significantly increased and was higher compared to that of fruit in Bag 2 without clove oil during shelf life after 42 and 56 days of storage.

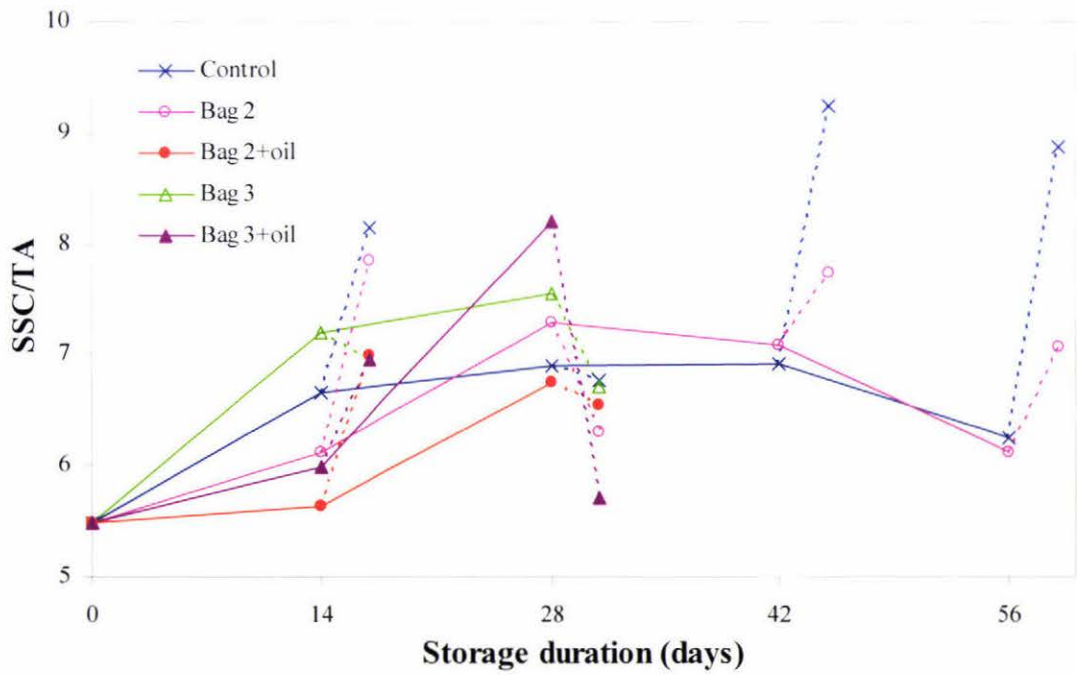

Figure 5-19. The SSC/TA ratio of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.45, n = 16 fruit)

5.3.5. Fruit stem measures

5.3.5.1. Moisture content

The moisture content in the fruit stem was affected by the combination of storage duration and shelf life ($P<0.05$). Clove oil did not affect the moisture content. The moisture content in the stem of all treatments was similar during cold storage with or without shelf life (Figure 5-20). The moisture content in the stem decreased during the first 14 days of cold storage, remained constant thereafter, and started to decline after 42 days of cold storage. During shelf life, the loss in stem moisture significantly increased after 28 days of storage onwards.

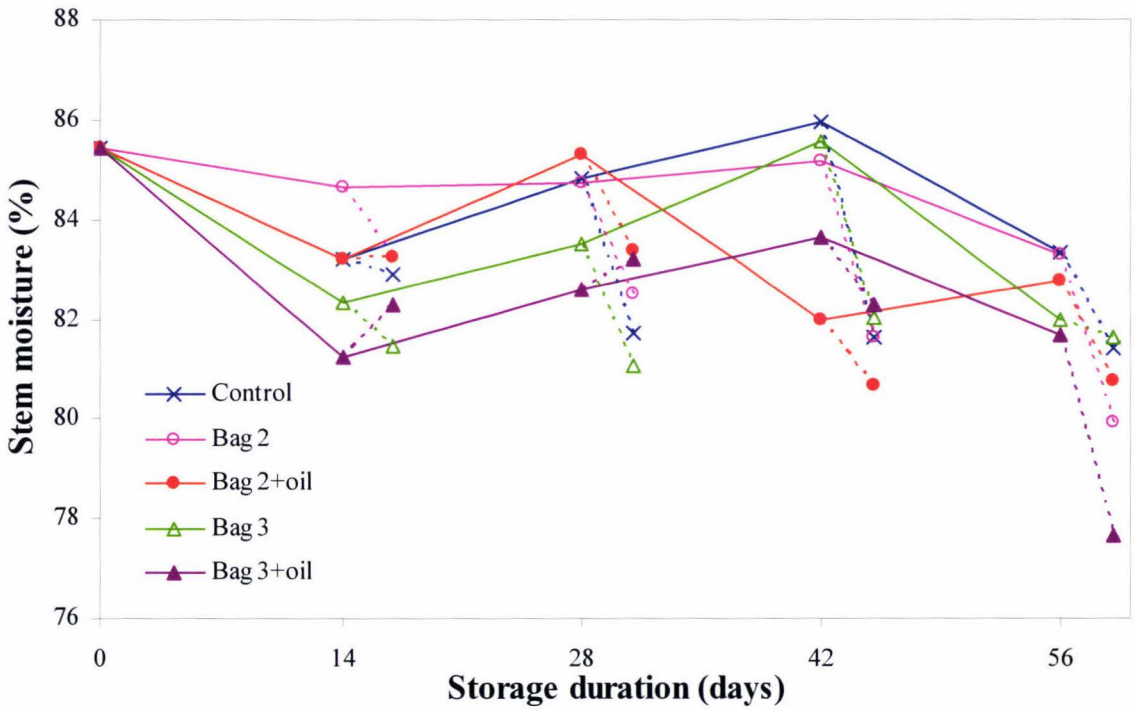

Figure 5-20. Moisture content in the stems of the control and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 3.45, n = 5 stems)

5.3.5.2. Chlorophyll content

The chlorophyll content of the fruit stem depended on the combination of packaging and clove oil ($P<0.05$). The chlorophyll content in the stem of all treatments was not significantly different during the cold storage period except after 56 days of cold storage,

when the chlorophyll content of Bag 3 with clove oil was significantly higher than that of the control fruit (Figure 5-21). However, the chlorophyll content in the stem of the control fruit seemed to decrease gradually with storage duration, while that of packaged fruit with or without clove oil remained constant.

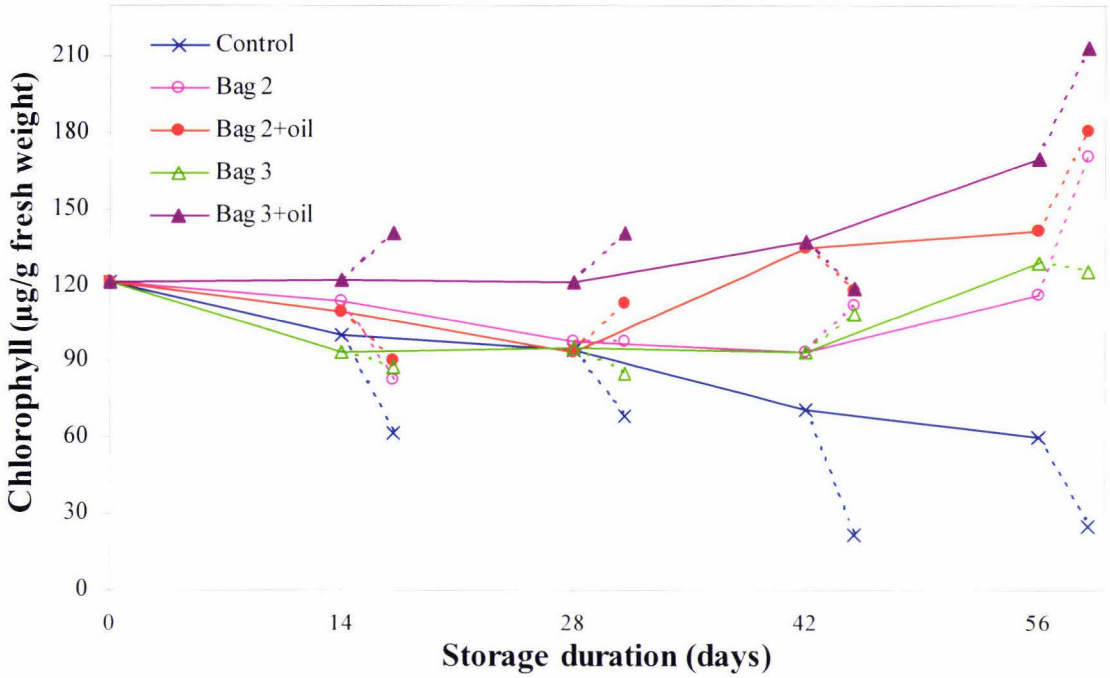

Figure 5-21. Chlorophyll content of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 14.97, n = 5 stems)

During shelf life, the chlorophyll content of all treatments was similar after 14 and 28 days of storage (Figure 5-21). During shelf life following 42 and 56 days of storage, the chlorophyll content in the stem of packaged fruit with or without clove oil was similar and insignificantly changed, whereas that of the control fruit insignificantly decreased, resulting in a significantly higher chlorophyll content in the stem of packaged fruit with or without clove oil compared to the control fruit.

5.3.5.3. Polyphenol oxidase activity

The polyphenol oxidase (PPO) activity depended on the combination of packaging, shelf life, and clove oil ($P<0.05$). The PPO activity of all treatments was similar and gradually increased during 42 days of cold storage, and thereafter it decreased for stems of fruit in

Bag 3 without clove oil and Bag 2 with clove oil, remained constant for Bag 2 without clove oil and Bag 3 with clove oil, and increased for the control fruit (Figure 5-22). During shelf life, the PPO activity of all treatments increased except after 42 days of storage, when a decrease was seen for all treatments possibly due to measurement uncertainty. During shelf life after 56 days of storage, the PPO activity of fruit in Bag 3 with clove oil and the control fruit did not significantly change, whereas that of the other treatments increased.

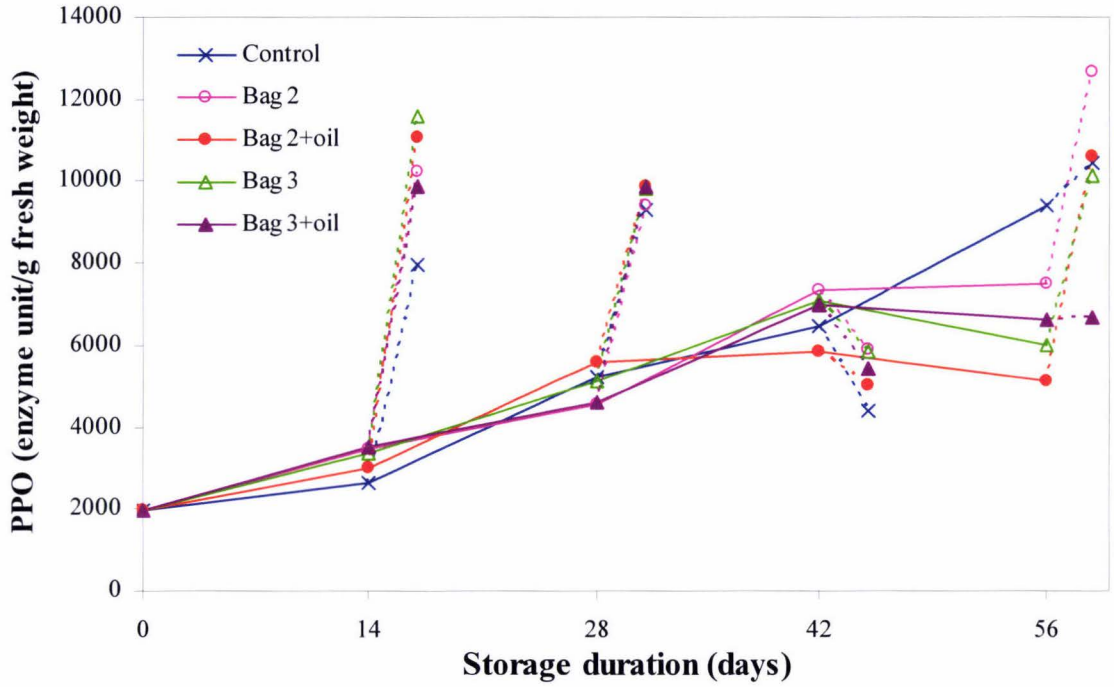

Figure 5-22. Polyphenol oxidase activity in the stem of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 615.49, n = 5 stems)

5.3.6. Sensory test

The sensory test was visually scored for fruit discoloration, calyx yellowing and blackening, stem yellowing and blackening, and stem-end rots. Only a few instances of stem-end rots occurred, within acceptable limits for commercial fruit, with a slightly higher occurrence after shelf life.

The development of discoloration on the fruit surface was affected by the combination of packaging, storage duration, and clove oil ($P<0.05$). Discoloration quickly occurred on the surface of packaged fruit with added clove oil, especially in Bag 3, and was unacceptable

after 28 days of cold storage (Figure 5-23). The discoloration of the control and packaged fruit without added clove oil occurred similarly after 28 days of cold storage with a lower score compared to packaged fruit with added clove oil. Although the appearance of fruit in Bag 3 was acceptable after 28 days of cold storage, bleeding in the locule was observed and considered unacceptable for consumption and sale. Fruit in Bag 2 without clove oil was observed to shrivel with loss of colour on the fruit surface after 42 days of cold storage, while this was not observed for the control fruit.

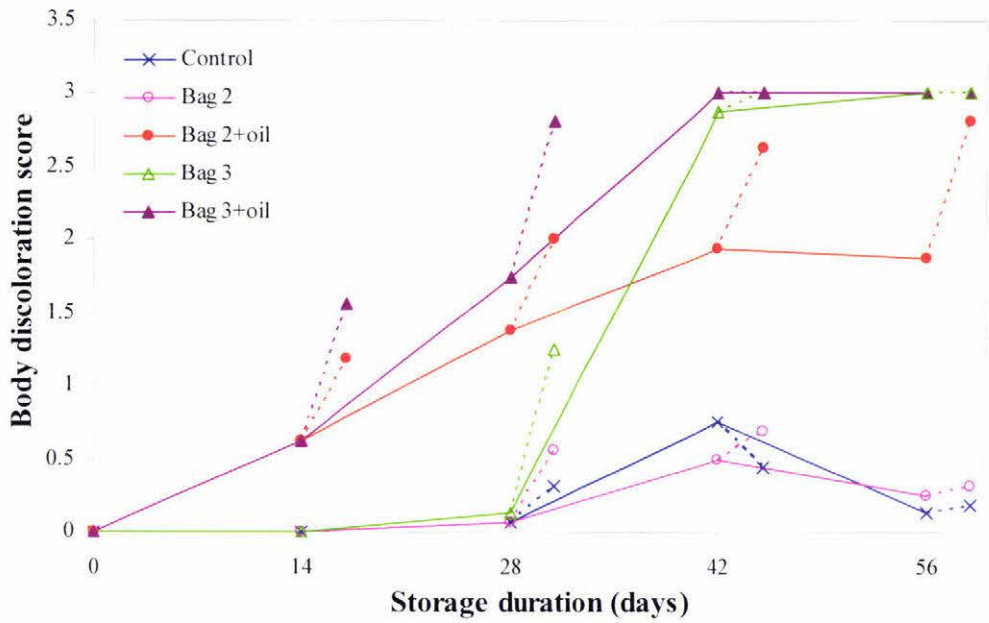

Figure 5-23. The score of discoloration on the fruit surface of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. = 0.14, n = 16 fruit)

Shelf life did not affect the colour of the control fruit and fruit in Bag 2 without clove oil, but it caused an insignificant increase in discoloration of fruit in Bags 2 and 3 with clove oil and a significant increase for fruit in Bag 3 without clove oil being unacceptable after 14 and 28 days of storage, respectively (Figure 5-23).

Calyx lifting and blackening depended on the combination of packaging, storage duration, and shelf life ($P<0.05$). Clove oil did not affect calyx lifting, but slightly affected calyx blackening. Calyx lifting of all treatments could be detected after 42 days of cold storage and increased thereafter (Figure 5-24). Calyx lifting scores were similar for all treatments during storage at 4°C.

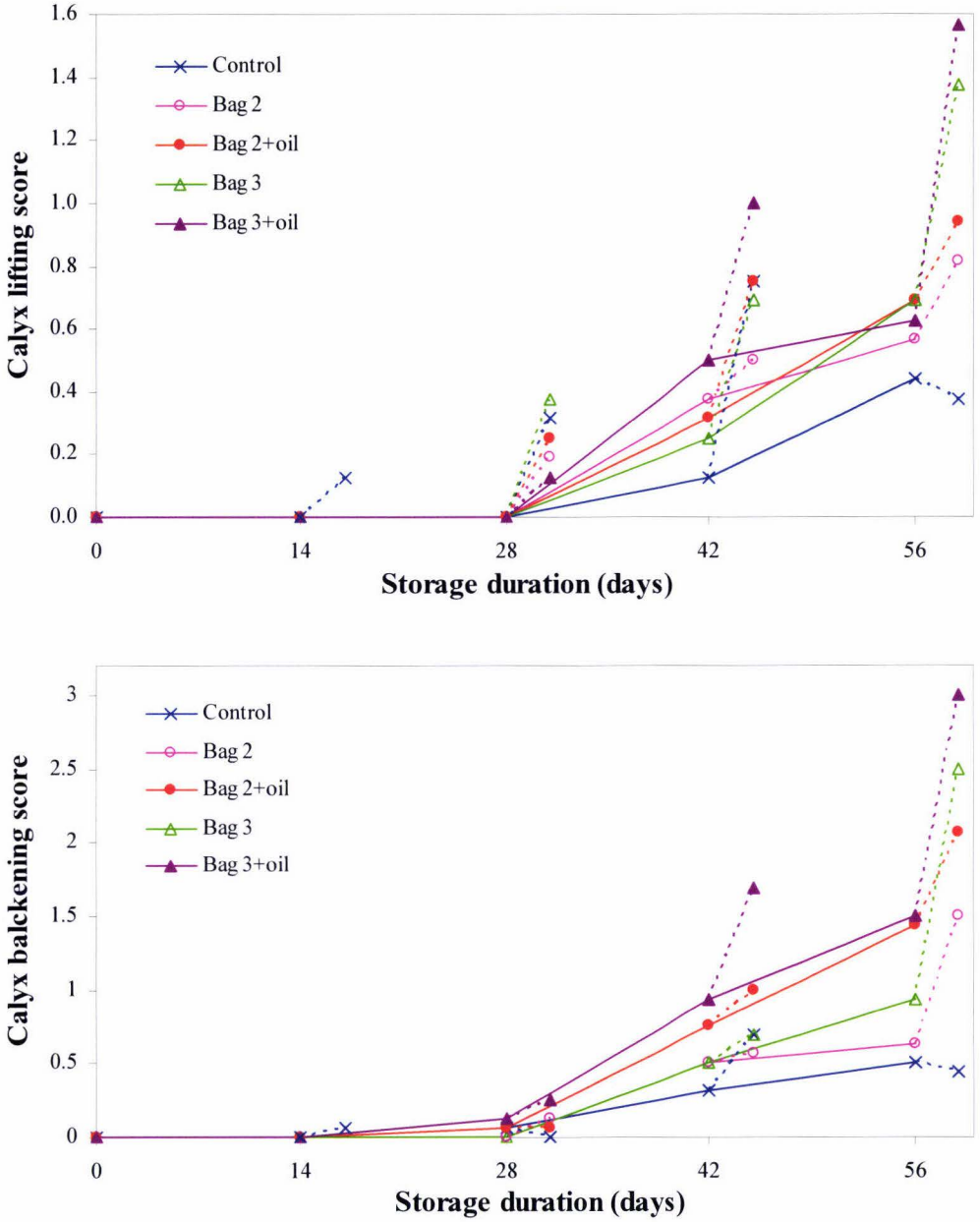

Figure 5-24. Calyx lifting and blackening scores of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. of calyx lifting and blackening = 0.05 and 0.1, respectively, n = 16 fruit)

Shelf life caused an increase in calyx lifting resulting in the occurrence of calyx lifting of the control fruit and fruit in all packaging after 14 and 28 days of storage, respectively. The calyx lifting of fruit in Bag 3 with clove oil was undesirable during shelf life following 56 days of storage, while that of the other treatments was not significantly different and was

still acceptable with the highest score (1.38) for fruit in Bag 3 without clove oil followed by fruit in Bag 2 with or without clove oil, and the lowest (0.38) for the control fruit.

Calyx blackening of the control fruit and fruit in Bags 2 and 3 with clove oil developed similarly after 28 days of cold storage and thereafter quickly increased for packaged fruit with added clove oil, and increased more slowly for the control fruit (Figure 5-24). Calyx blackening of fruit in packaging without clove oil could be detected after 42 days of cold storage with an intermediate score between packaged fruit with added clove oil and the control fruit, and thereafter it gradually increased for fruit in Bag 3 without clove oil, while it remained constant for fruit in Bag 2 without clove oil having a similar score as the control fruit at the end of storage period. After 56 days of cold storage, the calyx blackening of fruit in Bags 2 and 3 with clove oil was undesirable, whereas that of the other treatments was not significantly different with acceptability. Shelf life did not affect calyx blackening during the first 42 days of storage except for Bag 3 with clove oil, where calyx blackening significantly increased and was unacceptable after 42 days with three days of shelf life. After 56 days of storage with three days of shelf life, only calyx blackening of the control fruit was acceptable, whereas that of fruit in all packaging treatments was not.

Stem yellowing depended on the combination of packaging and shelf life, while stem blackening was affected by the combination of packaging, storage duration, and shelf life ($P<0.05$). Stem blackening developed in all treatments after 28 days of cold storage except in Bag 2 without clove oil, where stem blackening was detected after 42 days of cold storage (Figure 5-25). Stem blackening of all treatments increased with storage duration with a faster development for fruit in packaging with clove oil, especially in Bag 3. Stem blackening of fruit in Bags 2 and 3 with clove oil was unacceptable after 42 days of cold storage, while that of the other treatments was acceptable. After 56 days of cold storage, stem blackening of fruit in Bags 2 and 3 without clove oil was more severe than that of the control fruit, but it was still acceptable. During shelf life, the stem blackening of all treatments increased except Bag 3 with clove oil and the control after 28 and 56 days of storage, respectively. The stem blackening of fruit in Bags 3 and 2 without clove oil was

unacceptable during shelf life following 42 and 56 days of storage, respectively, while that of the control fruit was acceptable at the end of storage period with three days of shelf life.

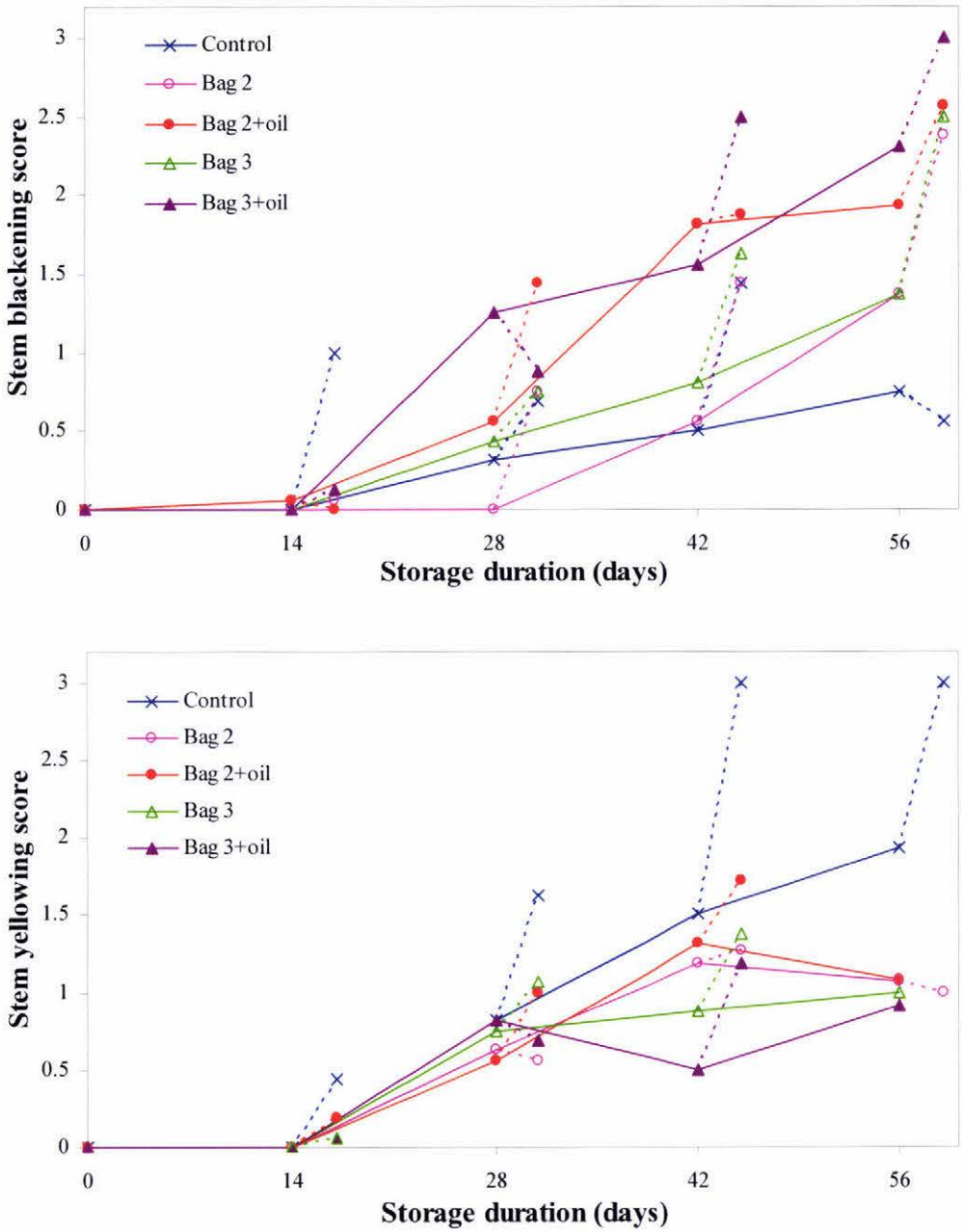

Figure 5-25. Stem blackening and yellowing score of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C without shelf life (—), and with 3 days of shelf life at 20°C (---). (s.e. of stem blackening and yellowing = 0.11 and 0.09, respectively, n = 16 fruit)

Stem yellowing of all treatments occurred similarly after 28 days of cold storage and gradually increased for the control fruit having the highest and unacceptable score after 42 days of cold storage (Figure 5-25). The stem yellowing of the packaged fruit with or without added clove oil slightly changed after 28 days of cold storage but remained acceptable. However, the stem yellowing of packaged fruit with added clove oil was difficult to detect after 42 days of cold storage as the stem developed blackening. During shelf life, the stem yellowing of the control fruit was undesirable after 28 days of storage, but it did not significantly change for packaged fruit with or without clove oil. The stems of the fruit in Bag 3 without clove oil and both Bags 2 and 3 with clove oil were all black and could not be assessed for stem yellowing during shelf life following 56 days of storage.

5.4. Discussion

5.4.1. Weight loss

Weight loss is mainly due to loss of water and responsible for loss of saleable weight. As little as 5% of weight loss will cause fruit quality deterioration and a shrivelled appearance (Wills *et al.*, 1998). In tamarillo fruit, the weight loss gradually increased with storage duration and only 0.5% loss at the end of storage period (34 days) (Schotsmans *et al.*, 2005), which is different from this study where the weight loss of all treatments was constant during cold storage with 0.3% loss for the control, fruit in Bags 2 and 3 without clove oil, and fruit in Bag 2 with clove oil, and 0.6% loss for fruit in Bag 3 with clove oil. The weight loss of all treatments increased up to 1% during shelf life except for Bag 2 without clove oil, where the weight loss was still lower than 1%.

Bag 3 (5.63×10^{-12} mol m s^{-1} m^{-2} Pa^{-1}) has a higher permeability to water vapour compared to Bag 2 (1.56×10^{-12} mol m s^{-1} m^{-2} Pa^{-1}), resulting in a faster water movement through Bag 3 than Bag 2. Additionally, the weight loss of fruit surrounding by high humidity is mainly due to respiration (Maguire *et al.*, 2001). Fruit in Bag 3 also had a higher respiration rate (0.40 μmol CO_2 kg^{-1} s^{-1}) than fruit in Bag 2 (0.20 μmol CO_2 kg^{-1} s^{-1}), leading to faster carbon and water loss from the fruit (Wills *et al.*, 1998). Hence, both higher water vapour

permeability of Bag 3 and fruit metabolism caused a higher weight loss of fruit in Bag 3 compared to fruit in Bag 2. However, fruit in Bag 3 had a higher weight loss than the control fruit possibly due to a significantly higher respiration rate of fruit in Bag 3. In general, fruit under unsealed polyliner have a higher rate of water loss compared to those under closed packaging as an unsealed polyliner provides more air movement around the fruit leading to a decrease in relative humidity and an increase in water vapour pressure deficit, and finally water loss (Wills *et al.*, 1998). Possibly, a higher respiration rate of tamarillo fruit under high relative humidity inside Bag 3 influenced the weight loss more than the effect of the polyliner. Bag 2 and the control caused a similar weight loss of tamarillo fruit during cold storage, but fruit in Bag 2 had a lower weight loss compared to the control after removal from cold storage to room temperature although fruit in Bag 2 had a higher respiration rate, indicating that Bag 2 reduced the weight loss during shelf life better than the control. The faster weight loss during shelf life is due to an increase in temperature which would increase transpiration and respiration (Maguire *et al.*, 2001) as well as permeability of the packaging material (Kader *et al.*, 1989) creating a lower relative humidity.

Addition of other antimicrobial substances like cinnamon or cinnamaldehyde did not affect water vapour permeability of edible film (Rojas-Grau *et al.*, 2007). Hence, the addition of clove oil in this study would not affect the permeability to water vapour of the packaging, and the weight loss of fruit in the packaging with clove oil was attributed to the effects of packaging materials and clove oil on fruit metabolism. In this study, the addition of clove oil to the packaging seemed to increase the weight loss of tamarillo fruit compared to the packaging without clove oil, which is very different from previous findings in sweet cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006) where the addition of eugenol to MAP reduced weight loss of fruits through decreased transpiration even though the mechanism is unknown. However, strawberry and tomato fruits exposed to eucalyptus or cinnamon oil vapours had a higher weight loss than untreated fruits, especially when transferred to room temperature, due to an increase in a respiration rate (Tzortzakis, 2007), whereas the addition of clove oil did not affect the respiration rate of tamarillo in both Bags 2 and 3. Possibly, fruit in the packaging with added clove oil may

have a higher transpiration rate compared to fruit in the packaging without clove oil, resulting in a higher weight loss.

5.4.2. Gas related measures

5.4.2.1. Respiration

Tamarillo is a non-climacteric fruit, its respiration and ethylene production do not quickly increase during ripening (Sale & Pringle, 1999). Respiration (measured as CO_2 production) of tamarillo fruit slowly decreases after harvest and starts to increase at the onset of senescence (Pratt & Reid, 1976). In this study, the CO_2 production rate of the control fruit remained constant at below 0.10 μmol CO_2 kg^{-1} s^{-1} during the entire storage period, whereas that of packaged fruit increased during the first 14 days of storage and remained constant thereafter. The higher respiration rate of the packaged fruit might be due to the way respiration rate measurements were done. The fruit were removed from the bags to measure respiration rate under normal atmosphere. Hence, the fruit might not have been in physiological equilibrium. The CO_2 production rate of fruit in Bag 3 was higher than that of the control fruit, but it was insignificantly higher compared to fruit in Bag 2. The CO_2 production rate of fruit in Bag 2 was insignificantly higher than that of the control fruit during 42 days of cold storage, but it became significant after 56 days of cold storage.

The respiration quotient (RQ), which is used to characterise the balance between aerobic and anaerobic respiration, and remained constant at around 0.8 during cold storage for the control fruit indicating aerobic respiration. The higher RQ of packaged fruit during cold storage corresponded to an increase in the CO_2 production rate with the development of bleeding in the locule. During shelf life at room temperature, the CO_2 production rate of packaged fruit increased two to three times and six to eight times for the control fruit resulting in an increase in RQ of all treatments. Within the same *Solanaceae* family, the RQ of capsicum fruit with aerobic respiration ranges between 0.89-1.81 (Blanke & Holthe, 1997). Hence, the RQ of the control fruit ranging between 1.5 and 2.3 during shelf life may indicate aerobic respiration, while the RQ of packaged fruit was more than 3 during shelf life after 28 days of storage for Bag 3 and after 42 days for Bag 2, possibly indicating a shift to anaerobic respiration. An increase in the RQ of the control fruit when stored at

room temperature indicated an accelerated respiratory metabolism leading to a lower O_2 availability inside the fruit (Lippert & Blanke, 2004). Hence, tamarillo fruit started ripening faster when stored at room temperature.

The development of low O_2 and high CO_2 concentrations in MAP is expected to slow down the metabolism of the fruit and inhibit micro-organism growth (Hertog, 2003). As in tomato and bell pepper (Burton, 1982), the main gas transport path in tamarillo is through the stem. However, during long exposure to low O_2 and high CO_2 concentrations inside the packaging, the tamarillo stem is closed thus preventing gas transport even more leading to reduced internal O_2 and increased CO_2, and subsequently anaerobic respiration in the tissue (Gran & Beaudry, 1993) as also found in tomatoes and bell peppers (Burton, 1982). This explains the higher rate of CO_2 production and RQ of packaged fruit compared to the control fruit. In general, the packaging in this study neither retarded respiration of tamarillo fruit nor extended shelf life.

Clove oil has been shown to reduce CO_2 production of yeast (Conner *et al.*, 1984) as well as the application of peppermint essential oil as weed control inhibits cucumber root respiration by reducing O_2 uptake (Mucciarelli *et al.*, 2001), which are different from this study where the addition of clove oil to MAP did not affect either CO_2 production or O_2 consumption rates of tamarillo.

5.4.2.2. Gas composition in the bags

During cold storage at 4°C, Bags 2 and 3 had low O_2 concentrations, 3-4% and 1-2%, respectively as well as high CO_2 concentrations, 4-7% and 7-10%, respectively. The lower O_2 and higher CO_2 concentration in Bag 3 compared to Bag 2 related to their characteristic, Bag 3 having the lower permeability of the two. The depleted O_2 and enriched CO_2 in the packaging mainly results from the gas exchange characteristic of the packaged fruit coupled with the packaging material applied (Chen *et al.*, 2000). During shelf life at room temperature, the O_2 concentrations of Bags 2 and 3 slightly increased, although insignificant, whereas the CO_2 concentrations of both Bags 2 and 3 doubled. The insignificant increase in O_2 concentration was possibly due to an increase in CO_2 concentration in the bags suppressing O_2 consumption and/or an increase in permeability

of the bags to O_2 with an increase in temperature (Kader *et al.*, 1989). In general, a change in temperature influences the respiratory metabolism of the fruit and also the rate of gases penetrating through the packaging material (Hertog, 2003), but the respiratory mechanism of fruit is more sensitive to a change in temperature than the permeability of packaging film (Kader *et al.*, 1989). Hence, the different atmosphere developing inside the packaging of tamarillo fruit during storage at 4°C and 20°C may indicate higher activation energy for tamarillo fruit respiration compared to that for film permeability to gas exchange.

The addition of clove oil to the packaging in this study did not affect O_2 and CO_2 concentrations similar to results found in cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006). Addition of other antimicrobial substrate like cinnamon oil or cinnamaldehyde did not affect oxygen permeability of edible film (Rojas-Grau *et al.*, 2007). Hence, the development of bleeding in the locule of tamarillo fruit in the packaging with or without clove oil can be attributed to the high CO_2 and low O_2 concentrations. The development of bleeding in the locule of fruit in Bag 3 within 28 days of storage at 4°C may indicate that the low O_2 (1-2%) and high CO_2 (7-10%) levels could have induced anaerobic respiration, whereas the slightly higher O_2 (3-4%) and lower CO_2 (4-7%) levels in Bag 2 would induce anaerobic respiration later, within 42 days of storage. Nevertheless, neither Bag 2 nor Bag 3 could prolong postharvest life of tamarillo compared to the control. The development of discoloration on the surface of the fruit base in Bag 3 without clove oil during shelf life after 28 days of cold storage and the shrivelling of fruit with lost skin colour in Bag 2 without clove oil after 42 of cold storage were similar to low O_2 (0.5 or 1%) or high CO_2 (80%) injury in tomato (Klieber *et al.*, 1996).

5.4.2.3. Ethylene production

Tamarillo fruit produces a small amount of ethylene after harvest and rapidly increases ethylene production at the onset of senescence (Pratt & Reid, 1976). Ethylene production is decreased by low temperatures (Zamorano *et al.*, 1994) as seen by the lower production at 4°C and higher production at 20°C. An increase in ethylene production accelerates fruit ripening (Pratt & Reid, 1976; Prohens *et al.*, 1996). Therefore, ripening of tamarillo is delayed by cold storage and hastened when fruit is warmed up again during shelf life. In

this study, the control fruit had a lower level of ethylene production compared to fruit in packaging. The higher ethylene production rate of packaged fruit was related to a higher respiration rate, this corroborates previous findings in tamarillo (Pratt & Reid, 1976) where an increase in the respiration rate (CO_2 production rate) corresponded to an increase in the ethylene production rate.

Ethylene concentration in all packaging, especially in Bag 2, increased with storage duration, and rapidly increased during shelf life. This was related to continued production of ethylene of packaged fruit. The increase in ethylene concentration during shelf life is due to a higher temperature as it accelerates fruit metabolism (Hertog, 2003).

In table grape, the addition of eugenol reduced ethylene concentration in MAP during cold storage (Valverde *et al.*, 2005) which is different from this study where the addition of clove oil affected neither the ethylene production rate of tamarillo nor ethylene concentration in the packaging.

5.4.2.4. Eugenol concentration

As an antibacterial additive, eugenol has been shown to improve postharvest quality of cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006) keeping their stems healthy. In this study, the effect of eugenol on the quality of tamarillo fruit and stem was studied in the form of eugenol released from a clove oil sachet as clove oil is composed of 85% eugenol (Burt, 2004). The eugenol release was slow during cold storage at 4°C, but increased during shelf life at room temperature (20°C). The eugenol concentration inside the packaging increased during the first 14 days of storage and decreased after 42 days of storage. In cherry (Serrano *et al.*, 2005), 1 ml pure eugenol was applied on gauze and put inside MAP with the size 15×20 cm and its release did not cause any disorders; it had only positive effects on fruit quality like delaying weight loss and colour changes, retarding a change of SSC/TA ratio, and maintaining firmness. This is very different from this study where discoloration developed on the fruit surface in the packaging with added clove oil after only 14 days of cold storage and became unacceptable after 28 days of cold storage, while it was not observed for the control fruit and fruit in the packaging without clove oil. This may be due to the hydroxyl group on eugenol. The

hydroxyl group interacts with the proteins in the membrane hence disrupting the cytoplasmic membrane (Burt, 2004). As a result, cell membranes lose their structure, and subsequently the hydrophobic compound of eugenol can disrupt the membrane lipids resulting in more leakage. Eugenol is also found to cause cytotoxicity on rat and mouse hepatocytes (Burkey *et al.*, 2000) and to inhibit mitochondrial respiration of rat liver (Cotmore *et al.*, 1979). Moreover, the 15×21 cm bag for the tamarillo fruit was possibly too small and 0.76 ml clove oil in a sachet may be too high. Hence, the atmosphere inside the packaging is quickly saturated with a high eugenol concentration in a short time resulting in an ill effect on the fruit skin.

5.4.3. Non destructive measures

5.4.3.1. Colour

The colour development of tamarillo fruit is commercially used to assess fruit maturity in New Zealand (Sale & Pringle, 1999). A decrease in lightness and yellowness, and an increase in redness of the tamarillo fruit peel after harvest indicate fruit ripening (Mwithiga *et al.*, 2007). The addition of eugenol to MAP successfully delays the changes of lightness, redness, yellowness, chroma, and hue angle in cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006). This is very different from what we found in tamarillo where a yellow colour developed on the fruit skin in the packaging with added clove oil after only 14 days of cold storage and was unacceptable after 28 days of cold storage. This discoloration was confirmed by an increase in hue angle during shelf life following 28 days of storage indicating a change in colour from red to yellow.

The healthy fruit in Bag 2 without clove oil had a constant lightness during storage and like the control fruit redness, yellowness, and chroma decreased after 28 days of storage. The decline in chroma with a decrease in redness and yellowness of the control fruit and fruit in Bag 2 reflects a decreasing intensity of red and yellow. The hue angle of the control fruit and fruit in Bag 2 was similar and did not change during storage and shelf life compared to the initial fruit. However, fruit in Bag 2 lost skin colour and shrivelled after 42 days of cold storage and fruit in Bag 3 without clove oil developed an unacceptable

yellow colour and higher hue angle on the fruit surface at the fruit base after shelf life following 28 days of storage.

Therefore, the packaging with or without the addition of clove oil in this study did not delay ripening of tamarillo fruit, improve colour development or extend shelf life compared to the control.

5.4.3.2. Fruit texture

Texture of tamarillo fruit was measured by two different techniques and expressed as stiffness and compression firmness. Stiffness is measured as the acoustic impulse response of a fruit after tapping it with a light plastic rod, and compression firmness is measured as the compression force by recording the maximum force required to compress the fruit for 2 mm. Stiffness and compression firmness decrease with a decrease in fruit firmness as found in tomato fruit (Hertog *et al.*, 2004a), which is similar to this study where stiffness and compression firmness of tamarillo decreased with an increase in storage duration. Stiffness and compression firmness are similarly influenced by temperature, water vapour pressure deficit, and relative humidity, factors that affect water loss. Stiffness and compression firmness are higher at lower temperature (Hertog *et al.*, 2004a) with a higher stiffness and compression firmness of tamarillo at 4°C and lower at 20°C. Moreover, the loss in stiffness also relates to an increase in mass loss (weight loss) as found in apple (De Belie *et al.*, 1999). The gradual decrease in stiffness and compression firmness of the control fruit with constant weight loss during cold storage was related to an increase in ripeness as the texture of tamarillo changes with an increase in ripeness (Mwithiga *et al.*, 2007). As fruit ripens, the cell wall swells with an increase in enzyme activities, pectin methyl esterase (PME) and polygalacturonase (PG), resulting in softening (MacRae *et al.*, 1990). The enzyme activities also depend on temperature with a higher temperature corresponding to higher activities, accelerating fruit softening (Wills *et al.*, 1998) as also seen in a higher loss of stiffness and compression firmness of the control fruit during shelf life storage in this study. The higher temperature also accelerates water loss from fruit resulting in a loss of cell turgor related to a decrease in stiffness and compression firmness (Hertog *et al.*, 2004a). Fruit in Bags 2 and 3 had a similar weight loss during cold storage

with or without shelf life, but stiffness and compression firmness of fruit in Bag 3 decreased after 14 days of cold storage with a dramatic decrease during shelf life mainly due to the earlier development of bleeding in the locule. The fast decline of stiffness and compression firmness of fruit in Bag 2 after 28 days of cold storage resulted from an increase in bleeding in the locule bringing stiffness and compression firmness of fruit in Bag 2 to the same level of the control fruit after 42 and 56 days of cold storage, respectively. Although the control fruit and fruit in Bag 2 had a similar decrease in stiffness and compression firmness during shelf life following 42 and 56 days of storage, the decrease in stiffness and compression firmness of the control fruit resulted from an increase in ripeness and weight loss, while that of fruit in Bag 2 was due to an increase in the bleeding in the locule and weight loss. Concluding, Bags 2 and 3 in this study neither improved texture of tamarillo fruit during long term cold storage nor extended shelf life.

The addition of clove oil to MAP of cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006) delayed softening during storage and shelf life as the antibacterial compound reduced the activities of PG and PME, involved in cell wall degradation even though the mechanism is unknown. This is different from what we found in tamarillo where the addition of clove oil to packaging had a negative effect on fruit texture as the surface of fruit close to a clove oil sachet was notably softer as measured by touch and compared to a healthy surface of fruit in the same packaging. As an antibacterial additive, eugenol interacts with the proteins in the membranes resulting in a loss of cell compartmentation (Burt, 2004), and hence it could lead to the movement of fluid from inside the cell to the intercellular space causing a loss of cell turgor, and subsequently tissue softening. Concluding, the addition of clove oil to the packaging in this study caused an ill effect and did not intensify the effect of packaging on firmness.

5.4.4. Juice characteristics

5.4.4.1. Soluble solids content

The soluble solids content (SSC) of tamarillo fruit increases during ripening (El-Zeftawi *et al.*, 1988; Mwithiga *et al.*, 2007). The SSC range in this study was 10-11°brix during storage with or without shelf life similar to previous findings (Schotsmans *et al.*, 2005)

where the SSC of red tamarillo ranged between 11-12°brix during storage and shelf life. In this study, SSC of the control and packaged fruit was not significantly different and slightly changed during cold storage. Hence, the packaging in this study did not enhance the SSC of tamarillo fruit compared with the control. In general, SSC decreases during cold storage and shelf life in most fruits. This is a result of the use of these soluble solids as a substrate for the general metabolism of the fruit that is separated from the tree and thus from other substrates for the biochemical reactions like fruit respiration where fruit converts sugars to carbon dioxide and energy (Wills *et al.*, 1998). The increased temperature normally results in a higher general metabolism and reduction of SSC (Wills *et al.*, 1998). However, in this study, SSC remained constant after the transfer from cold storage to room temperature which seems contradictory to the increase in respiration rate during shelf life. However, during aerobic respiration, some fruit can utilise organic acids as substrates, evident in RQ values higher than 1 (Robertson, 1993). This is possibly the case for tamarillo fruit, where the RQ was typically high during shelf life and is confirmed by the decrease in TA during shelf life after longer storage.

In cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006), the addition of eugenol to MAP delayed an increase in SSC compared to untreated fruit, which is similar to this study where the addition of clove oil to the packaging slightly decreased SSC during 28 days of cold storage.

5.4.4.2. pH and titratable acidity of the juice

An increase in pH and decrease in TA is found in most fruit during storage and is a result of the use of acids as a substrate for the general metabolism of the fruit like the tricarboxylic acid cycle, a reaction in the respiratory system (Wills *et al.*, 1998). In general, pH of tamarillo increases (Mwithiga *et al.*, 2007) and TA decreases (El-Zeftawi *et al.*, 1988) during fruit ripening. During cold storage at 4°C, pH and TA of the control fruit were constant, and increased and decreased, respectively during shelf life indicating that the ripening of tamarillo was slowed down during cold storage and accelerated during shelf life. Packaging did not affect pH and TA of tamarillo compared to the control. The increase in pH and the reduction in TA of all treatments during shelf life were related to an

increase in fruit metabolism due to an increase in temperature (Wills *et al.*, 1998) as seen a higher CO_2 production rate. During shelf life following 42 and 56 days of storage, TA of fruit in Bag 2 decreased slower, but was insignificantly higher, than that of the control fruit showing that Bag 2 possibly delayed the reduction of organic acids during shelf life following long term storage.

In cherry (Serrano *et al.*, 2005) and table grape (Valverde *et al.*, 2005; Valero *et al.*, 2006), the addition of eugenol to MAP delayed the loss of organic acids resulting in slower ripening, which is different from findings in strawberry and tomato fruits (Tzortzakis, 2007) and tamarillo in this study where pH and TA of fruits exposed to the essential oil did not differ compared to untreated fruits.

5.4.4.3. The ratio of SSC to TA

The SSC/TA ratio is a parameter to define maturity or ripening (Wills *et al.*, 1998). The SSC/TA ratio increased for all treatments during 14 days of storage at 4°C due to an increase in SSC and decrease in TA. After 28 days of storage, the SSC/TA ratio of the control fruit and fruit in Bag 3 without clove oil remained constant, while it increased for fruit in Bag 2 with or without clove oil and Bag 3 with clove oil due to a decrease in TA. The SSC/TA ratio of the control fruit and fruit in Bags 2 and 3 without clove oil was similar during cold storage due to no significant differences in SSC and TA ranging from 10-11°brix and 1.5-1.9 g citric/100 ml juice, respectively.

Shelf life caused an increase in the SSC/TA ratio of all treatments during storage except after 28 days of storage, when a decrease in the SSC/TA ratio was seen due to a decrease in SSC and increase in TA. The increase in the SSC/TA ratio during shelf life was mainly due to a lower TA. Using the SSC/TA ratio as a predictor of tamarillo fruit flavour, the packaging, especially Bag 2, did not improve fruit flavour compared to the control during cold storage, but slowed down a change in the SSC/TA ratio during shelf life following long term cold storage by delaying the loss in TA.

The addition of eugenol to MAP of table grape reduced the SSC/TA ratio mainly by delaying the loss of organic acids (Valero *et al.*, 2006) contrary to findings in strawberry

and tomato fruits (Tzortzakis, 2007) and tamarillo where the SSC/TA ratio did not change during exposure to essential oil vapour compared to untreated fruits.

5.4.5. Fruit stem measures

5.4.5.1. Moisture content

The healthy and intact green stem of tamarillo fruit influences consumer and marketing perception (Sale & Pringle, 1999). For cherry fruit, a healthy green stem is correlated to higher stem moisture content (Drake *et al.*, 1988). The discoloration of fruit stem has been related to an increase in ripeness (Clayton *et al.*, 2003) and dehydration (Schick & Toivonen, 2002). The loss in stem moisture results from an increase in temperature and a decrease in relative humidity, and subsequently the cells in the stem tissue lose their integrity and compartmentation leading to the interaction between polyphenol oxidase and phenolic substrates and finally tissue browning (Schick & Toivonen, 2002). However, the loss in stem moisture under polyliner (the control) and in the packaging in this study was equally slowed down during storage at 4°C, but accelerated at higher temperature (20°C). Thus, the packaging in this study did not enhance the moisture content in the stem of tamarillo fruit better than the polyliner.

The addition of clove oil to packaging caused a fast development of blackening on the tamarillo stem compared to the other treatments although the moisture content in the stem was not significantly different from the others, which is very different from previous findings in cherry (Serrano *et al.*, 2005) where the addition of eugenol to MAP decreased dehydration and stem browning with a higher green colour at the end of storage period compared with untreated fruit. Eugenol is thought to reduce transpiration even though the mechanism is unknown. However, this was not supported by the findings of this study. Thus, the addition of clove oil to the packaging neither enhanced the stem moisture content nor improved stem colour.

5.4.5.2. Chlorophyll content

The chlorophyll content in fruits and vegetables is related to the green colour (Fonseca *et al.*, 2005). A decrease in chlorophyll content during ripening or senescence unmasks other colours. Hence, the occurrence of stem discoloration possibly relates to the change in chlorophyll content of its tissue. Although the overall mechanism of chlorophyll degradation is not understood, the two critical factors causing chlorophyll degradation are the presence of light and/or oxygen (Brown *et al.*, 1991). Also, the enzymes chlorophyllase and peroxidase, and activated oxygen like peroxide have been related to chlorophyll degradation. In tamarillo, the chlorophyll content in the stem of the control fruit decreased gradually, although insignificantly, with an increase in storage duration similar to results found in Galega kale (Fonseca *et al.*, 2005), Bok Choy (Lu, 2007), and broccoli (Serrano *et al.*, 2006). The chlorophyll content of the stem of packaged fruit remained constant during cold storage and was higher than for the control fruit after long term storage as low O_2 and high CO_2 concentration in the packaging slows down photooxidative bleaching or chlorophyll peroxidase (Brown *et al.*, 1991), and reduces the activity of chlorophyllase (Guevara *et al.*, 2003), involved in the degradation of chlorophyll.

During shelf life at room temperature, the chlorophyll content in the stem insignificantly decreased for the control fruit but remained constant for the packaged fruit. The decrease in the chlorophyll content in the stem of the control fruit possibly related to an increased activity of chlorophyll degrading enzymes due to an increase in temperature (Hendry *et al.*, 1987), and an increase in photodegradation due to increased light (Brown *et al.*, 1991). In the packaging this was counteracted by the low O_2 concentration in the bags. Low O_2 concentrations can suppress chlorophyll degradation as shown in Galega kale (Fonseca *et al.*, 2005), Bok Choy (Lu, 2007), and broccoli (Serrano *et al.*, 2006). Hence, the packaging in this study retarded the loss in the chlorophyll content in the stem of tamarillo fruit during long term storage and extended shelf life.

The addition of clove oil to the packaging, especially Bag 3, apparently caused a higher chlorophyll content in the stem of tamarillo compared to the packaging without added clove oil as the antioxidant activity of eugenol released from the clove oil possibly reduced

chlorophyll degradation (Serrano *et al.*, 2005). Eugenol has been found to prevent peroxidation (Lederer *et al.*, 2004), in which singlet oxygen like peroxide degrades chlorophyll (Brown *et al.*, 1991), leading to the inhibition of chlorophyll degradation. Hence, the eugenol released through a clove oil sachet in this study may intensify the effect of packaging on reducing the chlorophyll degradation in the stem. Nevertheless, the fruit stem in the packaging with clove oil in this study had the highest incidence of stem and calyx blackening contrary to the results found in cherry stem in packaging with added pure eugenol (Serrano *et al.*, 2005), where the stem browning was lowest. Thus, the addition of clove oil to the packaging in this study maintained chlorophyll content in the stem but did not prevent the calyx and stem discoloration.

5.4.5.3. Polyphenol oxidase activity

Polyphenol oxidase (PPO) activity has been related to enzymatic browning and polymerizes phenolic compounds causing browning in fruit and vegetable tissues (Vamos-Vigyazo, 1981). In this study, the PPO activity of all treatments increased similarly during storage at 4°C and quickly increased during shelf life as an increased temperature accelerated the PPO activity (Martinez & Whitaker, 1995). The increase in the PPO activity coincided with the development of calyx and stem blackening in this study similar to an increase in the PPO activity with the development of surface browning of strawberry (Nunes *et al.*, 2005), tissue browning of loquat fruit (Cai *et al.*, 2006) and peach (Brandelli & Lopes, 2005), and browning of mango peel (John *et al.*, 2002). However, the calyx and stem of fruit in packaging with added clove oil developed blackening faster compared to the other treatments contrary to the results found in cherry (Serrano *et al.*, 2005) where the addition of pure eugenol to MAP delayed stem browning of the fruit. Eugenol can delay enzymatic browning because the benzene derivatives of eugenol can effectively prevent oxidation (Ruberto & Baratta, 2000), resulting in the inhibition of brown polymers formation. The addition of clove oil in this study may have been overdosed and have disrupted cell compartmentation enabling substrates to interact with PPO (Nunes *et al.*, 2005) thus increasing stem browning/blackening.

Controlled atmosphere and MAP seem to slow down tissue browning as O_2 is required for the oxidation of substrates to produce brown polymers (Vamos-Vigyazo, 1981) and CO_2 is a competitive inhibitor of PPO (Deng *et al.*, 2006). Nevertheless, the stem of packaged fruit had similar PPO activity to that of the control fruit, but the sensory score of calyx and stem blackening was higher, meaning that blackening was worse, in the packaged fruit compared to the control fruit after long term storage and during shelf life storage. These results are similar to findings in bamboo shoot (Shen *et al.*, 2006) where MAP (2% of O_2 and 5% of CO_2) did not inhibit the PPO activity; however, it did prevent browning on the bamboo shoot by inhibiting the accumulation of malondialdehyde and reducing the activity of peroxidase and phenylalanin ammonialyase. Possibly, the blackening of calyx and stem of the packaged fruit in this study may have been due to the low O_2 and high CO_2 injury (Kader *et al.*, 1989). The results in this study demonstrate that the packaging with or without clove oil did not inhibit the PPO activity in comparison with the control.

5.4.6. Sensory test

The fast development of fruit discoloration, calyx blackening, and stem blackening of fruit in packaging with clove oil during cold storage with or without shelf life indicated that the addition of clove oil neither enhanced nor maintained fruit and stem quality. Without the addition of clove oil, fruit in Bags 2 and 3 had similar calyx lifting and blackening, and stem blackening and yellowing during cold storage. A gradual increase in calyx and stem blackening of fruit in the packaging was not related to chlorophyll content as it remained constant during the cold storage period and was higher compared to the control fruit after long term storage. Bag 3 caused unacceptable discoloration on the fruit surface after 42 days of storage potentially due to low O_2 and high CO_2 injuries, while Bag 2 did not. Unlike for the control, shelf life accelerated calyx and stem blackening of fruit in Bags 2 and 3 and made the fruit unacceptable following 56 days of storage. Bags 2 and 3 did maintain the stem quality of tamarillo during cold storage without extending shelf life, but Bag 3 did not prolong fruit appearance. Although Bag 2 was better than Bag 3 in maintaining fruit quality and stem colour to the end of the cold storage period, fruit in Bag 2 had a higher incidence of calyx lifting, and blackening of both calyx and stem compared to the control fruit. Additionally, after 42 days of cold storage, fruit in Bag 2 was shrivelled

and had lost colour which was not seen for the control fruit. The development of stem yellowing of the control fruit was the only problem causing unacceptability and was due to chlorophyll degradation, while packaged fruit, especially with clove oil, had a lower incidence of stem yellowing due to a higher chlorophyll content. Therefore, the control would be the best treatment for long term storage with additional shelf life to maintain tamarillo fruit appearance with acceptable stem blackening, but it did not inhibit stem yellowing. The packaging without clove oil retarded stem yellowing with acceptable stem blackening during 56 days of storage, but it did not maintain the quality of the fruit for long term storage and did not extend shelf life. The incidence of stem end rot was low and acceptable during cold storage with or without shelf life in this study.

5.5. Conclusion

The application of two types of packaging (low permeability; Bag 3; BL-WO-23339, and high permeability; Bag 2; MK-WO-23339) with or without the addition of clove oil was investigated as a technique to extend postharvest life of tamarillo fruit and stem. Tamarillo fruit in a cardboard box with plastic tray and polyliner (the control) had low ethylene production during storage at 4°C. Weight loss, respiration rate, lightness, hue angle, SSC, pH, TA, and SSC/TA ratio were constant, and redness, yellowness, chroma, stiffness, and compression firmness decreased. The low permeability of Bag 3 caused the highest CO_2 production rate and accumulation of CO_2 and the lowest O_2 and ethylene concentrations with the development of bleeding in the locule and discoloration on the fruit surface at the fruit base after only 28 days of cold storage and becoming more intense during shelf life. This rules out Bag 3 as an appropriate packaging to extend the postharvest life of tamarillo fruit. However, Bag 2 with the higher permeability did not improve the quality of tamarillo fruit either, with weight loss, redness, yellowness, chroma, hue angle, stiffness, compression firmness, SSC, pH, TA, and the SSC/TA ratio being similar to the control fruit after long term cold storage with or without shelf life. Also, fruit in Bag 2 shrivelled, lost skin colour and developed bleeding in the locule after 42 days of cold storage. Packaging slowed down the development of stem yellowing but the incidence of calyx lifting, calyx blackening, and stem blackening was higher compared to the control fruit.

This shows that the packaging did not provide any advantage in maintaining the quality of the fruit, but it did retard only the stem yellowing.

The addition of clove oil to the packaging focused on the effect of eugenol release on tamarillo fruit and stem. The addition of clove oil did not cause a different atmosphere to develop inside the packaging nor did it change the quality of the fruit except for an increase in weight loss and a decrease in SSC. However, the fruit in the packaging with clove oil developed unacceptable discoloration on the fruit surface after only 28 days of cold storage, becoming more intense during shelf life. The stem of the fruit in the packaging with clove oil had a higher incidence of calyx and stem blackening in comparison with the control fruit and fruit in packaging without clove oil. Thus, the addition of clove oil did not enhance the quality of tamarillo fruit and stem. Nevertheless, further study is necessary to elucidate the effect of clove oil in the packaging on the quality of tamarillo fruit and stem since the amount of clove oil added to the packaging might have been too high.

To determine the mechanism of discoloration of tamarillo fruit stem during storage, moisture content, chlorophyll degradation, and PPO activity were studied and related to the levels of yellowing and blackening. The moisture content in the stem was similar between packaged fruit with or without added clove oil and the control fruit. Chlorophyll degradation was related to the development of stem yellowing and delayed by the packaging, especially with added clove oil, during long term storage and shelf life. The higher chlorophyll content in the fruit stem in the packaging may reveal a higher green colour, but the greater incidence of blackening masked the green colour. An increase in PPO activity was a reasonable parameter to predict stem blackening of tamarillo fruit as it increased with the development of calyx and stem blackening as found in the control fruit during storage and shelf life. Nevertheless, the PPO activity was not inhibited by the packaging with or without added clove oil. The higher incidence of calyx and stem blackening of packaged fruit may have been due to the depleted O_2 and enriched CO_2 atmosphere and the clove oil disrupting the cell membranes resulting in an interaction between phenolic substrates and PPO, and subsequently calyx and stem blackening.

CHAPTER 6

CONCLUSION

6.1. Passionfruit

The changes in quality parameters during storage and shelf life for purple passionfruit were as expected in line with changes that occur in other fruit: during storage at 8°C weight loss, a^* (redness), pH, and sweetness increased; respiration rate, lightness, chroma, stiffness, and compression firmness were constant; and water vapour permeance, ethylene production, b^* (yellowness), hue angle, pulp yield, SSC, TA, and sourness decreased. Upon removal from cold storage to room temperature (20°C), weight loss, respiration rate, ethylene production, redness, chroma, stiffness, pulp yield, pH, and sweetness increased; hue angle, lightness, compression firmness, SSC, TA, and sourness decreased; and water vapour permeance and yellowness were constant.

Compression firmness was a better predictor to assess firmness change of passionfruit compared to stiffness as compression firmness reduced during cold storage and shelf life. TA was a good tool to predict eating quality as the reduction in TA corresponded to an increase in sensory sweetness and a decrease in sensory sourness. The SSC/TA ratio may be a better tool to predict the eating quality of purple passionfruit since it incorporated both flavour determining aspects, with the best flavour around 10-11.

Waxing did not provide any advantage to the fruit with the quality of waxed fruit being similar to that of control fruit for water vapour permeance, water loss, redness, stiffness, compression firmness, pulp yield, SSC, pH, TA, and sweetness. Moreover, shrivelling was not prevented by waxing the fruit. The modified atmosphere packaging controlled shrivelling and extended storage life with the packaging material playing an important part in the quality retention of the fruit. The lowest gas permeability of Bag 1 caused the highest accumulation of CO_2 and C_2H_4, and the lowest level of O_2 and resulted in the development of bleeding, red spots, and fungal growth within 20 days of storage, indicating that Bag 1 is not an appropriate packaging to extend the postharvest life of

passionfruit. Bags 2, 3, and 4 had similar O_2 and C_2H_4 concentrations during cold storage. However, more CO_2 accumulated in Bag 3 and fruit was more red and sour, had higher, stiffness and TA, and lower hue angle, compression firmness, SSC, pH, SSC/TA ratio and sweetness compared to fruit in Bags 2 and 4. Although fruit in Bag 3 looked healthy after removal from cold storage, fungal growth and red discoloration on the fruit surface appeared during shelf life after 28 days of storage. Hence, Bag 3 does not maintain the quality of passionfruit either. Fruit quality in Bags 2 and 4 was similar with no difference in weight loss, redness, yellowness, chroma, hue angle, stiffness, compression firmness, pulp yield, SSC, pH, TA, sweetness, and sourness. However, Bag 4 had more ethylene accumulation, although insignificant, which could be the reason for the development of an off-flavour during shelf life after 42 days of storage, whereas the fruit in Bag 2 had no off-flavour.

Because the insignificantly higher ethylene concentration in Bag 4 was the only difference between Bags 2 and 4, an ethylene scavenger ($KMnO_4$) was added to Bag 4 in a second trial and fruit quality in Bag 4 with the ethylene absorber was compared with fruit in Bag 2 without the ethylene absorber. The addition of the ethylene scavenger did not affect the levels of O_2 and CO_2 inside the packaging, but it brought the C_2H_4 concentration in Bag 4 to the same level as in Bag 2 although levels of C_2H_2 were much lower than in the first trial. The quality of fruit in Bag 4, expressed as weight loss, colour, firmness, pulp yield, SSC, pH, TA, the SSC/TA ratio, sweetness, and sourness, was also brought to the same level as fruit in Bag 2 during long term storage. Moreover, the juice of fruit in Bag 4 with the ethylene absorber did not develop an off-flavour. However, shelf life of fruit in Bag 2 at room temperature after 70 days of storage was less than four days as the fruit developed large indentations and a white power-like substrate on the fruit surface. In contrast, 60% of fruit in Bag 4 with the ethylene absorber was healthy with lower redness and SSC/TA ratio, higher hue angle and TA, and constant lightness, yellowness, chroma, firmness, pulp yield, SSC, pH, sweetness, and sourness during seven days of shelf life following 70 days of storage. Thus, the addition of an ethylene absorber to the packaging may have a major effect on extending shelf life at room temperature following long term cold storage though whether this is due to ethylene absorption is uncertain.

From the results of these trials, we can conclude that modified atmosphere storage would provide a certain benefit for long term storage of passionfruit. However, special care has to be given to the choice of the atmosphere inside the packaging as undesirable atmosphere can lead to bleeding, discoloration, development of off-flavours and general bad fruit quality during long term storage. The addition of an ethylene scavenger improved the quality and extended the shelf life of passionfruit after long term storage.

6.2. Tamarillo

Red tamarillo in a cardboard box with plastic tray and polyliner (the control) had low ethylene production and during cold storage at 4°C, weight loss, respiration rate, lightness, hue angle, SSC, pH, TA, and SSC/TA ratio were constant, and redness, yellowness, chroma, stiffness, and compression firmness decreased. After transfer to room temperature, weight loss, respiration rate, ethylene production rate, pH, and SSC/TA ratio increased, redness, yellowness, chroma, stiffness, compression firmness, and TA decreased, and lightness, hue angle, and SSC were constant.

The lower permeability of Bag 3 compared to Bag 2 resulted in a higher CO_2 production rate and thus accumulation of CO_2 and the lowest O_2 and ethylene concentrations in Bag 3. This resulted in the development of bleeding in the locule and yellow discoloration on the fruit surface at the fruit base after only 28 days of storage at 4°C which intensified during shelf life. This rules out Bag 3 as an appropriate packaging to extend the storage life of tamarillo. However, Bag 2 with the higher permeability did not improve the quality of tamarillo either compared to the control fruit with no measurable difference in weight loss, redness, yellowness, chroma, hue angle, stiffness, compression firmness, SSC, pH, and the SSC/TA ratio during long term cold storage with or without shelf life. Fruit in Bag 2 also shrivelled, lost skin colour, and developed bleeding in the locule after 42 days of cold storage, whereas this did not happen to the control fruit, indicating that fruit no longer tolerated the modified atmosphere (3-4% O_2 and 4-7% CO_2) inside the packaging. From these results it seems that modified atmosphere packaging is not a good option for tamarillo and the current method of adding a polyliner to the cardboard tray is the best method.

The appearance of the fruit stem and calyx was judged by assessing calyx lifting and blackening, and stem yellowing and blackening. Bags 2 and 3 similarly slowed down the development of stem yellowing, but did not reduce the incidence of calyx lifting and blackening, and stem blackening compared the control. The higher incidence of calyx lifting and blackening, and stem blackening of packaged fruit may have been due to low O_2 and high CO_2 injury. This shows that even though the packaging did not provide any advantage in maintaining the quality of tamarillo fruit, it did retard only the stem yellowing.

The addition of clove oil to the packaging focused on the effect of eugenol release. The addition of clove oil neither affected the atmosphere developed inside the packaging nor did it change fruit behaviour except for an increase in weight loss and a decrease in SSC. However, it caused unacceptable discoloration on the fruit surface after only 28 days of storage at 4°C, being more intense during shelf life. The stem of fruit in the packaging with clove oil had a higher incidence of calyx and stem blackening compared to the control fruit and fruit in packaging without clove oil possibly due to an overdose of clove oil coupled with depleted O_2 and enriched CO_2 atmosphere inside the packaging. Concluding, the addition of clove oil to the packaging in this study did not improve the quality of tamarillo fruit and stem.

The mechanism of stem discolouration was investigated; moisture content, chlorophyll content, and the activity of PPO were evaluated. The moisture content of the stem was similar between packaged fruit with or without added clove oil and the control fruit during cold storage with or without shelf life. The chlorophyll content in the stem of the control fruit gradually decreased during storage and this coincided with the development of stem yellowing, while the chlorophyll content in the stem of packaged fruit with or without added clove oil remained constant. The higher chlorophyll content in the stem of packaged fruit may reveal a higher green colour, but the greater incidence of blackening masked the green colour. The PPO activity increased with the development of calyx and stem blackening as found in the control fruit during storage and shelf life. However, the packaging with or without the added clove oil did not decrease PPO activity. The low O_2 and high CO_2 levels, and an overdose of clove oil may have disrupted the cell membrane

and led to the interaction between phenolic substrates and PPO, and subsequently an increase in calyx and stem blackening.

6.3. Recommendations and future work

The storage issues for purple passionfruit and red tamarillo are very different and so are the solutions. Where for purple passionfruit the issue is shrivelling, for tamarillo fruit the main problem is stem discoloration. This necessitates different storage solutions, as was found in the research presented here. For purple passionfruit, MAP seems to be adding value by preventing shrivelling, whereas this was not the case for red tamarillo where MAP was detrimental for the quality of the fruit and did not prevent stem discoloration.

With the information available from this research, compression firmness, TA, and the SSC/TA ratio are the best parameters to assess quality changes of purple passionfruit during storage. Modified atmosphere packaging is advisable for long term cold storage since it minimised shrivelling while also maintaining other quality aspects. However, care should be taken that a packaging film with a high oxygen transmission rate is chosen. In our research this was found in Bag 2. The addition of an ethylene absorber could provide an additional increase in postharvest life of passionfruit.

However, modified atmosphere packaging is not a viable option for storage of red tamarillo fruit as Bags 2 and 3 did not extend the storage life of red tamarillo fruit and stem. The current method of a cardboard box with plastic tray and polyliner is the best method to maintain the quality of tamarillo fruit and stem. The addition of clove oil at the concentration we chose does not extend the storage life of tamarillo fruit and stem nor does it inhibit stem discoloration. The development of stem yellowing and blackening is related to chlorophyll degradation and an increase in the PPO activity, respectively.

Based on the overall results obtained in this study, the following areas of further research are suggested:

- since the addition of an ethylene scavenger to Bag 4 in the passionfruit trials brought the quality of the fruit to the same level as for Bag 2, it would be

interesting to investigate whether the addition of adding an ethylene absorber to the optimal MAP during storage of purple passionfruit would provide further quality retention and longer storage life;

- currently there are little or no objective quality standards for purple passionfruit and red tamarillo. To ensure consistent good quality for the consumers in the target market the most favourable range needs to be determined for the key quality aspects like compression firmness, SSC, TA, SSC/TA and stem quality for the development of both harvest and export standards;

- in the present work, several MAP films were tested and the best out of those was chosen. It would be advisable to try and determine the optimum O_2/CO_2 concentration for purple passionfruit as well as red tamarillo and develop a packaging film and packaging dimensions based on those optimal concentrations;

- the eugenol concentration used in this study might have been too high, leading to toxicity. Since there were clear benefits for cherry stem (Serrano *et al.*, 2005) and grape rachis (Valverde *et al.*, 2005), further study into the effects of lower concentrations of clove oil on the quality of tamarillo stem is recommended;

- although the development of stem yellowing and blackening was related to chlorophyll degradation and an increase in the PPO activity, further investigation of the development of stem blackening related to the chemical composition (e.g. peroxidase and phenolic content) of the tamarillo stem is needed.

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APPENDICES

Appendix A: The preparation of saturated silica gel with clove oil

1. The aluminium moisture can was weighed and approximately 1.2 g of silica gel (Grade 40, 6-12 mesh, Davison Chemical, Baltimore, Maryland) was added.

2. The moisture can containing the silica gel was dried in an oven at 110°C for ten hours.

3. Then, the moisture can was cooled in a desiccator for three hours.

4. The cooled moisture can was weighed and this weight was recorded as the gross weight of the dry moisture can containing the silica gel.

5. The dried weight of silica gel was obtained by subtracting the known weight of the moisture can from the gross weight.

6. The dried silica gel of each moisture can was transferred to a 50 ml borosilicate flask.

7. 3 ml of clove oil was then added to each flask and the flask was covered by a flask cap.

8. The flask was then shaken at 40 rpm for three days at 20°C.

9. The saturated silica gel was transferred to a vacuum filter to remove the excess clove oil from the silica gel.

10. The saturated silica gel was transferred to the moisture can and then weighed. The weight of saturated silica gel was obtained by subtracting the known moisture can weight from the moisture can with the saturated silica gel.

11. The saturated silica gel was then transferred to a 4×5 cm sachet (paper-like) (Tyvek® brand spunbonded olefin, DuPont, Wilmington, DE) that was immediately sealed.

Appendix B: Calculation of water vapour permeance

P'_{H_2O} was estimated using Eq 9.

$$P'_{H_2O} = \frac{r'_{H_2O}}{A\Delta P_{H_2O}} \quad \text{Eq 9}$$

where

P'_{H_2O} = the fruit skin permeance to water vapour (mol s^{-1} m^{-2} Pa^{-1})

r'_{H_2O} = the rate of water loss (mol s^{-1})

A = the surface area of the fruit (m^2)

ΔP_{H_2O} = the difference in partial pressure between the fruit and the environment (Pa)

The rate of water loss was estimated using Eq 10.

$$r'_{H_2O} = \frac{(M_{initial} - M_{final}) - (12 r_{CO_2} M_{final})}{18t} \quad \text{Eq 10}$$

where

$M_{initial}$ = the initial weight of the fruit (g)

M_{final} = the final weight of the fruit (g)

r_{CO_2} = the respiration rate at storage or room temperature (mol g^{-1} s^{-1})

t = time (s)

ΔP_{H_2O} was calculated using the following equations (Eq 11 and Eq 12).

$$\Delta P_{H_2O} = P^f_{H_2O} - P^e_{H_2O} \quad \text{Eq 11}$$

$$P^e_{H_2O} = P^{sat}_{H_2O}(T_w) - \gamma(T_e - T_w) \quad \text{Eq 12}$$

where

$P^f_{H_2O}$ = the partial pressure of water vapour in the fruit (Pa)

$P^e_{H_2O}$ = the partial pressure of water vapour of the environment (Pa)

$P^{sat}_{H_2O}(T_w)$ = the saturated water vapour pressure at the wet bulb temperature (T_w) (Pa)

γ = the psychometric constant (67 Pa $°C^{-1}$)

T_e = the air (dry bulb) temperature (°C)

$P_{H_2O}^{sat}(T_w)$ was calculated using Eq 13.

$$P_{H_2O}^{sat}(T_w) = 611\exp^{\left(17.27\left(\frac{T_w}{T_w+237.3}\right)\right)} \quad \text{Eq 13}$$

$P_{H_2O}^{f}$ was calculated using Eq 14.

$$P_{H_2O}^{f} = 611\exp^{\left(17.27\left(\frac{T_f}{T_f+237.3}\right)\right)} \quad \text{Eq 14}$$

where

T_f = the fruit temperature directly under the skin of the fruit (°C)

Fruit surface area was estimated from fruit weight using Eq 15 as proposed by Maguire *et al.* (2001).

$$A = 0.058\left(\frac{M_{final}}{1000}\right)^{0.685} \quad \text{Eq 15}$$

Appendix C: Calculation of rates of CO_2 production, O_2 consumption, and C_2H_4 production

The respiration rate, expressed as CO_2 production (r_{CO_2}) or O_2 consumption (r_{O_2}), and the ethylene production (C_2H_4) rate were calculated as moles of gas *i* (produced CO_2, consumed O_2, or produced C_2H_4) per fruit mass per time by using Eq 16.

$$r_i = \frac{V_{net} P_{net}}{RTM_f t} \qquad \text{Eq 16}$$

where

r_i = the specific rate of exchange of gas *i* (mol kg^{-1} s^{-1})

V_{net} = the free volume in the jar calculated as the difference in volume between the fruit and the jar (m^3)

P_{net} = net partial pressure of gas *i* as the difference between the partial pressure quantified when the fruit was placed in the jar and a certain period after placing the fruit in the sealed jar (Pa)

R = the universal gas constant (8.3145 Pa m^3 mol^{-1} K^{-1})

T = temperature (K)

M_f = the fruit mass (kg)

t = time (s)

To determine the fruit volume (Mohsenin, 1986), a container filled with water is placed on a balance and the balance is tarred, an individual fruit is completely submerged into the water ensuring the fruit does not touch the bottom or sides of the container. The resulting weight represents the weight of the water displaced by the fruit. Using the density of water this can then be used to determine the volume of water that was displaced and hence the volume of the fruit, using Eq 17.

$$V_f = \frac{W_w}{\rho_w} \qquad \text{Eq 17}$$

where

V_f = the volume of the fruit (m^3)

W_w = the weight of displaced water (kg)

ρ_w = the density of water at 20°C (998.20 kg m^{-3})

Appendix D: Calculation of eugenol concentration

Eugenol concentration (mol m^{-3}) was calculated using Eq 18.

$$C^{Eug} = \frac{K_{GC} A_{GC}}{Vol_{inj}} \qquad \text{Eq 18}$$

where

C^{Eug} = Eugenol concentration (mol m^{-3})

K_{GC} = Detector response or slope of eugenol standard curve as shown in Figure A- 1

A_{GC} = Area of gas chromatogram according to injected volume of sample (area)

Vol_{inj} = Injected volume of sample (m^3)

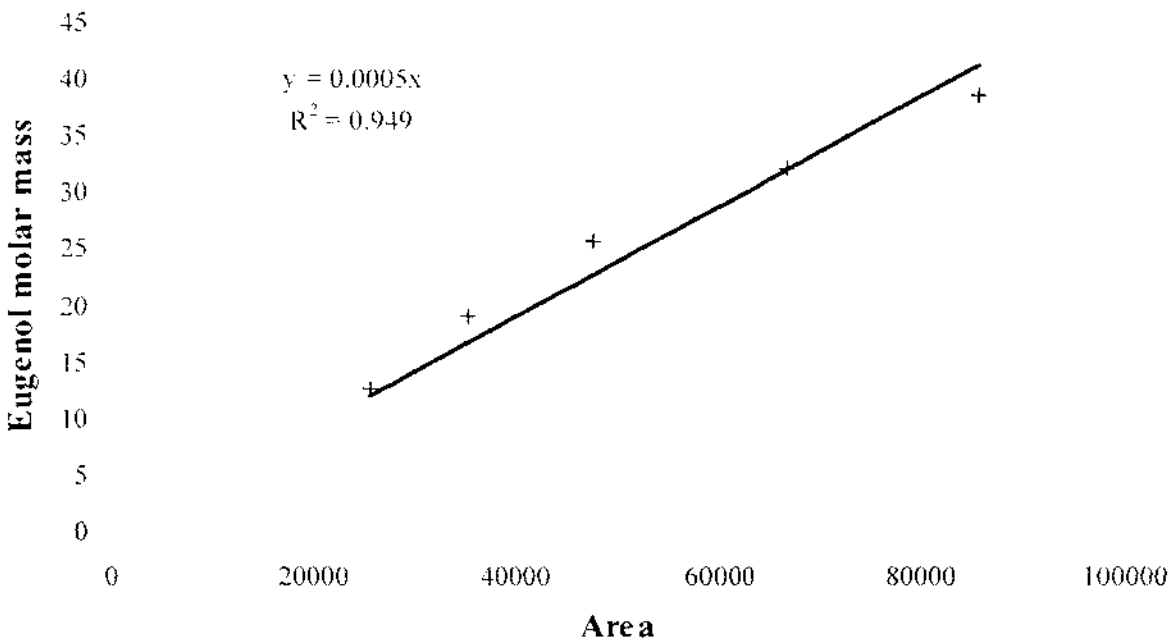


Figure A- 1. Standard curve of eugenol

Appendix E: The appearance scales developed by HortResearch

Figure A- 2. Calyx lifting

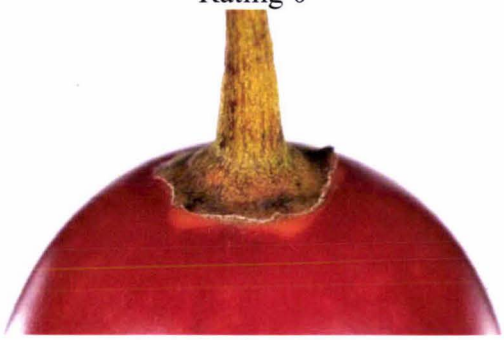
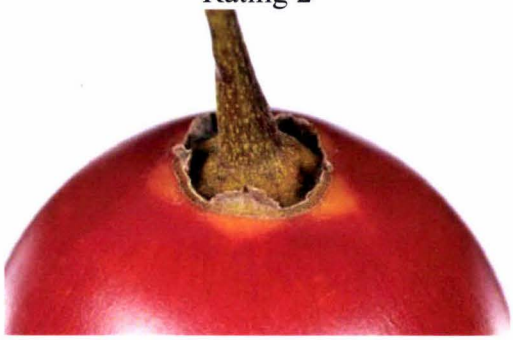
Photo	Description
 Rating 0	All calyx edges flat on fruit surface
 Rating 1	1-2 lobes lifting, slightly
 Rating 2	2-4 lobes lifting, moderately
 Rating 3	More than 3 lobes lifted fully

Figure A- 3. Calyx blackening

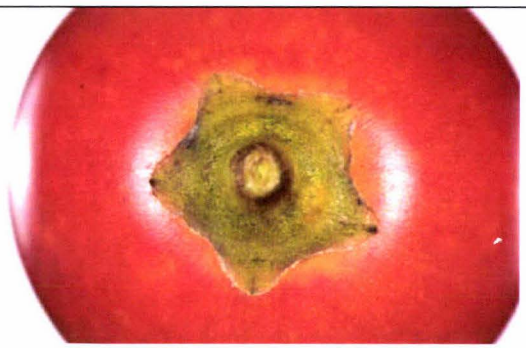
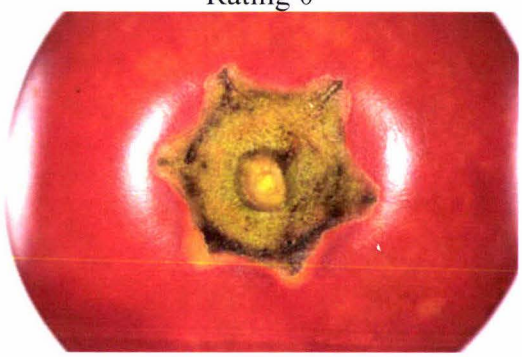
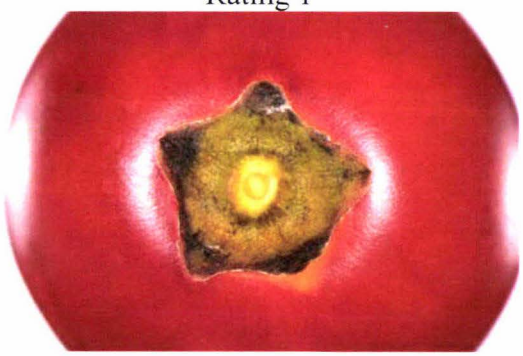
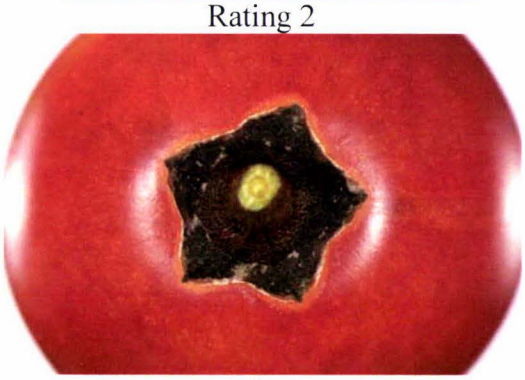
Photo	Description
 Rating 0	Healthy and green calyx
 Rating 1	Less than 25% of calyx black/brown
 Rating 2	25-50% of calyx black/brown
 Rating 3	More than 90% of calyx black/brown

Figure A- 4. Body disorders and discoloration

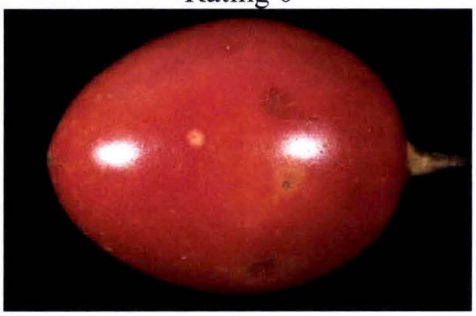
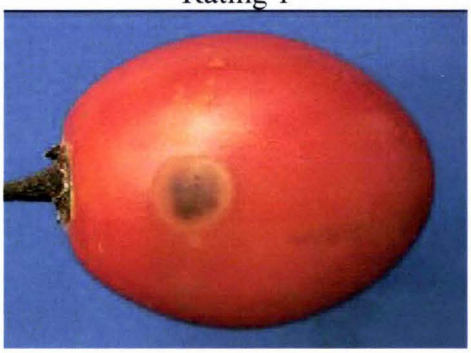
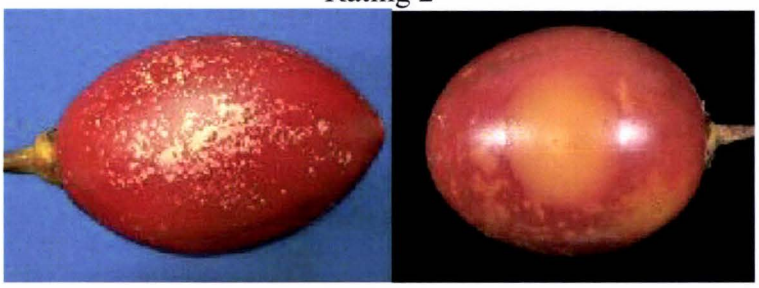
Photo	Description
 Rating 0	No disorders and discoloration present on body
 Rating 1	Slight: 1-3 spots with diameter <2 mm
 Rating 2	Moderate: 1-2 spots diameter 2 -10 mm or 3-10 rots diameter < 2 mm
 Rating 3	Severe: 1-2 spots diameter > 10mm or >10 spots diameter < 2 mm

Figure A- 5. Stem end rots

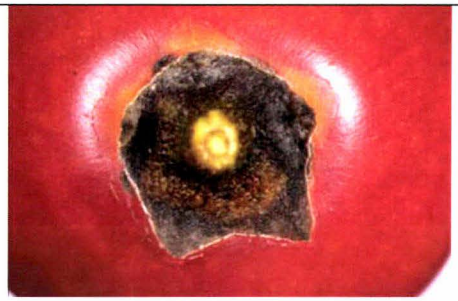
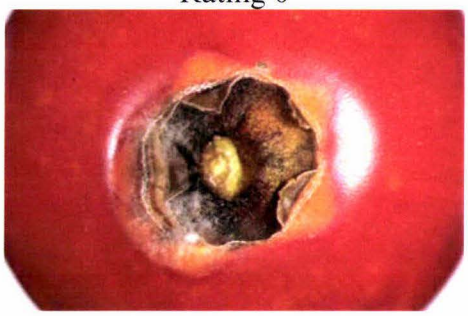
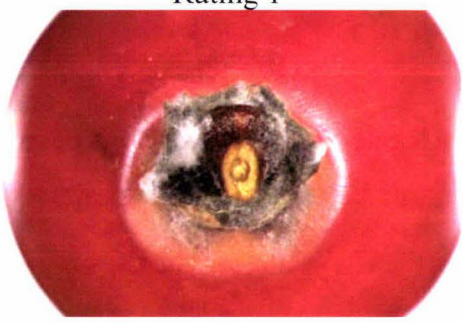
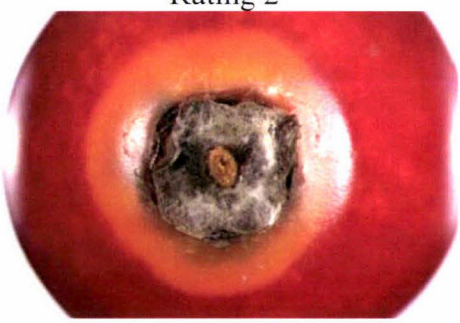
Photo	Description
 Rating 0	No rots around calyx
 Rating 1	Less than 25% of circumference around with rot
 Rating 2	50% of circumference around with rot
 Rating 3	More than 90% of rot around the calyx

Figure A- 6. Stem yellowing

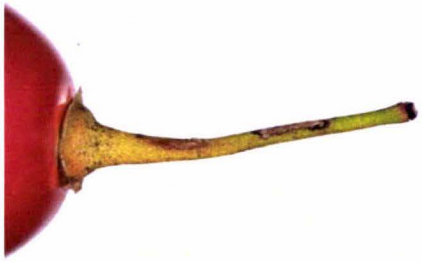
Photo	Description
 Rating 0	No yellowing
 Rating 1	Less than 25% of yellowing
 Rating 2	25-50% of yellowing
 Rating 3	More than 90% of yellowing

Figure A- 7. Stem blackening

Photo	Description
 Rating 0	No blackening
 Rating 1	Less than 25% of blackening
 Rating 2	25-50% of blackening
 Rating 3	More than 90% of blackening

Appendix F: The measurement and calculation of the plastic packaging film permeability to water vapour

A plastic film sample was prepared with an 8×8 cm size and weighed. Distilled water (≈ 5 ml) was added to an aluminium moisture can consisting of a body, a hollow lid, and a sample support (a porous aluminium plate) with known weight (Figure A- 8).

Figure A- 8. Aluminium moisture can

The sample was placed on the sample support, on the body of the aluminium moisture can and secured with the lid. The system was placed on a balance and the weight was dynamically logged for 48 hours (Figure A- 9). The external relative humidity of the ambient air was measured continuously using a RH logger (Tinytalk®II, Gemini Data Loggers, UK). The internal container relative humidity was maintained at nearly 100% as the nature of the closed system.

Figure A- 9. The measurement of the permeability of the plastic packaging film to water vapour

Permeability to water vapour was estimated using Eq 19.

$$P'_{H_2O} = \frac{r'_{H_2O} L_{film}}{A_{film} \Delta P_{H_2O}} \quad \text{Eq 19}$$

where

P'_{H_2O} = the film permeability to water vapour (mol m s^{-1} m^{-2} Pa^{-1})

r'_{H_2O} = The rate of water loss (mol s^{-1})

L_{film} = the thickness of the plastic packaging film (m)

A_{film} = the surface area of the plastic packaging film (m^2)

ΔP_{H_2O} = the difference in partial pressure between the inside of the aluminium can and the environment (Pa)

ΔP_{H_2O} was calculated using the following equations (Eq 20 and Eq 21).

$$\Delta P_{H_2O} = P^{sat}_{H_2O} - P^{e}_{H_2O} \quad \text{Eq 20}$$

$$P^{e}_{H_2O} = 611 \exp^{\left(17.27 \frac{T}{237.3 + T}\right)} \frac{RH}{100} \quad \text{Eq 21}$$

where

$P^{sat}_{H_2O}$ = the saturated water vapour pressure inside the aluminium can (Pa)

$P^{e}_{H_2O}$ = the partial pressure of water vapour of the environment (Pa)

T_e = the air temperature (°C)

RH = relative humidity (%)

Appendix G: Analyses of ethylene production of passionfruit

Table A- 1. Influence of waxing, cold storage at 8°C (days), and shelf life at 20°C (days) on ethylene production rate (C_2H_4)

Treatment		C_2H_4 production (nmol kg^{-1} s^{-1})
Factor (F)	control	1.28*
	wax	1.11
Storage duration (Sd)	20	1.77a
	28	1.23b
	42	0.66c
Shelf life (Sl)	0	0.34*
	7	2.09
Interaction	F × Sd	NS
	F × Sl	NS
	Sd × Sl	*
	F × Sd × Sl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 2. Influence of packaging (Bags 2, 3, and 4), cold storage at 8°C (days), and shelf life at 20°C (days) on ethylene concentration (C_2H_4) in packaging

Treatment		C_2H_4 concentration (ppm)
Packaging (P)	Bag 2	97.79b
	Bag 3	108.88b
	Bag 4	158.81a
Storage duration (Sd)	20	226.99a
	28	68.21b
	42	24.47b
Shelf life (Sl)	0	91.40*
	7	164.33
Interactions	P × Sd	NS
	P × Sl	NS
	Sd × Sl	*
	P × Sd × Sl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Appendix H: Summary of analyses of postharvest quality attributes of tamarillo provided in Table A- 3 to Table A- 16

Table A- 3. Influence of packaging, cold storage at 4°C (days), shelf life at of 20°C (days), and clove oil on weight loss

Factors		Weight loss (%)
Packaging (P)	Control	0.62b
	Bag 2	0.54c
	Bag 3	0.69a
Storage duration (Sd)	14	0.62^{NS}
	28	0.61
	42	0.55
	56	0.60
Shelf life (Sl)	0	0.37*
	3	0.84
Clove oil (Cl)	No	0.55*
	Yes	0.72
Interactions	P × Sd, P × Sl, Sd × Sl, P × Sd × Sl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl	*
	P × Cl, Sd × Cl, Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 4. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on CO_2 production rate (rCO_2)

Factors		rCO_2 (μmol kg^{-1} s^{-1})
Packaging (P)	Control	0.24c
	Bag 2	0.38b
	Bag 3	0.50a
Storage duration (Sd)	0	0.10c
	14	0.37b
	28	0.38b
	42	0.35b
	56	0.44a
Shelf life (Sl)	0	0.20*
	3	0.56
Clove oil (Cl)	No	0.33NS
	Yes	0.42
Interactions	P × Sd, Sd × Sl	*
	P × Sl, P × Cl, Sd × Cl, Sl × Cl, P × Sd × Sl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 5. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on O_2, CO_2, and ethylene (C_2H_4) concentrations in packaging

Factors		O_2 (%)	CO_2 (%)	C_2H_4 (ppm)
Packaging (P)	Bag 2	4.10*	8.27*	34.44*
	Bag 3	1.42	10.72	6.06
Storage duration (Sd)	14	3.44a	9.00c	0.90c
	28	2.04c	11.02a	7.36c
	42	2.30bc	9.55b	28.04b
	56	2.50b	9.11c	39.19a
Shelf life (Sl)	0	2.54NS	7.25*	3.55*
	3	2.63	11.74	31.41
Clove oil (Cl)	No	2.61NS	9.79NS	22.96NS
	Yes	2.58	9.51	13.99
Interactions	P × Sd, P × Sl, Sd × Sl, Sd × Cl, P × Sd × Sl	*	*	*
	Sl × Cl, P × Sd × Cl, Sd × Sl × Cl	*	NS	NS
	P × Sl × Cl	*	*	NS
	P × Cl, P × Sd × Sl × Cl	NS	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 6. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on ethylene production rate (C_2H_4)

Factors		C_2H_4 production rate (nmol kg^{-1} s^{-1})
Packaging (P)	Control	0.02b
	Bag 2	0.09a
	Bag 3	0.01b
Storage duration (Sd)	14	0.01d
	28	0.03c
	42	0.08b
	56	0.18a
Shelf life (Sl)	0	0.01*
	3	0.09
Clove oil (Cl)	No	0.01NS
	Yes	0.03
Interactions	P × Sd, P × Sl, Sd × Sl, P × Sd × Sl	*
	P × Cl, Sd × Cl, Sl × Cl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 7. Influence of packaging, cold storage at 4°C (days), and shelf life at 20°C (days) on eugenol concentration in packaging

Factors		Eugenol concentration (mmol m^{-3})
Packaging (P)	MK	0.07NS
	BL	0.07
Storage duration (Sd)	14	0.04b
	28	0.11a
	42	0.09a
	56	0.05b
Shelf life (Sl)	0	0.04*
	3	0.10
Interactions	P × Sd, P × Sl	*
	Sd × Sl, P × Sd × Sl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 8. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*), and hue angle (h°)

Factors		L^*	a^*	b^*	C^*	h°
Packaging (P)	Control	30.96b	21.81a	7.39b	23.03a	18.62^{NS}
	Bag 2	31.56a	22.10a	7.76a	23.43a	19.27
	Bag 3	31.26ab	20.66b	7.22b	21.93b	19.26
Storage duration (Sd)	0	30.98b	25.74a	8.56a	27.14a	18.32bc
	14	31.77a	21.23c	7.08b	22.40c	18.36c
	28	30.99b	22.16b	8.00a	23.59b	19.87a
	42	30.92b	21.55bc	7.50b	22.82bc	19.08b
	56	31.31b	20.20d	7.00b	21.39d	18.99bc
Shelf life (Sl)	0	30.86^*	23.42^*	7.95^*	24.75^*	18.65^*
	3	31.75	19.67	7.01	20.90	19.51
Clove oil (Cl)	No	31.21^*	22.22^{NS}	7.46^*	23.45^{NS}	18.45^*
	Yes	31.61	21.36	7.80	22.78	20.09
Interactions	P × Sd	*	*	*	*	*
	P × Sl	NS	NS	*	NS	*
	Sd × Sl	*	*	NS	*	*
	Sd × Cl, Sl × Cl, P × Sl × Cl	NS	NS	NS	NS	*
	P × Sd × Sl	*	NS	*	NS	*
	Sd × Sl × Cl	*	NS	NS	NS	*
	P × Cl, P × Sd × Cl, P × Sd × Sl × Cl	NS	NS	NS	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly ($P<0.05$)

Table A- 9. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on stiffness and compression firmness

Factors		Stiffness ($10^6 Hz^2 g^{2/3}$)	Compression (N)
Packaging (P)	Control	6.21ab	11.66b
	Bag 2	6.46a	13.39a
	Bag 3	6.01b	13.40a
Storage duration (Sd)	0	7.94a	14.89a
	14	7.84a	14.59a
	28	5.98b	13.38b
	42	4.95c	10.95c
	56	3.90d	8.64d
Shelf life (Sl)	0	8.46*	16.40*
	3	3.91	9.06
Clove oil (Cl)	No	7.18NS	14.31*
	Yes	6.61	13.55
Interactions	P × Sl, P × Sd × Sl	*	*
	Sd × Sl	NS	*
	P × Sd, P × Cl, Sd × Cl, Sl × Cl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly ($P<0.05$)

Table A- 10. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on the moisture content of the fruit stem

Factors		Moisture content (%)
Packaging (P)	Control	83.37a
	Bag 2	82.90a
	Bag 3	82.14b
Storage duration (Sd)	0	85.44a
	14	82.77b
	28	83.28b
	42	83.05b
	56	81.44c
Shelf life (Sl)	0	83.64*
	3	81.73
Clove oil (Cl)	No	83.00NS
	Yes	82.24
Interactions	P × Sd, Sd × Sl	*
	P × Sl, P × Cl, Sd × Cl, Sl × Cl, P × Sd × Sl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 11. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on chlorophyll content of the fruit stem

Factors		Chlorophyll (µg/g fresh weight)
Packaging (P)	Control	69.51b
	Bag 2	116.64a
	Bag 3	123.86a
Storage duration (Sd)	0	121.09ab
	14	100.32b
	28	100.75b
	42	100.96b
	56	133.21a
Shelf life (Sl)	0	109.99^{NS}
	3	108.19
Clove oil (Cl)	No	93.22^{*}
	Yes	133.94
Interactions	P × Sd, P × Sl, Sd × Sl, P × Cl	*
	Sd × Cl, Sl × Cl, P × Sd × Sl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 12. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on the activity of polyphenol oxidase (PPO)

Factors		PPO (enzyme unit/g fresh weight)
Packaging (P)	Control	6426.22^{NS}
	Bag 2	7315.05
	Bag 3	6925.21
Storage duration (Sd)	0	1955.13d
	14	6495.25c
	28	7335.87b
	42	6018.26c
	56	8505.93a
Shelf life (Sl)	0	5298.62*
	3	8757.10
Clove oil (Cl)	No	7070.01*
	Yes	6808.47
Interactions	P × Sd, P × Sl, Sd × Sl, Sd × Cl, P × Sd × Sl, P × Sl × Cl	*
	P × Cl, Sl × Cl, P × Sd × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)

Table A- 13. The score of stem-end rots of the control fruit and fruit in Bags 2 and 3 with or without clove oil during storage at 4°C (days) with and without 3 days of shelf life at 20°C

	Storage duration at 4°C (days)					Storage duration + 3 days of shelf life (days)			
Treatments	0	14	28	42	56	17	31	45	59
Control	0	0	0	0	0.19	0	0	0.25	0.06
Bag 2	0	0	0.06	0	0	0	0	0	0.38
Bag 2 +clove oil	0	0	0	0	0.06	0	0	0.13	0
Bag 3	0	0	0	0	0	0	0	0	0
Bag 3 +clove oil	0	0	0	0	0	0	0	0	0

Table A- 14. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on fruit discoloration score

Factors		Fruit discoloration score
Packaging (P)	Control	0.21c
	Bag 2	1.05b
	Bag 3	2.00a
Storage duration (Sd)	0	0.00d
	14	0.40c
	28	1.03b
	42	1.88a
	56	1.77a
Shelf life (Sl)	0	1.04*
	3	1.44
Clove oil (Cl)	No	0.70*
	Yes	2.07
Interactions	P × Sd, Sd × Sl, P × Cl, Sd × Cl, Sl × Cl, P × Sd × Cl	*
	P × Sl, P × Sd × Sl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly ($P<0.05$)

Table A- 15. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on the scores of calyx lifting and blackening

Factors		Calyx lifting	Calyx blackening
Packaging (P)	Control	0.24c	0.23c
	Bag 2	0.34b	0.54b
	Bag 3	0.45a	0.76a
Storage duration (Sd)	0	0.00cd	0.00c
	14	0.01d	0.01c
	28	0.13c	0.07c
	42	0.53b	0.76b
	56	0.81a	1.45a
Shelf life (Sl)	0	0.22*	0.39*
	3	0.51	0.73
Clove oil (Cl)	No	0.32NS	0.40*
	Yes	0.42	0.80
Interactions	P × Sd, P × Sl, Sd × Sl, P × Sd × Sl	*	*
	Sd × Cl	NS	*
	P × Cl, Sl × Cl, P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly ($P<0.05$)

Table A- 16. Influence of packaging, cold storage at 4°C (days), shelf life at 20°C (days), and clove oil on the scores of stem yellowing and blackening

Factors		Stem yellowing	Stem blackening
Packaging (P)	Control	1.37a	0.58c
	Bag 2	0.77b	1.05b
	Bag 3	0.66b	1.20a
Storage duration (Sd)	0	0.00c	0.00d
	14	0.08c	0.13d
	28	0.85b	0.71c
	42	1.38a	1.41b
	56	1.43a	1.88a
Shelf life (Sl)	0	0.71*	0.74*
	3	1.07	1.28
Clove oil (Cl)	No	0.97NS	0.77*
	Yes	0.71	1.37
Interactions	P × Sd, Sd × Sl	*	*
	P × Sl, P × Cl	*	NS
	Sd × Cl, Sl × Cl, P × Sd × Sl	NS	*
	P × Sd × Cl, P × Sl × Cl, Sd × Sl × Cl, P × Sd × Sl × Cl	NS	NS

NS, Non significant or *, significant (α=0.05). Values followed by different letters in a column differ significantly (P<0.05)